THE JOURNAL OF PHYSICAL CHEMISTRY B
A JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

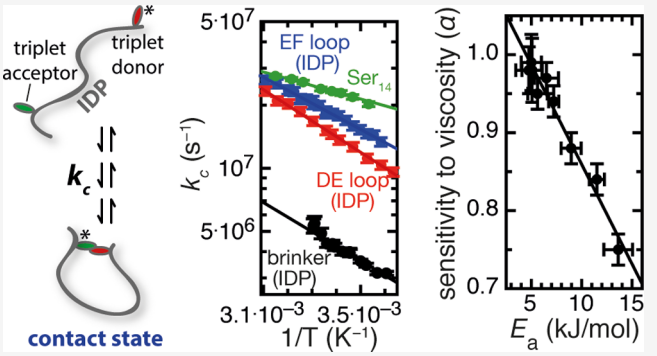
pubs.acs.org/JPCB
Article

Local and Large-Scale Conformational Dynamics in Unfolded Proteins and IDPs. II. Effect of Temperature and Internal Friction

Florian Krieger,[‖] Karin Stecher,[‖] Christian Nyffenegger, Michael Schleeger, and Thomas Kiefhaber*

Cite This: J. Phys. Chem. B 2023, 127, 8106−8115

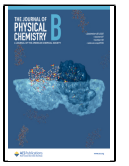
ABSTRACT: Internal dynamics of proteins are essential for protein folding and function. Dynamics in unfolded proteins are of particular interest since they are the basis for many cellular processes like folding, misfolding, aggregation, and amyloid formation and also determine the properties of intrinsically disordered proteins (IDPs). It is still an open question of what governs motions in unfolded proteins and whether they encounter major energy barriers. Here we use triplet−triplet energy transfer (TTET) in unfolded homopolypeptide chains and IDPs to characterize the barriers for local and long-range loop formation. The results show that the formation of short loops encounters major energy barriers with activation energies (E_a) up to 18 kJ/mol (corrected for effects of temperature on water viscosity) with very little dependence on amino acid sequence. For poly(Gly-Ser) and polySer chains the barrier decreases with increasing loop size and reaches a limiting value of 4.6 ± 0.4 kJ/mol for long and flexible chains. This observation is in accordance with the concept of internal friction encountered by chain motions due to steric effects, which is high for local motions and decreases with increasing loop size. Comparison with the results from the viscosity dependence of loop formation shows a negative correlation between E_a and the sensitivity of the reaction to solvent viscosity (α) in accordance with the Grote−Hynes theory of memory friction. The Arrhenius pre-exponential factor (A) also decreases with increasing loop size, indicating increased entropic costs for loop formation. Long-range loop formation in the investigated sequences derived from IDPs shows increased E_a and A compared with poly(Gly-Ser) and polySer chains. This increase is exclusively due to steric effects that cause additional internal friction, whereas intramolecular hydrogen bonds, dispersion forces, and charge interactions do not affect the activation parameters.



INTRODUCTION

Structural and dynamic properties of unfolded polypeptide chains are important for many cellular processes like protein folding, misfolding, aggregation, and amyloid formation. Furthermore, the cellular function and the coupled folding and binding reaction of intrinsically disordered proteins (IDPs) are linked to the structure and the internal dynamics of the unfolded state. It is still an open question whether the dynamics in unfolded polypeptide chains are limited by significant energy barriers (>kT) and whether barriers are different for local and large-scale motions. Internal polymer dynamics were postulated to encounter barriers caused by backbone bond rotations.[1] In agreement with this model, barriers for local bond rotations between 10 and 20 kJ/mol were reported for synthetic polymer chains in NMR and fluorescence anisotropy studies[2−7] and were also observed in MD simulations on the local bond rotations in short peptides.[8] Larger-scale dynamics in polypeptide chains may encounter additional barriers due to breakage/formation of intramolecular interactions like hydrogen bonds, electrostatic interactions and van der Waals interactions/dispersion forces. These interactions contain contributions from both the polypeptide backbone and the amino acid side chains and may be local and nonlocal. Thus, different kinds of barriers may oppose internal chain motions and thus create internal friction, which was first proposed by Kuhn and Kuhn.[1] They further postulated that effective barrier heights for backbone chain motions and thus effective internal friction for chain motions should decrease with increasing chain length since bond rotations become more likely.[9] On the other hand, increasing chain length leads to an increasing number of possible intramolecular interactions in unfolded polypeptide chains and may thus result in larger barrier heights and additional internal friction for polypeptide chain motions.

Received: June 16, 2023
Revised: August 15, 2023
Published: September 18, 2023



https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

Experiments on the synthetic linear polymers polysterene,[2] 1,2-polybutadiene,[3,4,6] and polyisoprene[5,7] as well as MD simulations on local dynamics in short peptides[8,10] further suggested that the observed barriers for local chain dynamics influence the coupling of chain dynamics to solvent motions and cause a weaker than $1/\eta$ viscosity dependence for these dynamics in accordance with the Grote−Hynes theory of memory friction.[11,12]

Previous experimental studies addressing the barrier heights for polymer chain dynamics mainly focused on the local dynamics of bond rotations[2−7] and on local segmental motions.[13−15] However, for the dynamics of unfolded proteins and protein folding, larger-scale motions leading to nonlocal intramolecular interactions through loop formation are important. We therefore determined effective activation energies and Arrhenius pre-exponential factors for both local and long-range chain motions in unfolded polypeptide chains by applying triplet−triplet energy transfer (TTET). We measured the dynamics of loop formation in polypeptide chains of different lengths and amino acid sequence. As described in the accompanying paper, TTET between a xanthone (Xan) donor and a naphthylalanine (Nal) acceptor is a fast and diffusion-controlled two electron transfer process that requires van der Waals contact between the triplet donor and acceptor (Dexter mechanism). It thus yields absolute rate constants for contact formation between the triplet donor and acceptor, which corresponds to the rate constant for loop closure (k_c). We applied TTET between Xan and Nal attached at specific sites on polypeptide chains to investigate the temperature dependence of loop formation in the same polypeptide chains, for which we determined the viscosity dependence in the accompanying paper. The results enable us to dissect the previously described effect of loop size and chain flexibility on the dynamics of loop formation[16] into contributions from activation energy, i.e., effective barrier heights and from chain entropy. The investigated chains include homopolymers consisting of either flexible poly-(glycine-serine) ($(Gly\text{-}Ser)_n$) or stiffer polyserine (Ser_n) chains of different length. In addition, we investigated fragments from the two IDPs parvalbumin and brinker domain and a short turn fragment from the GB1 hairpin to test whether steric effects and/or intramolecular interactions introduced by side chains create additional barriers for loop formation (for sequences and structures, see the accompanying paper). Comparing the results to the effect of solvent viscosity on loop formation described in the accompanying paper further enabled us to test the correlation between the effective activation energy for loop formation and its sensitivity to solvent viscosity. Our results reveal significant effective barriers for loop formation up to E_a = 18 kJ/mol for formation of short loops, which decrease with increasing chain length in homopolypeptide chains until a limiting lower value of about E_a = 4.6 ± 0.4 kJ/mol is reached for long and flexible chains. Additional steric effects introduced by side chains create additional barriers, whereas intramolecular interactions do not lead to an increase in the effective barrier height. Comparing the results to the viscosity dependence of loop formation shows that the effective barrier height correlates with a reduced sensitivity of the reaction to solvent viscosity, as predicted for simple barrier crossing reactions by the Grote−Hynes theory.[11]

MATERIALS AND METHODS

All peptides were synthesized, purified, and labeled as described in the accompanying paper. TTET experiments were performed as described in the accompanying paper. Transient triplet absorption decay data were collected using a Laser Flash Reaction Analyzer (LKS.60) from Applied Photophysics with a themostated cell holder. The temperature stability was ±0.1 °C. All measurements were performed in 10 mM potassium phosphate buffer, pH 7.0. The transient triplet absorbance of xanthone was measured at 590 nm. Typically 6−10 kinetic traces were averaged and analyzed using the software ProFit (Quantum Soft, Zürich, Switzerland). Error bars in the figures are given by standard deviation obtained from ProFit.

