Structural fluctuations of a helical polypeptide traversing a lipid bilayer

(time-resolved fluorescence anisotropy/molecular dynamics calculation/membrane polypeptide)

HORST VOGEL*†, LENNART NILSSON‡, RUDOLF RIGLER‡, KLAUS-PETER VOGES§, AND GÜNTHER JUNG§

*Biocenter, University of Basel, CH-4056 Basel, Switzerland; ‡Department of Medical Biophysics, Karolinska Institutet, S-10401 Stockholm, Sweden; and §Institut für Organische Chemie, Universität Tübingen, D-7400 Tübingen, Federal Republic of Germany

ABSTRACT

Time-resolved fluorescence anisotropy (FA) measurements are reported for five helical bilayer-spanning hенicosapetides, each containing one tryptophan at sequence position 1, 6, 11, 16, or 21. The FA decay reflects two molecular processes in all cases: local internal fluctuations of the tryptophan side chain with a relaxation time of 200–500 ps, and motions of the whole polypeptide molecule with a relaxation time of 9–10 ns. The amplitudes of the fast fluctuations are largest at the helix ends and decrease toward the center of the helix. A similar observation was made for the helical polypeptides dissolved in organic solvents showing that the mobility gradient along the polypeptide sequence is an inherent property of the polypeptide helix and not due to the lipid bilayer. However, the amplitudes of the fast fluctuations can be modulated by the physical state of the lipid bilayer. With decreasing temperature, the internal mobility of the tryptophan in all positions decreases with an abrupt change at the lipid-phase transition. Concomitant molecular dynamics calculations indicate a correlation between the fast FA decay and conformational fluctuations within the helix. According to the simulation, the conformation of the polypeptide is on average predominantly helical, but actually the molecule can fluctuate between a variety of different substructures. The conformational fluctuations are largest at the helix ends and provide the free space required for rotation of the indole ring around the tryptophan side chain bonds.

A number of experimental and theoretical studies on water-soluble proteins indicated the presence of protein structural fluctuations that may play an essential role for biological activity (1–3). However, in the particular case of membrane proteins, little information exists concerning such structural fluctuations of a polypeptide in a lipid bilayer (4–6). Here we report on structural fluctuations of membrane-incorporated synthetic polypeptides of the type H2N-(Ala-Ala-Ala-Ala), Trp-(Ala-Ala-Ala-Ala), or OMe (Aib, a-aminoisobutyric acid) with x = 0, 1, 2, 3, 4—i.e., 21-amino-acid-long hенicosapetides, containing a tryptophan at sequence position 1, 6, 11, 16, or 21 ([Trp-1], [Trp-6], [Trp-11], [Trp-16], and [Trp-21]). A recent study (7) indicates that these polypeptides insert into lipid bilayers and traverse the membrane in an a-helical conformation. The tryptophan residue was found to be located near the membrane surface in [Trp-1] and [Trp-21], around the center of the bilayer in [Trp-11], and at an intermediate depth of the lipid membrane in [Trp-6] and [Trp-16] (7). We have measured the time-resolved fluorescence anisotropy (FA) of the single tryptophan of each of the corresponding polypeptides in lipid vesicles. Such experiments yield direct information about polypeptide structural fluctuations that occur within the lifetime of the fluorophore (6, 8–10).

These structural fluctuations are composed of internal motions of the tryptophan residue, that of the polypeptide backbone, and the orientational fluctuations of the whole molecule in the lipid bilayer. The various kinds of motion are expected to occur on different time scales in a hierarchical order and, therefore, should be distinguished to a certain extent by time-resolved FA measurements. Using polypeptides with a tryptophan residue at different sequence positions, we can probe structural fluctuations along a polypeptide helix in a lipid bilayer. Time-resolved FA measurements were also performed with the same polypeptides dissolved in methanol. The hенicosapetides adopt a helical conformation in methanol as well (7), and so these experiments will help to distinguish between structural fluctuations of a polypeptide helix in organic solution and in a lipid bilayer. Furthermore, we have measured the circular dichroism (CD) of the various hенicosapetides in lipid vesicles and in planar multilayer membranes. These experiments yield information about the polypeptide secondary structure and the orientational fluctuations of the whole polypeptide helix in a lipid membrane (11).

