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# Dynamics of the peptide hormone motilin studied by time resolved fluorescence spectroscopy

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**Abstract.** Time resolved fluorescence was used to study the dynamics on the nanosecond and subnanosecond time scale of the peptide hormone motilin. The peptide is composed of 22 amino acid residues and has one tyrosine residue in position 7, which was used as an intrinsic fluorescence probe. The measurements show that two rotational correlation times, decreasing with increasing temperature, are needed to account for the fluorescence polarization anisotropy decay data. Viscosity measurements combined with the fluorescence measurements show that the rotational correlation times vary approximately as viscosity with temperature. The shorter rotational correlation time (0.08 ns in an aqueous solution with 30% hexafluoropropanol, HFP at 20 °C) should be related to internal movement of the tyrosine side chain in the peptide while the longer rotational correlation time (2.2 ns in 30% HFP at 20 °C) describes the motion of the whole peptide. In addition, the interaction of motilin or the derivative motilin (Y7F) –23W (with tyrosine substituted by phenylalanine and with a tryptophan fluorophore added to the C-terminal) with negatively charged phospholipid vesicles (DOPG) was studied. The results show the development of a long anisotropy decay time which reflects partial immobilization of the peptide by interaction with the vesicles.

**Key words:** Motilin – Fluorescence – Rotational correlation time

## Introduction

Motilin is a gastrointestinal peptide hormone whose primary function is to regulate the migrating motor complex in the gut. The peptide consists of 22 amino acids of which the first five are hydrophobic and the rest are mainly hydrophilic. The hormone binds to a membrane bound receptor, not yet purified, in the smooth muscle in the gastrointestinal tract. Earlier $^1$H-NMR measurements in a mixed aqueous solvent with 30% HFP have shown that residues 3–6 form a wide turn. A probably highly dynamic "hinge-like" region was found around Tyr 7. In the C-terminal part of the peptide a relatively stable $\alpha$-helix is formed between residues 9 and 20. HFP was used in the NMR study to stabilize the secondary structure of the peptide (Khan et al. 1990; Edmondson et al. 1991). As shown in a previous CD study, this HFP solution structure should be relevant for motilin in a negatively charged phospholipid environment, typical for a biomembrane (Backlund et al. 1994). The interaction between peptide and phospholipid membrane appears to be in part electrostatic in nature, since negatively charged vesicles induce secondary structure in the peptide, in contrast to neutral vesicles. However, although electrostatic forces may be required to attract and orient the peptide at the vesicle, the mechanism of structure induction may involve mainly the screening from interactions between water and the peptide, as has been suggested for the galanin peptide (De Loof et al. 1992).

Previous fluorescence studies have shown that the tyrosine fluorescence decay in motilin is described by more than one lifetime, probably reflecting the presence of rotamers that exchange slowly on the fluorescence decay time scale (Backlund and Gräslund 1992). In the present work we probe further into the dynamics of motilin as reflected by studies of the polarization anisotropy decay of the tyrosine fluorescence, under conditions of varying solvent and temperature. We also extend the studies to an active motilin analogue, motilin (Y7F) –23W, which has its fluorescent group (tryptophan) at the carboxyl terminal end of the peptide.

## Materials and methods

Porcine motilin was kindly provided by professor Viktor Mutt, Karolinska Institutet in Stockholm. The concentration used was typically 1.0 $10^{-4}$ M in 20 mM acetic acid (HAc) with a pH of about 3.5.

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Synthetic porcine motilin with the tyrosine in position 7 substituted by phenylalanine and with one additional amino acid, tryptophan, in position 23 was synthesized at the Dept. of Biophysics, Stockholm University. An in vitro assay of receptor binding ability showed that it is about as active as native porcine motilin. We thank Dr. T. L. Peeters, Katholieke Universiteit te Leuven, Belgium for performing the assay.

Sodium dodecyl sulphate (SDS) was obtained from BDH. 1,1,1,3,3,3-hexafluoro-2-isopropanol $(CF_3)_2CHOH$ (HFP) was obtained from Sigma Ltd.

1,2-dioleyl-sn-glycero-3-phosphoglycerol (DOPG) was provided by Avanti Polar Lipids (USA). The purity of the lipids was better than 99% as checked by thin layer chromatography. The lipid vesicles were prepared by sonication with a soniprep 150 (MSE, Scientific instruments, England) supplemented with an exponential microtip. The prepared DOPG vesicles were characterized by spin labelling and ESR to have a radius of about 110 Å (cf Backlund et al. 1994).

The viscosity of an aqueous solution containing 30% HFP was determined in the temperature range 5 °C to 56 °C with a capillary viscosimeter. For evaluation of the viscosity, the density of the 30% HFP aqueous solution was estimated to 1.2 $10^{-3}$ kg/dm$^3$. At 20 °C, assuming the water viscosity to be 1.0 $10^{-3}$ Ns/m$^2$, the viscosity of the 30% HFP solution was evaluated to be 2.0 $10^{-3}$ Ns/m$^2$.