RESULTS AND DISCUSSION

Temperature Dependence of the Probing Reaction. A suitable experimental system to measure the effect of the temperature on the dynamics of loop formation in unfolded polypeptide chains requires a probing reaction that is fully diffusion-controlled and thus does not contribute to the observed activation parameters. We therefore determined the activation energy for TTET between the triplet donor xanthonic acid (Xan) and the triplet acceptor 1-naphthylalanine (Nal). The bimolecular rate constant for intermolecular TTET between Xan and Nal was shown to be $k_T = 3.0 \times 10^9$ M^{-1} s^{-1} and increases to $k_T = 4.1 \times 10^9$ M^{-1} s^{-1} when the smaller naphthylacetic acid is used as an acceptor,[16,17] which is in agreement with the expected rate constants for a diffusion-controlled bimolecular reaction. The reaction was further shown to be fully viscosity dependent ($k_T \sim 1/\eta$; see the accompanying paper), as expected for a diffusion-controlled process. The activation energy for TTET was determined in bimolecular TTET experiments between Xan and Nal at different temperatures. Figure 1 shows an Arrhenius plot for the temperature dependence of the rate constant for intermolecular TTET from Xan to Nal (k_T) measured under

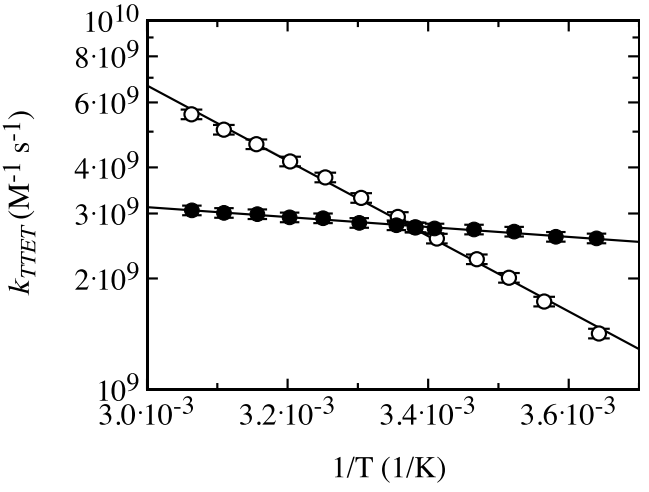


Figure 1. Arrhenius plot of the temperature dependence of bimolecular TTET from Xan to Nal. Both uncorrected (○) and viscosity-corrected (●) data are shown. An α value of 1 was used for viscosity correction, as observed in the viscosity dependence of bimolecular TTET (see the accompanying paper). A linear fit gives E_a = 19.5 ± 0.4 and 2.6 ± 0.4 kJ/mol for the uncorrected and viscosity-corrected data, respectively.

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

pseudo-first-order conditions, which yields an apparent activation energy (E_a^{app}) of 19.5 ± 0.4 kJ/mol according to the Arrhenius equation

$$k = A \cdot e^{-E_a/RT} \quad (1)$$

Since solvent viscosity changes with temperature, the experimentally determined bimolecular rate constants, k_{obs}, were corrected for the effect of temperature on water viscosity (η) by normalizing the rate constants to the reference temperature of 22.5 °C (η_0 = 0.94) using the relationship

$$k_{obs} = k_T \cdot \left(\frac{\eta}{\eta_0}\right)^{-\alpha} \quad (2)$$

We applied the experimentally determined $1/\eta$ viscosity dependence, i.e., α = 1, for the reaction as reported in the accompanying paper, to obtain the viscosity-corrected rate constants for TTET, k_T, at the different temperatures. This correction yields an activation energy (E_a) of 2.6 ± 0.4 kJ/mol which corresponds to RT (Figure 1) and is in accordance with a thermally activated diffusion-controlled process. This result together with the observed $1/\eta$ viscosity dependence shows that the TTET process itself is diffusion-controlled and thus does not contribute to the temperature dependence of the dynamics of intrachain loop formation described in the following.

Temperature Dependence of Loop Formation in Unfolded Polypeptide Chains. We performed intramolecular TTET experiments to determine the effect of temperature on the rate constant for loop formation (k_c) in unfolded polypeptide chains of different lengths and amino acid sequences. The triplet donor Xan was attached at the N-terminus, and the triplet acceptor Nal was attached near the C-terminus of the polypeptide chains (see Figure 2 of the accompanying paper). All polypeptide chains are unfolded as judged by circular dichroism (CD) spectra (see the accompanying paper). From the temperature dependence of loop formation we determined the activation energies (E_a) and the Arrhenius pre-exponential factor (A) in an Arrhenius plot according to eq 1. The rate constants measured at the different temperatures (k_{obs}) were corrected for the effect of temperature on solvent viscosity by applying eq 2 to yield the viscosity-corrected rate constants for loop formation, k_c, normalized to the reference viscosity η_0 = 0.94 cP. For each peptide the experimentally determined α values were used for correction (see the accompanying paper).

Effect of Chain Length and Backbone Flexibility on the Activation Parameters for Loop Formation. The effect of chain length and backbone flexibility on the activation parameters for loop formation was tested in poly(Gly-Ser) and polyserine homopolypeptide chains. Figure 2A shows the effect of temperature on the TTET kinetics between Xan and Nal for a long and flexible poly(glycine-serine) loop ($(Gly\text{-}Ser)_{14}$) measured by the decay in Xan triplet absorbance at 590 nm after excitation with a 4 ns laserflash at 355 nm. The TTET kinetics become faster with increasing temperature, which shows that loop formation is accelerated. The resulting Arrhenius plots for the uncorrected (k_{obs}) and viscosity-corrected (k_c) rate constants for loop formation (see eq 2) are shown in Figure 2B and compared to the respective Arrhenius plots for the short and flexible $(Gly\text{-}Ser)_1$ loop and the short but stiffer Ser_2 loop. All Arrhenius plots are linear, indicating the absence of changes in heat capacity for the reaction as expected for chain dynamics in a fully solvated polypeptide chain. The linearity of all Arrhenius plots over the entire temperature range further shows that the same rate-limiting process determines loop formation at all temperatures for a given peptide.[18−20] Comparison of the results from the Arrhenius plots reveals that the activation energy for loop formation depends on the amino acid sequence and chain length. Formation of the shorter $(Gly\text{-}Ser)_1$ loop has a larger activation energy (E_a = 14.2 ± 0.6 kJ/mol) than formation of the longer $(Gly\text{-}Ser)_{14}$ loop (E_a = 5.0 ± 0.6 kJ/mol). Comparison of loops with identical length shows that formation of the stiffer Ser_2 loop has a higher activation energy (E_a = 18.1 ± 0.5 kJ/mol) than formation of the more flexible $(Gly\text{-}Ser)_1$ loop.

The Arrhenius pre-exponential factor (A) also depends on chain length and on amino acid sequence. Formation of the

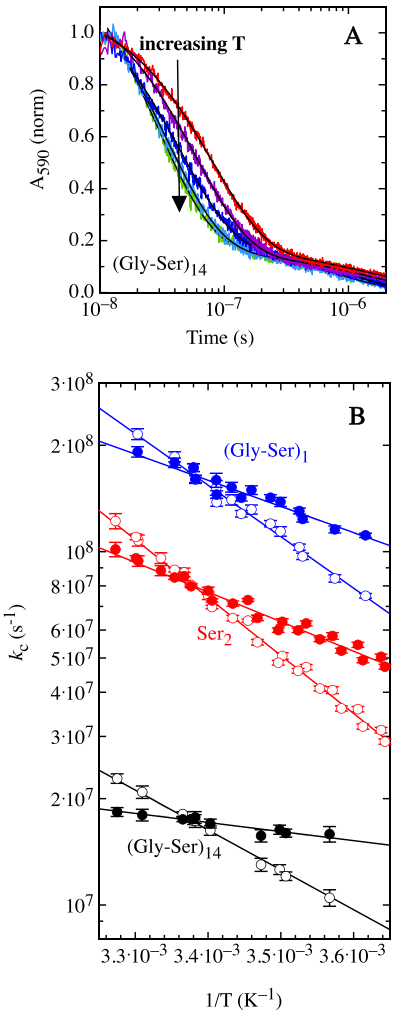


Figure 2. Effect of temperature on loop formation in various polypeptide chains as indicated. The rate constant for loop formation was measured in intramolecular TTET experiments between Xan and Nal (A). Panel B shows Arrhenius plots of the rate constant of loop formation (k_c) for different polypeptides. Both uncorrected (open circles) and viscosity-corrected rate constants (filled circles) are shown. The experimentally determined α values (see the accompanying paper) were used for viscosity correction. The uncorrected activation energies (E_a) are 21.6 ± 0 kJ/mol for $(GS)_{14}$, 27.9 ± 0.6 kJ/mol for $(GS)_1$, and 31.4 ± 0.3 kJ/mol for S_2. The viscosity-corrected activation energies and pre-exponential factors are shown in Figure 3.