It is tempting for this simple system to compare the experimentally determined time dependence of the tryptophan FA with that obtained from a molecular dynamics (MD) calculation. The goal of such simulations is to yield a reasonable explanation of the experimentally observed FA in terms of molecular fluctuations at an atomic level. Here we present as well the results of a MD calculation for the 22-amino-acid-long peptide H2N-Trp-(Ala-Ala-Ala-Ala)-Trp-(Ala-Ala-Ala-Ala), OMe, [Trp-1, -12], in methanol where the solvent was simulated by using Langevin dynamics taking into account an average frictional force (12). The two tryptophan residues at positions 1 and 12 are expected to reflect the motions of the corresponding fluorophores in [Trp-1] and [Trp-11] and were introduced simultaneously in one molecule to save computing time. Although the solvent molecules were not explicitly considered, the MD calculations should reflect the molecular fluctuations of the helical peptide in a first step reasonably well. We expect that the simulation is also relevant for the fluctuations of the polypeptide in a membrane, because the helix is practically totally inserted into the lipid bilayer without any considerable part being located in the water phase.

MATERIALS AND METHODS

Sample Preparation. The polypeptides were synthesized as described (7). Appropriate amounts of lipid and polypeptide were dissolved in methanol at a molar ratio of 100. After the methanol was evaporated, lipid vesicles were prepared by adding buffer (10 mM NaCl/1 mM Tris HCl, pH 7.4) to the dry samples followed by tip sonication for 5 min at 35°C. The

Abbreviations: Aib, α-aminoisobutyric acid; FA, fluorescence anisotropy; MD, molecular dynamics; [Myr]PtdCho, dimyristoylphosphatidylcholine; [Tet]PtdCho, ditetradecylphosphatidylcholine.

†To whom reprint requests should be addressed.

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final polypeptide concentration was 20 and 200 μM for fluorescence and CD measurements, respectively. Planar multibilayers were prepared as described (11) by spreading polypeptide-containing lipid vesicles in water on quartz glass plates, drying these samples in a vacuum desiccator, and finally rehydrating them in a water vapor atmosphere of 97% relative humidity. Pure lipid membranes were prepared as described above but without polypeptides.

**CD Measurements and Data Analysis.** The CD of a particular polypeptide in lipid vesicles and planar multibilayers was measured between 186 and 250 nm as described (11). The α-helix content of the polypeptide under study was derived by fitting the experimental CD spectrum of the vesicle preparation to a superposition of 15 reference proteins of known three-dimensional structure according to ref. 11. The orientational order parameter $S_n = \frac{3\cos^2\beta - 1}{2}$ of the polypeptide helix in the lipid membrane was determined from the difference between the CD spectra of a polypeptide in planar multilayers and lipid vesicles as outlined in ref. 11.

**FA Measurements and Data Evaluation.** Time-resolved fluorescence measurements were performed with time-correlated single photon counting as described (13). The excitation wavelength was 300 nm and emission was monitored through a WG 345-nm cut-off filter. The fluorescence of pure lipid membrane vesicles without polypeptides was measured separately and subtracted from the fluorescence observed in lipid-polypeptide samples. The subtracted fluorescence intensity was always <5% that of the polypeptide-containing lipid vesicles. The protocol for data collection as well as for data analysis of both total fluorescence intensity $i(t)$ and fluorescence anisotropy $r(t)$ was the same as recently reported (6, 13). $i(t)$ and $r(t)$ are described by a sum of exponential functions

\[ i(t) = \sum_{i=1}^{N} a_i \exp(-t/\tau_i) \]

\[ r(t) = \sum_{i=1}^{M} r_i \exp(-t/\phi_i) + r_\infty, \]

with $r(t)$ containing, in addition, a constant term $r_\infty$. For the analysis of the FA of a polypeptide in organic solution, $r_\infty$ was set to zero. The partial amplitudes $a_i$ of the sum, the average lifetime $\langle \tau \rangle$, and the initial anisotropy $r_0$ are derived as

\[ a_i = a_i / \sum_{i=1}^{N} a_i \]

\[ \langle \tau \rangle = \sum_{i=1}^{N} a_i \tau_i \]

\[ r_0 = \sum_{i=1}^{M} r_i + r_\infty. \]

The quality of the fit of the experimental fluorescence intensity and FA decays with the functions in Eq. 1 is expressed by $\chi^2$ and $\chi^2_r$, respectively (14).

**MD Calculation.** The MD simulation of the polypeptide [Trp-1, -12] was performed with the program CHARMM (12). As initial coordinates we chose those of an ideal α-helix because it has been shown (15) that a related peptide Boc-L-Ala-Ala-Ala-Glu(BOzl)-Ala-Ala-Ala-Ala-Ala-OMe adopts a right-handed α-helical conformation in the crystalline state. All atoms of the polypeptide were treated explicitly, except for aliphatic hydrogens, which were included with the carbon to which they are bound, as described (12). The MD calculation was performed for the peptide in vacuum with 2-fs time steps by using a Langevin algorithm and taking into account an average friction caused by the molecular motions of the polypeptide relative to its surroundings (16). The friction coefficient used, 30 ps$^{-1}$, corresponds to the viscosity of methanol at 15°C.

The molecular flexibility of the α-helix at different positions was assessed by a segmental order parameter $S_m$ (10),

\[ S_{hi}^2 = \lim_{t \to \infty} \langle r_2(h_i(0)\cdot h_i(t)) \rangle. \]

The angle brackets indicate a time average, $P_2[x]$ is the second-order Legendre polynomial, and the unit vector $h_i$ is directed between the Cα atom at position $i$ and $i + 4$. If the helix and its conformational fluctuations are assumed to be rotationally symmetric around the mean helix axis, this generalized order parameter corresponds to the usual order parameter $\langle P_2[\cos \beta] \rangle$, where $\beta$ is the angle between the helix segment axis and the mean helix axis.

Since the MD simulation is done for the molecule in vacuum with no net translation or rotation, the FA can be computed from the MD trajectory according to ref. 9.

\[ r(t) = 2/5 \langle P_2[\mu_x(0)\cdot \mu_x(t)] \rangle, \]

where $\mu_x(0)$ and $\mu_x(t)$ are the unit vectors directed along the tryptophan absorption and emission dipole moments at times 0 and $t$, respectively, with coordinate systems A and E fixed to the helix molecule (9). These vectors reorient because of internal motions of the chromophore and motions of the whole helix molecule. For a polypeptide α-helix inserted in a lipid bilayer, the internal motions can be assumed to be independent of the much slower motions of the whole helix so that a comparison can be made with the calculated FA due to the internal motions.

**RESULTS**

**CD Measurements.** Fig. 1 shows the CD spectra of [Trp-1] in vesicles and planar bilayers of 1,2-ditetradecyl-sn-glycero-3-phosphocholine (here referred to as ditetradecylphosphatidylcholine; [Tet2]PtdCho) at 35°C—i.e., in a fluid lipid membrane above the ordered ↔ fluid lipid-phase transition

![Fig. 1](image-url)
temperature \(T_2 = 30^\circ\text{C}\). From an analysis of spectrum A in Fig. 1, an \(\alpha\)-helix content of 77% is determined. The intensity of the CD signal at 222 nm stays constant in the investigated temperature range above \(T_2\), and changes abruptly at the lipid-phase transition (Fig. 1 Inset), reflecting an increase of the helix content to 85% in the ordered lipid membrane at 15°C.

The orientational order parameter of the polypeptide helix in the fluid lipid membrane is calculated to be \(S^2 = 0.89\) from the difference between the spectra of the vesicle (A) and planar membrane preparation (B) in Fig. 1. Corresponding spectra of the other peptides are similar to those in Fig. 1, with a helix content ranging between 70% and 85% and \(S^2\) between 0.7 and 0.9. We conclude that according to the applied methods the henicosapeptides in fluid lipid membranes adopt a predominantly helical conformation irrespective of the sequence position of the tryptophan. The helical part is oriented preferentially parallel to the membrane normal.

**FA Measurements.** The FA \(r(t)\) of [Trp-1], [Trp-6], and [Trp-11] in lipid vesicles of dimyristoylphosphatidylcholine ([Myr\(2\)]PtdCho) at 32°C \((T_2 = 23^\circ\text{C})\) are shown in Fig. 2. Corresponding values of the fits to \(i(t)\) and \(r(t)\) of the whole set of henicosapeptides in fluid lipid membranes are summarized in Table 1. At least three lifetimes are needed to adequately describe the fluorescence decay \(i(t)\). The time course of the FA can be fitted in all cases by a sum of two exponentials according to Eq. 1, reaching a residual value \(r_\infty\) at long times. The two relaxation times \(\phi_1\) and \(\phi_2\) differ by more than an order of magnitude, with the fast component ranging between 0.2 and 0.5 ns and the slow one between 9 and 10 ns. Due to this large difference, \(\phi_1\) and \(\phi_2\) are assumed to reflect two independent molecular motions. The amplitude \(r_1\) and the relaxation time \(\phi_1\) critically depend on the sequence position of the tryptophan (Fig. 2 and Table 1). When going from the ends to the center of the helix, \(r_1\) decreases while at the same time \(\phi_1\) increases; on the other hand, \(r_2\) and \(\phi_2\) remain fairly constant, as well as the initial anisotropy \(r_0\) ranging from 0.213 to 0.235 (Table 1). The residual anisotropy \(r_\infty\) shows that the reorientational motion of the tryptophan side chain irrespective of its sequence position is anisotropic and restricted on the time scale of the fluorescence experiment. The most reasonable explanation of this result is to assign \(\phi_1\) to the internal fluctuations of the tryptophan residue and \(\phi_2\) to fluctuations of the whole helix in a lipid bilayer. Since these motions decay apparently exponentially, the FA can be expressed as