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

shorter $(Gly\text{-}Ser)_1$ loop has a higher pre-exponential factor ($A = (4.6 \pm 0.3) \times 10^{10}$ s^{-1}) than formation of the longer $(Gly\text{-}Ser)_{14}$ loop ($A = (1.2 \pm 0.3) \times 10^{8}$ s^{-1}), and formation of the stiffer Ser_2 loop has an even higher pre-exponential factor ($A = (1.1 \pm 0.2) \times 10^{11}$ s^{-1}). Comparing the effect of chain length on E_a and A for all investigated poly(Gly-Ser) and polyserine loops confirms these findings (Figure 3A and B).

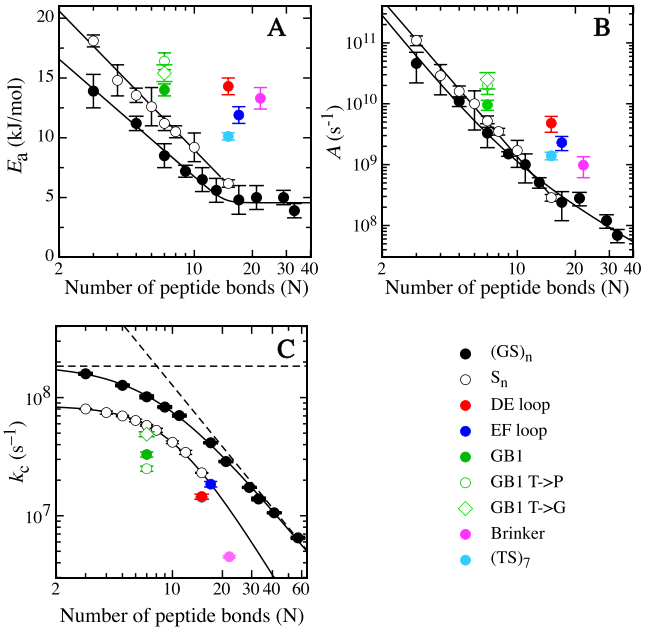


Figure 3. Effect of chain length and amino acid sequence on (A) the activation energy (E_a) and (B) Arrhenius pre-exponential factor (A) for loop formation in different polypeptide chains as indicated. For comparison, the effect of chain length and amino acid sequence on the rate constant for loop formation (k_c) for all polypeptide chains is shown in panel C.

Figure 3A shows that the activation energy for loop formation decreases with increasing chain length and reaches a limiting value of $E_a = 4.6 \pm 0.4$ kJ/mol for long poly(Gly-Ser) chains with $N > 17$. Formation of polyserine loops is associated with a higher E_a than poly(Gly-Ser) loops of the same length (Figure 3A). The effect of chain length and amino acid sequence on E_a can be explained based on the polymer properties of polypeptide chains. Formation of short loops occurs within the persistence length of the chain, where chain stiffness opposes loop formation mainly due to steric constraints. This leads to high barriers that create a large internal friction for the formation of short loops. This model is supported by the higher barriers observed for the formation of short polyserine loops compared to the more flexible poly(Gly-Ser) loops of identical length (Figure 3A). Chain stiffness decreases with increasing loop length, and thus, also the activation energy decreases up to a limiting loop length, which is reached when the chain behaves like a Gaussian chain with excluded volume. The decrease in E_a with loop size is slightly smaller for poly(Gly-Ser) chains ($E_a \sim N^{-1.15\pm0.3}$) compared to polyserine ($E_a \sim N^{-1.22\pm0.02}$; Figure 3A). The Gaussian limit is reached at $N > 17$ for the poly(Gly-Ser) chains, which is identical to the loop size above which the rate constant for loop formation scales with $N^{-1.73\pm0.02}$, compatible with the scaling of a Gaussian chain with excluded volume[16] (Figure 3C). In this Gaussian chain limit, loop formation exhibits a $1/\eta$ viscosity dependence ($\alpha = 1$ in eq 2) as expected for purely diffusive chain motions during loop closure (see the accompanying paper). However, despite a $1/\eta$ viscosity dependence, the activation energy for formation of long loops does not reach RT but shows a limiting value of 4.6 ± 0.4 kJ/mol. This result shows that the conformational search for the formation of long loops is not completely barrierless. However, long loops in flexible chains can form through motions that encounter only minor barriers compared to local chain motions and thus experience less internal friction than local loop formation. This result is in accordance with the predictions from Kuhn and Kuhn, who proposed that internal friction due to local barriers for polymer chain motions should decrease with increasing chain length.[1,9] The barriers observed for the formation of long and flexible poly(Gly-Ser) loops are within the range of barrier heights of 2−6 kJ/mol observed for single bond rotations in the peptide backbone in microwave spectra[21] and predictions based on theoretical considerations.[22]

Contributions from Chain Entropy to the Kinetics of Loop Closure. The Arrhenius pre-exponential factor (A) decreases with increasing loop length in both the poly(Gly-Ser) and the polyserine homopolypeptide chains (Figure 3B). A contains contributions from the activation entropy, $\Delta S^{0\ddagger}$, and from the maximum rate constant for loop formation in the absence of free energy barriers, k_0, according to

$$A = k_0 \cdot e^{\Delta S^{0\ddagger}/R} \tag{3}$$

Since k_0 for loop formation is not known, we did not attempt to determine absolute values for $\Delta S^{0\ddagger}$. However, since the elementary bond rotations underlying the dynamics of loop formation should be identical for all chain lengths in each homopolypeptide series, it can be assumed that k_0 is identical within the polyserine and poly(Gly-Ser) chains, respectively. Thus, the Arrhenius pre-exponential factor within each homopolypeptide series mainly reflects differences in entropic costs for loop formation. Figure 3B shows that A decreases with increasing loop length, which shows that the loss in conformational entropy upon loop formation increases with loop length due to the increased total conformational space. The scaling of the loss in conformational entropy with loop length is similar for short poly(Gly-Ser) and polyserine chains with $A \sim N^{-3.9\pm0.4}$ and $A \sim N^{-3.7\pm0.1}$, respectively. (Figure 3B) However, for long poly(Gly-Ser) chains the scaling factor decreases and can be described by $A \sim N^{-1.8}$, as expected for the entropy loss upon loop formation for a Gaussian chain with excluded volume.[23] This change in the scaling factor occurs at the same loop size ($N = 17$) at which the activation energy reaches its limiting value of 4.6 ± 0.4 kJ/mol (Figure 3A), where loop formation becomes fully viscosity-dependent ($k_c \sim 1/\eta$) and where k_c scales with $N^{-1.73\pm0.02}$ (Figure 3C).[16] This suggests that internal motions in poly(Gly-Ser) chains are well-described by a Gaussian chain with excluded volume above a segment size of about 17 amino acids and supports the assumption that the decreasing pre-exponential factor with increasing loop size mainly reflects the increased loss in conformational entropy. Comparing the different homopolymers shows that A is smaller for the more flexible poly(Gly-Ser) loops, which sample a larger conformational space compared to polyserine loops of identical length. A similar behavior has previously been observed for the formation of very short host−guest loops with the sequence Ser-Xaa-Ser

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

(Xaa = Pro, Ser, Gly), which showed a larger pre-exponential factor for the stiffer proline residue in the central position and a smaller pre-exponential factor for the more flexible glycine residue compared to serine.[13] However, the dynamics for elementary chain motions, k_0, are most likely different for glycine and proline compared to those of all other amino acids, which also contributes to A (eq 3).