\[
r(t) = 2/5 P_2(\cos \lambda)(1 - S^2) [\exp(-\tau/\phi_1) + S_1^2]\]

\[
[1 - S_2^2] [\exp(-\tau/\phi_2) + S_2^2],
\]

where \(\lambda\) is the angle between \(\mu_\phi\) and \(\mu_2\), and \(S_1\) and \(S_2\) are order parameters characterizing the internal and the whole helix fluctuations (9). The results are summarized in Fig. 3 (Left), showing the square of the order parameters \(S_1\) and \(S_2\), and in Fig. 3 (Right), showing \(\phi_1\) and \(\phi_2\), in each case as a function of the tryptophan sequence position.

To test the question of how the molecular motions of the helical polypeptides are influenced by the physical state of the lipid bilayer, we have measured the tryptophan FA of different henicosapeptides at various temperatures below and above the lipid-phase transition. Fig. 4 shows the temperature dependence of \(S_1^2\) and \(\phi_1\) of [Trp-6] in [Tet\(2\)]PtdCho vesicles when going through the lipid-phase transition. The results show the following general trend: \(S_1^2\) and \(\phi_1\), as well as \(\phi_2\), are high in the ordered lipid membrane phase, decrease slowly with increasing temperature, and change abruptly around the phase transition temperature toward low values in the fluid lipid phase. The change at \(T_2\) is more pronounced for \(S_1^2\) and \(\phi_1\) than for \(S_2^2\) and \(\phi_2\). Fig. 4 also contains the data of [Trp-1] and [Trp-11] in [Tet\(2\)]PtdCho vesicles below \(T_2\) at 15°C and above \(T_2\) at 32°C. The values of \(S_1^2\) and \(\phi_1\) are low at location 1 and increase at the helix center, whereas \(S_2^2\) and \(\phi_2\) remain at fairly constant values for all sequence positions, indicating a very similar behavior as for the case of [Myr\(2\)]PtdCho.

The question arises as to whether the variation of the fast components \(S_1^2\) and \(\phi_1\), with the sequence position of the tryptophan, is an intrinsic property of a polypeptide helix or is due to incorporating the helix into a lipid bilayer. We therefore


can be fitted in all cases by a sum of two exponentials according to Eq. 1, reaching a residual value \(r_\infty\) at long times. The two relaxation times \(\phi_1\) and \(\phi_2\) differ by more than an order of magnitude, with the fast component ranging between .

**Table 1.** Fit parameters to the fluorescence intensity and FA decays of different henicosapeptides in [Myr\(2\)]PtdCho

<table>
<thead>
<tr>
<th>[Trp-1]</th>
<th>[Trp-6]</th>
<th>[Trp-11]</th>
<th>[Trp-16]</th>
<th>[Trp-21]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a_1)</td>
<td>0.32</td>
<td>0.21</td>
<td>0.34</td>
<td>0.26</td>
</tr>
<tr>
<td>(\tau_1)</td>
<td>0.29</td>
<td>0.42</td>
<td>0.45</td>
<td>0.48</td>
</tr>
<tr>
<td>(a_2)</td>
<td>0.31</td>
<td>0.31</td>
<td>0.43</td>
<td>0.50</td>
</tr>
<tr>
<td>(\tau_2)</td>
<td>2.2</td>
<td>3.0</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>(a_3)</td>
<td>0.37</td>
<td>0.48</td>
<td>0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>(\tau_3)</td>
<td>5.9</td>
<td>6.4</td>
<td>6.1</td>
<td>9.1</td>
</tr>
<tr>
<td>(\langle r \rangle)</td>
<td>3.0</td>
<td>4.1</td>
<td>2.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