Effect of Temperature on Chain Stiffness. The effect of temperature on the rate constants for the formation of loops of different lengths gives additional information on the effect of temperature on chain stiffness. At the reference temperature of 22.5 °C the dynamics of loop formation can be described by the dynamics of a Gaussian chain above a loop size of about 17 amino acids for the poly(Gly-Ser) loops. Below this loop size, chain stiffness contributes to the dynamics of loop closure, and formation of very short loops is only weakly dependent on loop size for the poly(Gly-Ser) loops and virtually independent of loop size for the polyserine chains. Comparing the effect of loop size on the experimentally observed rate constant for loop formation (k_{obs}) at different temperatures shows that the limiting loop size, at which the Gaussian limit is reached, is reduced with increasing temperatures both for the poly(Gly-Ser) (Figure 4A) and for the polyserine loops (Figure 4B) indicating that chain stiffness decreases with increasing temperature. A similar effect was observed in the presence of 6 M GdmCl, which is a good solvent for the polypeptide chain and leads to increased chain flexibility compared to water.[16] The effect of temperature on the length dependence of the rate constant for loop formation (k_{obs}) contains contributions from the effect of temperature on chain stiffness and also from changes in solvent viscosity. The activation energy is higher for the formation of shorter loops, and thus, loop formation is more strongly accelerated with increasing temperature for short loops compared to long loops. On the other hand, shorter loops show a weaker viscosity dependence (see the accompanying paper) and thus decreasing solvent viscosity with increasing temperature has a smaller effect on the formation of shorter loops. Parts C and D of Figure 4 show the effect of chain length on the viscosity-corrected rate constants for loop formation (k_c), which reflects the effect of the temperature on the intrinsic chain properties. Temperature has only a little effect on internal, large-scale chain motions within the Gaussian chain limit, but it reduces chain stiffness by accelerating local dynamics within the persistence length, due to their high activation energies. Figure 4 further shows that the scaling of k_{obs} with the loop size for poly(Gly-Ser) chains in the Gaussian limit is also slightly affected by temperature (Figure 4F). The scaling of k_{obs} with loop size can be described by equation 4

$$k_{obs} = \frac{1}{1/k_{lim} + 1/(k_0 \cdot N^m)} \quad (4)$$

where m reflects the scaling exponent, k_{lim} is the length independent rate constant for formation of short loops, and k_0 is the maximum rate constant for zero loop size extrapolated from the Gaussian limit. The viscosity-corrected Arrhenius plots for the effect of temperature on k_{lim} give activation parameters of E_a = 17.7 ± 1.8 kJ/mol (A = (2.6 ± 1.9) × 10^{11} s^{-1}) and 17.0 ± 1.3 kJ/mol (A = (8.0 ± 4.4) × 10^{10} s^{-1}) for the poly(Gly-Ser) and polyserine chains, respectively (Figure 4E). The scaling exponent, m, decreases from −1.82 ± 0.06 at 275.65 K to −1.66 ± 0.05 at 315.65 K (Figure 4F), which indicates decreasing contributions from the excluded volume

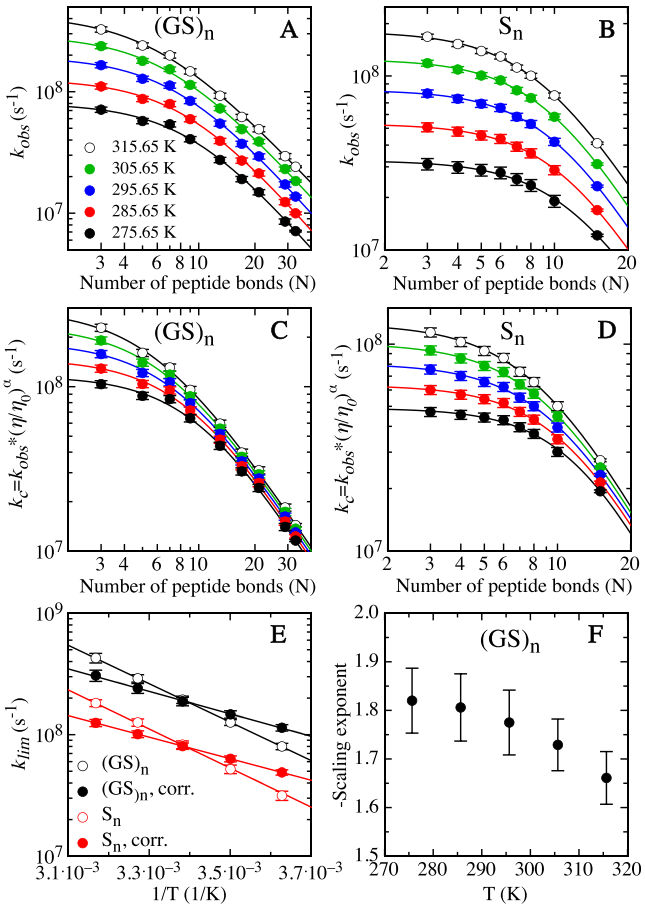


Figure 4. Effect of temperature on the scaling of rate constant for loop formation (k_{obs}) with chain length for poly(Gly-Ser) (A) and polySer (B) chains. Panels C and D show the respective viscosity-corrected rate constants (k_c), which reflect the effect of temperature on intrinsic chain properties. Panel E shows uncorrected and viscosity-corrected Arrhenius plots for the length-independent limiting rate constant for loop formation (k_{lim}) that is reached for the formation of short loops (eq 4).[16] The Arrhenius fit according to eq 1 yields values of E_a = 30.3 ± 1.6 kJ/mol, A = (4.4 ± 2.9) × 10^{13} s^{-1} and E_a = 17.7 ± 1.8 kJ/mol, A = (2.6 ± 1.9) × 10^{11} s^{-1}, respectively, for the uncorrected and viscosity-corrected Arrhenius plots for poly(Gly-Ser) and respective values of E_a = 31.7 ± 1.7 kJ/mol, A = (3.3 ± 2.2) × 10^{13} s^{-1} and E_a = 17.0 ± 1.3 kJ/mol, A = (8.0 ± 4.4) × 10^{10} s^{-1} for polySer. Panel D displays the effect of temperature on the scaling exponent for the effect of loop size on k_{obs} (eq 4).

effect to the scaling exponent due to weaker intramolecular interactions with increasing temperature. This result is in accordance with theoretical work by Flory, who showed that the excluded volume of a polymer chain contains contributions from intramolecular interactions, which decrease with increasing temperature.[24] Even at the highest temperature applied in this study (315.65 K) the scaling exponent does not reach the value of −1.5, which is expected for an ideal chain at Θ-temperature. This result shows that the Θ-point for poly(Gly-Ser) chains lies at even higher temperatures.

Barriers and Entropic Costs for the Formation of Natural Protein Loops. To test for contributions from interactions and steric effects introduced by side chains, we determined the activation parameters for loop formation in several unfolded polypeptide chains of different length and amino acid sequence that were derived from natural proteins and compared them with the properties of the poly(Gly-Ser)

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

and polyserine chains that only form backbone−backbone and backbone−serine hydrogen bonds. We studied two peptides derived from carp parvalbumin (EF loop and DE loop), a fragment from the DNA-binding domain of the brinker protein and the turn region of hairpin 1 from protein G (GB1 hairpin; for sequences and protein structures, see the accompanying paper). Carp parvalbumin and the brinker domain are IDPs that are unfolded in the absence of their binding partners, Ca^{2+} and DNA, respectively. Loop formation in all natural sequences shows higher activation energies and larger Arrhenius pre-exponential factors compared to the poly(Ser) and poly(Gly-Ser) chains of the same length (Figures 3 and 5).

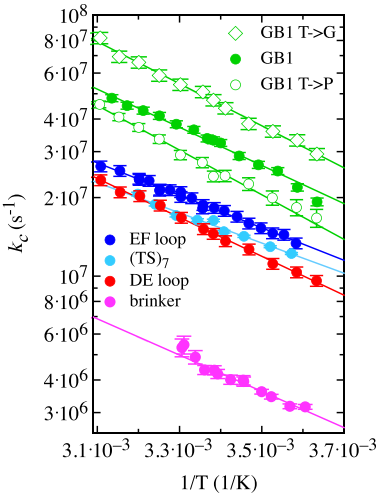


Figure 5. Arrhenius plots for the viscosity-corrected rate constant of loop formation (k_c) for different polypeptide chains and IDP fragments, as indicated. The Arrhenius parameters obtained from the fits are shown in Figure 3.