**Values in parentheses are SDs.** \(\tau\) and \(\phi\) values are in ns.

Fig. 2. Time dependence of the FA of [Trp-1], [Trp-6], and [Trp-11] in lipid vesicles of [Myr\(2\)]PtdCho at 32°C.
FIG. 3. (Left) Squares of the order parameters $S_1$ (●) and $S_2$ (○) of the different henicosapeptides in lipid vesicles of [Myr$_2$]PtdCho at 32°C, as calculated with Eq. 5 from the data of Table 1. (Right) Corresponding relaxation times $\phi_1$ (●) and $\phi_2$ (○) of the tryptophan anisotropy from Table 1. For comparison, data of $S_1^z$ and $\phi_1$ from FA measurements of the peptides in methanol (■) at 15°C are shown in Left and Right, respectively. Typical SDs according to the fit of the time dependence of the FA are included.

performed FA measurements on the different peptides dissolved in methanol. In such solutions at 15°C, the time dependence of the tryptophan FA can be described by two relaxation times with a fast component at 0.1–0.2 ns and a slow component $\phi_2$ at ~0.6 ns. The relaxation time for the rotational motion of a whole henicosapeptide molecule with spherical shape would be 0.4 ns in methanol. It is reasonable, therefore, to assume that the measured values of $\phi_2$ reflect mainly the rotational motion of the whole henicosapeptide molecules in the solution, and $\phi_1$ values reflect the internal motions of the tryptophan residue, although the two processes may be coupled to some extent because of their similar time scales.

Interestingly, the values of the fast component $S_1^z$ and $\phi_2$, derived from tryptophan FA, show a qualitatively similar dependence on sequence position of the tryptophan for the peptides dissolved in methanol and inserted into lipid membranes (Fig. 3). The values of the fast process are, however, considerably lower for the samples in methanol than in fluid lipid membranes.

**MD Calculation.** Fig. 5 shows the FA of [Trp-1, -12] calculated from the MD simulations with Eq. 4. According to ref. 9, the absorption dipole was set at $-38^\circ$ from the long axis of the tryptophan indole ring and the emission dipole at either $-15^\circ$ or $-60^\circ$. For Trp-1, the FA decays to a limit of 0.2 for $\mu_e$ at $-60^\circ$ in 10–20 ps. For Trp-12 with $\mu_e$ assumed to be at $-15^\circ$ gives a FA which in the present calculation still has not leveled out in the 50 ps that could be considered reliable in the calculation of the correlation functions from a 170-ps piece of the trajectory. The amplitude of the FA decay with $\mu_e$ at $-60^\circ$ is about twice as large in Trp-1 as in Trp-12, which is in qualitative agreement with the corresponding experimental observation. The fluctuations occurring toward the end of the correlation functions in Fig. 5 are probably due to rare events that have not been completely averaged out in the 170-ps-long simulation and therefore cannot be interpreted.

To determine the time dependence of the conformational fluctuations within the $\alpha$-helix, we calculated the correlation function $P_2(h_1(0), h_1(i))$. The results for the positions $i = 1, 3, 5, 9$ are shown in Fig. 6. The correlation function for $i = 1$ shows a decay within 30–40 ps to a constant value of 0.73. The amplitude of the decay is considerably smaller in all other sequence positions and, furthermore, decreases when going toward the helix center ($S_{h1}^z = 0.73, S_{h3}^z = 0.87, S_{h5}^z = 0.91, S_{h9}^z = 0.93$).

**DISCUSSION**

Within the FA experiments, two molecular motions of the polypeptide in lipid membranes can be distinguished, a fast...
process due to internal motions of a tryptophan residue and a slow process reflecting fluctuations of the whole helix in a lipid bilayer. The amplitude and relaxation time of the fast process depend on the position within the helix. The largest amplitudes and the shortest relaxation times are found at the helix ends.

Because a similar observation was made with the helical henicosapeptides in organic solution, the motional gradient is an inherent property of an α-helix and is not due to surrounding effects of the lipid bilayer on the polypeptide. If the henicosapeptides were to adopt an ideal helix as do related polypeptides in the crystalline state (15), no variation of the internal motions of the tryptophan residue at least in the central helix part ([1tr-6], [1tr-11], [1tr-16]) would be expected, contrary to the experimental finding. The most reasonable explanation of the gradient in internal motion is a conformational flexibility of the polypeptide molecule. Although predominantly helical, the molecule can adopt a variety of conformations. According to the FA experiments, the deviation from an α-helix is largest at the helix ends, which explains why the helix content as determined by CD is considerably below 100% in lipid bilayers and in organic solvents, compared to the nearly ideal α-helical structure in crystals of related polypeptides. In lipid membranes, the decrease of the internal mobility of the tryptophan side chains with decreasing temperature (Fig. 4) is paralleled by an increase of the helix content of the henicosapeptides (Fig. 1 Inset) in accord with the consideration above.