This indicates that side-chain interactions or side-chain sterics create additional barriers for loop formation and lead to a more restricted conformational space compared to poly(Gly-Ser) and polyserine chains. The viscosity-corrected activation energies for loop formation are very similar for all four protein fragments with values between 12.4 ± 0.7 kJ/mol for the parvalbumin EF loop and 15.7 ± 0.8 kJ/mol for the GB1 turn (Figures 3 and 5), although the fragments vary in size between 6 amino acids (GB1 turn) and 21 amino acids (brinker fragment) and have different amino acid compositions. The lowest activation energy for loop formation is found for the parvalbumin EF loop, which is likely due to the large fraction of glycine residues (4 Gly; 25%) that are equally distributed throughout the sequence. This fraction of Gly residues is larger compared to the GB1 turn (no Gly), the DE loop (1 Gly; 7%), and the brinker fragment (2 Gly within the N-terminal three residues; 9.5%). This interpretation is in agreement with our earlier results on the effect of single glycine and proline residues on the activation energy for loop formation in Ser-Xaa-Ser host−guest peptides, which showed that Gly at position Xaa decreases and Pro increases the activation energy for loop closure compared to Ser.[13] The similar activation energies for all peptides further show that electrostatic interactions do not contribute significantly to the barriers for loop formation. The brinker fragment is part of the DNA binding region of the brinker domain and is highly positively charged (+7), containing 4 Arg and 3 Lys residues out of 21 amino acids (33%), which should lead to electrostatic repulsion. However, loop formation in the brinker fragment (E_a = 13.3 ± 1.0 kJ/mol) and in the parvalbumin DE (net charge = 0: 3−/3+; E_a = 14.3 ± 0.7 kJ/mol) and EF loops (net charge = −3: 5−/2+; E_a = 12.4 ± 0.7 kJ/mol) shows similar activation energies. This suggests that electrostatic repulsion due to a large number of positively charged amino acids, which is typically observed for the major class of DNA and RNA binding IDPs, does not have a major influence on the barriers for loop formation. Comparison of the two fragments from parvalbumin further reveals that electrostatic attraction between opposite charges does not affect the barriers for internal chain motions. In summary, these results show that side-chain interactions and/or steric effects induced by large and bulky side chains create additional barriers and thus introduce additional internal friction for segmental chain motions in long chains compared to poly(Gly-Ser) and polyserine chains. This additional internal friction introduced by larger side chains depends only very little on the chain length and amino acid sequence.

Figure 3B shows that the Arrhenius pre-exponential factor, A, decreases with increasing loop size for the natural loop sequences, as expected for an increased conformational space that is sampled during loop closure for longer chains. However, the pre-exponential factors for formation of the natural loops are much larger than the pre-exponential factors for poly(Gly-Ser) and polyserine loops of the same length, which suggests that sterics or interactions introduced by side chains reduce the conformational space available for the polypeptide chain. This reduced conformational space accelerates the conformational search and opposes the effect from increased barrier heights on the rate constant for loop closure. It is unlikely that the increased pre-exponential factor is due to accelerated internal dynamics (larger k_0) in the protein-derived peptides, which contain large and bulky side chains.

Contributions from Intramolecular Interactions and Side-Chain Sterics to the Activation Parameters for Loop Formation. The barriers for loop formation may contain contributions from the formation and breakage of intramolecular interactions. It was shown that poly(Gly-Ser) chains form intramolecular hydrogen bonds in water,[25,26] which leads to chain compaction and indicates the presence of intramolecular backbone hydrogen bonds as a general feature of unfolded polypeptide chains in water.[25] Breakage and formation of these intramolecular hydrogen bonds and of backbone/water hydrogen bonds during chain motions were proposed to add to internal friction by creating barriers.[27] To test whether the residual barrier for loop formation in long and flexible chains (E_a = 4.6 ± 4 kJ/mol) and the additional barriers observed in the natural protein loops arise from weak intramolecular interactions or from steric effects, we determined the effect of denaturants on the activation parameters. Denaturants like GdmCl and urea weaken non-covalent intramolecular interactions and thus represent a better solvent for polypeptide chains than water. For long poly(Gly-Ser) chains it was shown that GdmCl competes for intramolecular backbone hydrogen bonds which leads to more expanded polypeptide chains.[25] Thus, in the presence of high concentrations of denaturants, the contributions from intramolecular backbone hydrogen bonds and weak side-chain interactions to the activation energy should be eliminated or at least reduced. Figure 6 compares viscosity-corrected Arrhenius plots for the $(Gly\text{-}Ser)_1$ and $(Gly\text{-}Ser)_{16}$ peptides with the parvalbumin DE and EF loops and the GB1 hairpin in water

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

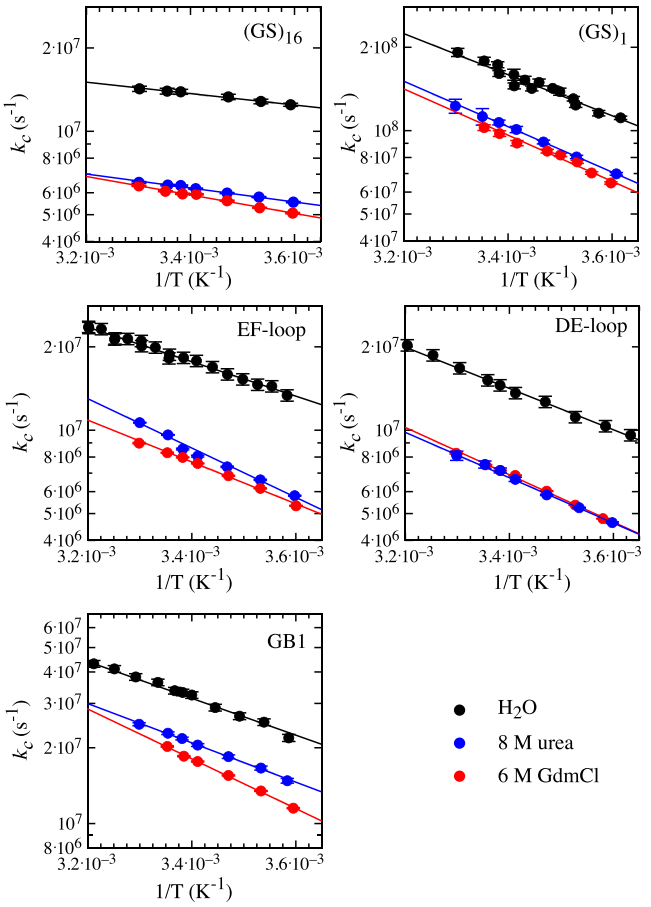


Figure 6. Effect of the presence of 8 M urea or 6 M GdmCl on the Arrhenius plots for the viscosity-corrected rate constant of loop formation (k_c) for different polypeptide chains, as indicated. The Arrhenius parameters obtained from the fits are shown in Figure 7.