This model is supported by MD simulations, which assign the calculated FA decay to the experimentally observed fast FA decay. Within the simulation, the decay of the FA correlates with the decay of the conformational fluctuations within the helix (Figs. 5 and 6). However, the values of the corresponding $S_{ij}$ are too small to be fully responsible for the amplitudes of the calculated FA decay. The main effect stems, therefore, from rotations of the tryptophan ring around the side chain bonds. Although the conformational flexibility of the helix contributes directly only to a small part of the total internal mobility of the tryptophan residue, it offers the free space for. According, thereby, the rotational motions of the indole ring around the $C_{\beta}-C_{\alpha}$ and $C_{\alpha}-C_{\beta}$ bonds in the side chain. An explanation for the difference between calculated and measured relaxation times can be that the molecular nature of the solvent has been neglected in the MD simulation, which only included average frictional effects. It is interesting in this context to note that the internal mobility of the lipid molecules is highest at the chain ends in the center of the bilayer and lowest at the glycerol backbone region (17), just the opposite of the internal mobility of a polypeptide helix in a membrane. Our finding of the positional dependence of the conformational fluctuations within a helix is in accord with a recent MD simulation for a decaglycine helix in vacuum (18), where the mean square amplitudes of the backbone atomic fluctuations were evaluated to be larger at the ends than at the center of the helix.

The slow relaxation process observed in FA experiments shows no dependence on the tryptophan sequence position and therefore reflects fluctuations of the whole polypeptide molecule in a lipid membrane. In principle, one or a combination of at least three processes may play a role: (i) twisting and bending of the helix, (ii) rigid-body fluctuations of the whole helix around the membrane and membrane normal, and (iii) rotational motions of the polypeptide molecule around the helix axis. The relaxation time of the rigid-body fluctuations of the helix can be estimated according to the formalism developed for describing the orientational fluctuations of lipid molecules in a membrane as $\phi_{\alpha} = 6\pi \nu V/F KT$ (19). The constant $\nu$ depends on the rotational order parameter of the helix and adopts a value of $\alpha = 0.087$ for $S_{ij} = 0.8$ (19). Modeling a helical henicosapeptide as a rotational ellipsoid with the main axis of 30 Å and the short axis of 9 Å (16), the product between the molecular volume $\nu$ and the shape factor $f_\nu$ is $1.16 \times 10^{-20}$ cm$^3$. The difficulty arises as to how to assign the correct value for the viscosity $\eta$. From FA measurements of lipid probes in fluid lipid bilayers, a viscosity of $\eta_\nu \approx 0.2 \, p$ was derived for rotation or wobbling around an axis perpendicular to the membrane normal (19). However, for the lateral diffusion of lipids and proteins, a viscosity of $\eta_\nu = 1\, p$ was obtained (20). The rotational diffusion of proteins around a fixed axis preferentially parallel to the membrane normal is correlated to a viscosity $\eta_\nu = 1-4\, p$ (21). Different molecular motions seem to sense different viscosities. Bending motions of a polypeptide helix may be coupled to the orientational fluctuations of the lipid molecules, and therefore $\eta_\nu$ is the appropriate viscosity. On the other hand, rigid-body fluctuations of a bilayer-traversing polypeptide helix can only occur if at the same time lipid molecules perform translational motion in the membrane plane. But orientational fluctuations of the lipid molecules may play a role as well. With a lipid viscosity ranging between reported values of $\eta_\nu$ and $\eta_\nu$, a rotational time of $\phi_{\alpha} = 30-150\, ns$ is calculated. For the rotation of the polypeptide around the helix axis time in the range of 100 ns is estimated according to ref. 21 by using the reported values of $\eta_\nu$. Furthermore, the different helix motions can be coupled to some extent. MD calculations taking into account the surrounding lipid bilayer may yield a better understanding of the dynamics of a helical membrane protein. It will be interesting to test whether fluctuations as observed here for membrane-spanning polypeptide helices are important for biological functions of membrane proteins such as in transport processes (4) or channel activity (6).

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