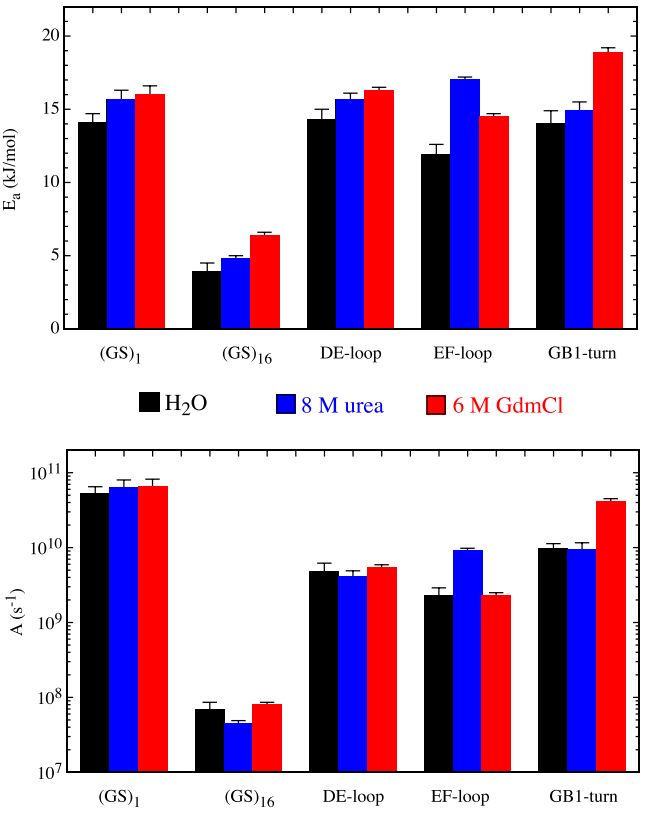


Figure 7. Effect of the presence of 8 M urea or 6 M GdmCl on the Arrhenius activation energy (E_a) and the Arrhenius pre-exponential factor (A) for loop formation in different polypeptide chains, as indicated.

and in the presence of 6 M GdmCl or 8 M urea. For all peptides loop formation slows down in the presence of denaturants, in accordance with our earlier results on polySer and poly(Gly-Ser) chains.[28] However, neither GdmCl nor urea leads to a decrease in the activation energy for any of the loops studied. Rather, E_a for loop formation slightly increases in the presence of 6 M GdmCl or 8 M urea for all loops (Figure 7). Except for the EF loop, the increase in E_a is slightly more pronounced in the presence of GdmCl compared to urea. This result suggests that breakage and formation of intramolecular backbone hydrogen bonds and of weak side-chain interactions does not create barriers for segmental chain motions and does not lead to additional internal friction, independent of chain length and amino acid sequence. Also, in accordance with the results discussed above, electrostatic interactions do not create barriers for chain dynamics. A 6 M GdmCl solution represents high salt conditions and leads to screening of charges, which should reduce barriers created by electrostatic repulsion or attraction. However, the activation energies for loop formation are even slightly higher in the presence of 6 M GdmCl compared to 8 M urea and water. The weaker increase in E_a by urea compared to GdmCl is in agreement with the weaker denaturing power of urea compared to GdmCl, which indicates that weak binding of the denaturants to the polypeptide chain leads to the observed slight increase in E_a.

The Arrhenius pre-exponential factor is also only slightly affected by the presence of GdmCl or urea (Figure 7). It is slightly increased in 6 M GdmCl compared to water, whereas 8 M urea has very little or no effect on A, except in the EF loop, where urea leads to an increase in A. This result is surprising since FRET experiments showed that GdmCl leads to an increase in the average end-to-end distance in a $(Gly\text{-}Ser)_{14}$ loop.[25] The slight increase in A suggests that the shift in population to more expanded conformations induced by GdmCl does not lead to a larger conformational space available for the chain during loop closure. This result rather indicates that, although more extended conformations are preferred in the presence of GdmCl, the overall accessible conformational space becomes even slightly smaller. Alternatively GdmCl may increase k_0 in the Arrhenius prefactor (eq 3), by increasing chain flexibility.

The effect of denaturants on the activation parameters shows that weak intramolecular interactions do not contribute to the observed activation energies for loop closure. Consequently, the major origin of barriers leading to internal friction is local steric effects in the polypeptide backbone induced by large and bulky side chains that hinder backbone rotations. To test this model we determined the activation parameters for loop formation in a homopolypeptide chain consisting of threonine and serine $(Thr\text{-}Ser)_7$. Thr has a larger side chain compared to Ser and Gly and contains both an −OH group, as Ser, and an additional methyl group at the C_β-atom. Thus, it is sterically more demanding than Gly or Ser which should introduce additional steric effects on chain motions. Figure 5 shows the Arrhenius plot for the formation of the $(Thr\text{-}Ser)_7$ loop, which gives a viscosity-corrected activation energy of E_a = 10.9 ± 0.3 kJ/mol and an Arrhenius pre-exponential factor of A = (1.3 ±

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

0.2) × 10^9 s^{-1}. Both values are significantly larger compared to the respective values for polyserine and poly(Gly-Ser) of the same length and only slightly smaller than those of the natural loop sequences (Figure 3), which confirms the conclusion that steric effects introduced by bulky side chains are the major origin for barriers in segmental motions both for local and long-range segmental motion in polypeptide chains and lead to additional internal friction.

Correlation between the Activation Energy of Loop Formation and Its Sensitivity to Solvent Viscosity. The effect of solvent viscosity on loop formation shows that the formation of long loops is determined by a purely diffusive search driven by coupling of solvent motions to chain motions leading to a $1/\eta$ viscosity dependence ($\alpha = 1$). The formation of short loops in poly(Gly-Ser) and in polyserine chains and loop formation in all natural sequences, in contrast, displays a weaker coupling of solvent motions to chain motions ($\alpha < 1$). NMR and fluorescence studies on local dynamics around individual bonds in synthetic polymers[2−7] and MD simulations on local bond rotations in short peptides[8] showed that the presence of energy barriers decreases the sensitivity of a reaction to solvent viscosity in accordance with predictions from the Grote−Hynes theory.[11] This suggests that also the reduced sensitivity of loop formation to solvent viscosity ($\alpha < 1$) in polypeptide chains may be due to the barriers reported in the work presented here. However, loop formation in the unfolded state occurs in an ensemble of rapidly interconverting conformations and it is not clear whether the Grote−Hynes theory also applies for these more complex dynamics. Figure 8A shows the correlation between the activation energy and the α value for the series of poly(Gly-Ser) loops. In accordance with the Grote−Hynes theory, the decrease in α value is correlated with the increase in activation energy with a correlation coefficient of $r = -0.98$ and a slope of $d\alpha/dE_a = -(2.6 \pm 0.3) \times 10^{-2}$ $(kJ/mol)^{-1}$ (Figure 8A). The polyserine loops show a weaker dependence of α on E_a compared to the flexible poly(Gly-Ser) loops, but there is also a correlation between α and E_a for the polyserine series ($r = -0.98$) with $d\alpha/dE_a = -(1.1 \pm 0.3) \times 10^{-2}$ $(kJ/mol)^{-1}$ (Figure 8B). The α values for the longer natural loops fall close to the correlation plot for polyserine, but the short GB-turn shows a slightly weaker effect of E_a on α (Figure 8C). These results show that also more complex local and long-range dynamics of loop formation display Grote−Hynes behavior, although they are not limited by crossing of a single barrier. The different slopes in the correlation between E_a and α for poly(Gly-Ser), the less flexible polySer chains, and the long natural loops show that the extent by which increasing barrier height decreases coupling of solvent motions to chain motions depends on amino acid sequence. Polyserine and longer natural sequences show a similar correlation between α and E_a that argues for local backbone dynamics as the major determinant for the effect of E_a on the coupling of chain motions to solvent motions. The GB1 hairpin does not fall onto the correlation plot between α and E_a for polySer and the IDPs. It shows a slightly stronger coupling of solvent motions to the dynamics of loop formation, given its high activation energy. We tested whether changes in local backbone flexibility in the GB1 hairpin have an effect on the correlation between α and E_a by replacing Thr49 of the GB1 hairpin by either Pro or Gly, which are the two most common amino acids in hairpin turns. As shown in the accompanying paper, Pro at position 49 slows down loop formation, whereas Gly leads to faster loop formation. However, the sensitivity to solvent viscosity is identical for all three loops with α values of 0.9 (see the accompanying paper). The temperature dependence of loop formation in the three GB1 variants reveals that both the T → P and the T → G variants of the GB1 hairpin show higher activation energies for loop formation than the wild-type turn with respective values of 16.4 ± 0.7 and 15.4 ± 0.7 kJ/mol, compared to 13.6 ± 0.4 kJ/mol for the wild-type turn (Figures 3A and 5). This effect is surprising, since Gly was shown to decrease the activation energy for loop formation in short serine-based host−guest peptides, whereas Pro increases the activation energy.[13] The increased activation energy is accompanied by an increased pre-exponential factor, both for the T → P and the T → G replacement with values of (2.0 ± 0.6) × 10^{10} s^{-1} and (2.5 ± 0.8) × 10^{10} s^{-1}, respectively, compared to (8.6 ± 1.5) × 10^9 s^{-1} for the wild-type turn (Figure 3B). Based on their effect on energetically favorable phi/psi angles Gly should lead to an increased loss in conformational space upon loop formation and thus to a lower pre-exponential factor, whereas Pro should lead to a reduced loss in conformational space upon loop formation, which thus results in the observed larger pre-exponential factor. The increased pre-exponential factor for the T → G variant may be due to effects on k_0, which should be larger around the more flexible Gly.

The three GB1 turn variants have identical α values but differ in their activation energies for loop formation, which shows that the correlation between the sensitivity to solvent viscosity and the activation energy depends on the local structural and dynamic features of the polypeptide chain. Surprisingly, both the more flexible Gly and the stiffer Pro lead to an even weaker effect of activation energy on the sensitivity

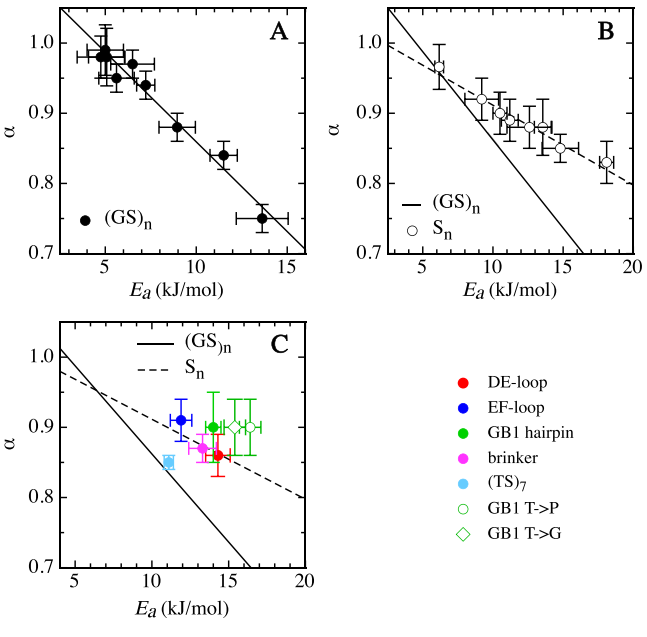


Figure 8. Correlation between the activation energy (E_a) for loop formation and the sensitivity of the reaction to solvent viscosity (α) for different polypeptide chains and IDP fragments, as indicated. The solid and dashed lines represent results from linear fits to the poly(Gly-Ser) and polySer data, respectively. The fits yielded slopes of $d\alpha/dE_a = -(2.6 \pm 0.3) \times 10^{-2}$ $(kJ/mol)^{-1}$ ($r = -0.98$) and $d\alpha/dE_a = -(1.1 \pm 0.3) \times 10^{-2}$ $(kJ/mol)^{-1}$ ($r = -0.98$) for poly(GS) and polyS, respectively.

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

to solvent viscosity compared to that of the wild-type GB1 turn.

CONCLUSIONS

Our results reveal that loop formation in unfolded proteins encounters energy barriers that create internal friction, which opposes chain motions. The height of the effective barriers depends on the loop size and amino acid sequence. Local dynamics that lead to the formation of short loops encounter viscosity-corrected barriers of up to E_a = 18 kJ/mol. This activation energy is almost independent of amino acid sequence and is in the same range as barrier heights observed for local internal dynamics in several synthetic polymers determined by NMR and fluorescence relaxation experiments.[2−7] This similarity suggests that the formation of short loops within the persistence length of the polypeptide chain is mainly limited by barriers of local bond rotations, which creates high internal friction for loop formation. In poly(Gly-Ser) and polyserine homopolymers the effective activation energy for loop formation decreases with increasing loop length and reaches a limiting value of E_a = 4.6 ± 0.4 kJ/mol for long and flexible poly(Gly-Ser) peptides that behave like Gaussian chains. This decrease in internal friction with increasing polymer length was predicted by Kuhn and Kuhn[1] and demonstrates the presence of pathways with low effective barriers for loop formation in long and flexible chains within the Gaussian limit. However, the residual barrier of E_a = 4.6 kJ/mol is larger than RT, which shows that, even in the Gaussian limit, loop formation is not completely barrierless. The results on loop formation in IDP fragments show that long and bulky side chains introduce additional barriers and thus create additional internal friction for the formation of long loops compared to poly(Gly-Ser) and polyserine chains. GdmCl and urea do not affect the barriers for long-range loop formation in the investigated IDP fragments indicating that the increased internal friction is purely due to steric effects and does not contain contributions from rearrangement of weak intramolecular interactions like H-bonds, dispersion forces, or electrostatic interactions, which were postulated to create internal friction in polypeptide dynamics.[27,29,30] The higher activation energies and the resulting stronger temperature dependence for loop formation in IDPs compared to long poly(Gly-Ser) and poly(Ser) chains (Figure 3) result in a reduction of the contributions from steric interactions to k_c with increasing temperature and lead to increased chain flexibility and thus to reduced relative internal friction at high temperatures. These results in combination with high activation energies found for native state dynamics suggest that chain motions at any stage during the protein folding process encounter significant energy barriers, which is not in agreement with a downhill folding scenario that has been proposed for some proteins.

Activation energies for internal chain dynamics have also been reported for an IDP domain of a nucleoprotein from Sendai virus measured by NMR.[14] These studies reported fast-time-scale motions in the range of 1 ns, which were assigned to local bond rotations within the Ramachandran space, which is in accordance with fluorescence studies on α-synuclein, which also revealed rotational dynamics of fluorophores on the time scale of 1 ns.[15] These bond rotations show low activation energies of about 5 kJ/mol throughout the chain.[14] These values are, however, not viscosity-corrected. The NMR studies reported additional motions on the 5−25 ns scale, which is comparable to the time scale we observed for the formation of short loops (Figure 3C). These motions were assigned to segmental chain dynamics and exhibited non-viscosity-corrected activation energies of 20−25 kJ/mol,[14] which is similar to the values we observed for the local dynamics of loop formation before viscosity correction (Figure 3).

The decreasing activation energy with increasing loop size in poly(Gly-Ser) and polySer chains is opposed by a concomitant decrease in the pre-exponential factor due to an increasing loss in conformational entropy upon loop formation. The scaling of the loss in conformational entropy with increasing loop size is stronger in the region of the persistence length of the chain with $A \sim N^{-3.9\pm0.4}$ and $A \sim N^{-3.7\pm0.1}$ for poly(Gly-Ser) and polyserine, respectively, and reduces to $A \sim N^{-1.8}$ for long poly(Gly-Ser) loops, as expected for a Gaussian chain with excluded volume. Loop formation in chains derived from IDPs exhibits a higher pre-exponential factor for loop formation compared to poly(Gly-Ser) and polyserine loops. In all chains investigated in this study, high concentrations of urea and GdmCl, which lead to a loss of intramolecular interactions, exhibit only minor effects on A. This indicates that intramolecular interactions introduced by side chains do not significantly reduce conformational space. Rather, steric effects introduced by large and bulky side chains in the IDPs lead to a decreased loss in conformational entropy upon loop formation.

The effect of temperature on the scaling of the rate constant for loop formation with loop size, N, shows that chain stiffness is reduced with increasing temperature for both the poly(Gly-Ser) and polySer chains (Figure 4) and that the scaling of k_c with loop size decreases from $k_c \sim N^{-1.82\pm0.06}$ at 275.65 K to $k_c \sim N^{-1.66\pm0.05}$ at 315.65 K for poly(Gly-Ser) indicating that excluded volume effects decrease with decreasing strength of intramolecular interactions, in accordance with theoretical work from Flory.[24]

Comparison of the results presented here with the results from the accompanying paper reveals an inverse correlation between the sensitivity of loop formation to solvent viscosity, α, and the effective activation energy, which is in accordance with the Grote−Hynes concept of memory friction.[11,12] The same negative correlation was observed for local bond rotations in experimental studies on synthetic homopolymers[2,4,5] and in computer simulations on short peptides.[8,10] This result shows that memory friction observed for local bond rotations also applies to more complex chain dynamics that include rotations of a large number of backbone bonds.

AUTHOR INFORMATION

Corresponding Author

Thomas Kiefhaber − *Martin-Luther-Universität Halle-Wittenberg, Institut für Biochemie und Biotechnologie, Abteilung Proteinbiochemie, 06120 Halle (Saale), Germany;* orcid.org/0009-0009-2429-764X; Phone: +49 345 552 4860; Email: t.kiefhaber@biochemtech.uni-halle.de

Authors

Florian Krieger − *Biozentrum der Universität Basel, CH-4056 Basel, Switzerland*

Karin Stecher − *Technische Universität München, Chemistry Department, D-85747 Garching, Germany*

Christian Nyffenegger − *Technische Universität München, Chemistry Department, D-85747 Garching, Germany*

Michael Schleeger − *Martin-Luther-Universität Halle-Wittenberg, Institut für Biochemie und Biotechnologie,*

https://doi.org/10.1021/acs.jpcb.3c04072
J. Phys. Chem. B 2023, 127, 8106−8115

Abteilung Proteinbiochemie, 06120 Halle (Saale), Germany; orcid.org/0000-0003-2388-9087

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jpcb.3c04072

Author Contributions

‖F.K., K.S.: These authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 863-A6, SFB/TRR 102-A10, INST 271/359-1 FUGG, and INST 271/345-1 FUGG).

■ REFERENCES

(1) Kuhn, W.; Kuhn, H. Bedeutung beschränkt freier Drehbarkeit für die Viskosität und Strömungsdoppelbrechung von Fadenmolekellösungen I. *Helvetica. Chem. Acta* **1945**, *28*, 1533−1579.

(2) Zhu, W.; Ediger, M. D. Viscosity Dependence of Polystyrene Local Dynamics in Dilute Solutions. *Macromolecules* **1997**, *30*, 1205−1210.

(3) Zhu, W.; Gisser, D. J.; Ediger, M. D. C-13 Nmr Measurements of Polybutadiene Local Dynamics in Dilute-Solution - Further Evidence for Non-Kramers Behavior. *J. of Polym. Sci. B Polym. Phys.* **1994**, *32* (13), 2251−2262.

(4) Adams, S.; Adolf, D. B. Viscosity dependence of the local segmental dynamics of anthracene-labeled 1,2-polybutadiene in dilute solutions. *Macromolecules* **1998**, *31* (17), 5794−5799.

(5) Adolf, D. B.; Ediger, M. D.; Kitano, T.; Ito, T. Viscosity dependence of the local segmental dynamics of antracene-labeled polyisoprene in dilute solutions. *Macromolecules* **1992**, *25* (2), 867−872.

(6) Zhu, W.; Ediger, M. D. Deuterium NMR characterization of 1,2-polybutadiene local dynamics in dilute solutions. *Macromolecules* **1995**, *28* (22), 7549−7557.

(7) Glowinkowski, S.; Gisser, D. J.; Ediger, M. D. Carbon-13 nuclear magnetic resonance measurements of local segmental dynamics of polyisoprene in dilute solution: nonlinear viscosity dependence. *Macromolecules* **1990**, *23* (14), 3520−3530.

(8) Zheng, W.; de Sancho, D.; Best, R. B. Modulation of folding internal friction by local and global barrier heights. *J. Phys. Chem. Lett.* **2016**, *7*, 1028−1034.

(9) Kuhn, W.; Kuhn, H. Modellmässige Deutung der inneren Viskosität (der Formzähigkeitskonstante) von Fadenmolekeln I. *Helv. Chim. Acta* **1946**, *29*, 609−626.

(10) de Sancho, D.; Sirur, A.; Best, R. B. Molecular origins of internal friction effects on protein-folding rates. *Nature Comm.* **2014**, *5*, 4307.

(11) Grote, R. F.; Hynes, J. T. The stable states picture of chemical reactions. II. Rate constants for condensed and gas phase reaction models. *J. Chem. Phys.* **1980**, *73* (6), 2715−2732.

(12) Bagchi, B.; Oxtoby, D. W. The effect of frequency dependent friction on isomerization dynamics in solution. *J. Phys. Chem.* **1983**, *78* (5), 2735−2741.

(13) Krieger, F.; Möglich, A.; Kiefhaber, T. Effect of proline and glycine residues on dynamics and barriers of loop formation in polypeptide chains. *J. Am. Chem. Soc.* **2005**, *127* (10), 3346−3352.

(14) Abyzov, A.; Salvi, N.; Schneider, R.; Maurin, D.; Ruigrok, R. W. H.; Jensen, M. R.; Blackledge, M. Identification of dynamic modes in an intrinsically disordered protein using temperature-dependent NMR relaxation. *J. Am. Chem. Soc.* **2016**, *138*, 6240−6251.

(15) Jain, N.; Naranq, D.; Bhasne, K.; Dalala, V.; Arya, S.; Bhattacharya, M.; Mukhopadhyah, S. Direct observation of the intrinsic backbone torsional mobility of disordered proteins. *Biophys. J.* **2016**, *111*, 768−774.

(16) Krieger, F.; Fierz, B.; Bieri, O.; Drewello, M.; Kiefhaber, T. Dynamics of unfolded polypeptide chains as model for the earliest steps in protein folding. *J. Mol. Biol.* **2003**, *332*, 265−274.

(17) Fierz, B.; Kiefhaber, T., Dynamics of unfolded polypeptide chains. In *Protein Folding Handbook*; Buchner, J., Kiefhaber, T., Eds.; Wiley-VCH: Weinheim, Germany, 2005; pp 805−851.

(18) Jencks, W. P. A primer for the Bema Hapothle. An empirical approach to the characterization of changing transition-state structures. *Chem. Rev.* **1985**, *85* (9), 511−527.

(19) Sánchez, I. E.; Kiefhaber, T. Non-linear rate-equilibrium free energy relationships and Hammond behavior in protein folding. *Biophys. Chem.* **2002**, *100* (1−3), 397−407.

(20) Sánchez, I. E.; Kiefhaber, T. Evidence for sequential barriers and obligatory intermediates in apparent two-state protein folding. *J. Mol. Biol.* **2003**, *325* (2), 367−376.

(21) Herschbach, D. R. *Bibliograqphy of hindered internal rotation and microwave spectra*; University of California: Berkeley, CA, 1962; pp 1−29.

(22) Brant, D. A.; Flory, P. J. The configuration of random polypeptide chains. II. Theory. *J. Am. Chem. Soc.* **1965**, *87*, 2791−2800.

(23) Szabo, A.; Schulten, K.; Schulten, Z. First passage time approach to diffusion controlled reactions. *J. Chem. Phys.* **1980**, *72*, 4350−4357.

(24) Flory, P. J. *Statistical Mechanics of Chain Molecules*; Hanser Publishers: Munich, Germany, 1969.

(25) Möglich, A.; Joder, K.; Kiefhaber, T. End-to-end distance distributions and intrachain diffusion constants in unfolded polypeptide chains indicate intramolecular hydrogen bond formation. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12394−12399.

(26) Neuweiler, H.; Löllmann, M.; Doose, S.; Sauer, M. Dynamics of unfolded polypeptide chains in crowded environment studied by fluorescence correlation spectroscopy. *J. Mol. Biol.* **2007**, *365* (3), 856−869.

(27) Erbaş, A.; Horinek, D.; Netz, R. R. Viscous friction of hydrogen-bonded matter. *J. Am. Chem. Soc.* **2012**, *134*, 623−630.

(28) Möglich, A.; Krieger, F.; Kiefhaber, T. Molecular basis for the effect of urea and guanidinium chloride on the dynamics of unfolded polypeptide chains. *J. Mol. Biol.* **2005**, *345* (1), 153−162.

(29) Schulz, J. C. F.; Schmidt, L.; Best, R. B.; Dzubiella, J.; Netz, R. R. Peptide chain dynamics in light and heavy water: zooming in on internal friction. *J. Am. Chem. Soc.* **2012**, *134*, 6273−6279.

(30) Sashi, P.; Ramakrishna, D.; Bhuyan, A. Dispersion forces and the molecular origin of internal friction in protein. *Biochemistry* **2016**, *55*, 4595−4602.