Time resolved fluorescence measurements were carried out with the single photon counting method as previously described (Rigler et al. 1987). A laser system consisting of an argon laser pumping a dye laser, including frequency doubling, was used as the light source for the measurements at different conditions of temperature/viscosity. The wavelength of the light reaching the sample was 280 nm or 300 nm, the excitation wavelength for tyrosine or tryptophan in motilin or its analogoue, respectively. The measurements were carried out using two cutoff filters, UG 11 and one of the following: WG 335, WG 305, WG 345, (dependent on the sample) on the emission side. The temperature (5–56 °C) was regulated with water circulating from a thermostated water bath.

Data were collected in four different ways (Claesens and Rigler 1986; MacKerell et al. 1987): laser light, dark current, the perpendicular intensity ($I_\perp$) and the magic angle intensity ($I_m$). The perpendicular and magic angle polarization direction is given relative to the polarization direction of the exciting light pulse. Each measurement took about one and a half hours, collecting data for 60 sec in every data set and then recycling 20 times.

The collected data were analyzed with a method described previously (MacKerell et al. 1987; Gräslund et al. 1992). The dark current counts were subtracted from the data before the analysis. The method is based on the concept of discrete fluorescence and polarization anisotropy decay components. Some important features are described here:

Fluorescence decays $I_m(t)$ were described as a sum of discrete exponential components

$$I_m(t) = \sum_i \alpha_i \exp(-t/\tau_i) \tag{1}$$

where $\tau_i$ is the fluorescence lifetime and $\alpha_i$ its amplitude.

The fluorescence polarization anisotropy decay of the system is a function of the rotational correlation times as well as of the fluorescence decay times. The rotational correlation times were evaluated assuming that each rotational component is associated with all decay components (previously determined).

Anisotropy decays were calculated from the unpolarized $I_m(t)$ and perpendicularly polarized $I_\perp(t)$ fluorescence emission components using the following relations (cf MacKerell et al. 1987):

$$\tilde{I}_m(t) = L(t) \otimes \sum_i \alpha_i \exp(-t/\tau_i) \tag{2}$$

$$\tilde{I}_\perp(t) = L(t) \otimes \left[ g \left( \sum_i \alpha_i \exp(-t/\tau_i) \left\{ 1 - \sum_j \beta_j \exp(-t/\rho_j) \right\} \right) \right] \tag{3}$$

where $L(t)$ is the instrument response, $g$ is a correction factor due to the difference in detector sensitivity in the different polarization directions ($g$ is approx. 1.05). $\otimes$ denotes a convolution, and the superscript $\tilde{}$ a convoluted quantity. $\beta_i$ is the zero-time anisotropy for component $j$ and $\rho_j$ is the corresponding rotational correlation time. The parameters ($\beta_i$, $\tau_i$, and $\rho_i$) were fitted to the measured ratio $I_\perp(t)/I_m(t)$:

$$\frac{\tilde{I}_\perp(t)}{\tilde{I}_m(t)} = g \left( 1 - \frac{L(t) \otimes \left[ \sum_i \alpha_i \exp(-t/\tau_i) \sum_j \beta_j \exp(-t/\rho_j) \right]}{L(t) \otimes \sum_i \alpha_i \exp(-t/\tau_i)} \right). \tag{4}$$

We normally first obtain the fluorescence lifetime parameters $\alpha_i$ and $\tau_i$ from fitting of (2) and then use this calculated function as denominator in (4) instead of the measured data. This is to avoid numerical problems with noise in the data, which sometimes can cause a zero to be measured. A $\chi^2$ value indicates goodness of the fit between model and experiments.

Theoretical calculations of the rotational correlation time were performed using the equation (Cantor and Schimmel 1980):

$$\tau_c = \frac{V\eta}{k \cdot T} \tag{5}$$

where $V$ is the Volume, $\eta$ the viscosity, $k$ the Boltzmann constant and $T$ the temperature. The colume was calculated assuming different shapes of the peptide molecule, such as a sphere or ellipsoid as will be described later.

## Results

Time resolved fluorescence spectroscopy studies were performed on motilin and its analogoue (motilin (Y7F) –23W) at different temperatures and in different solvents. Figures 1 and 2 show the fluorescence decay curves and

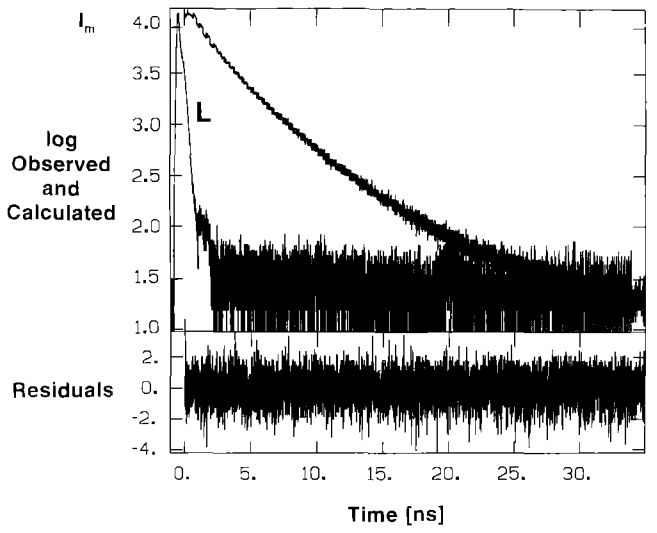


**Fig. 1.** Fluorescence decay curve (magic angle intensity $I_m$) of motilin at 5 °C. The motilin concentration was 1 $10^{-4}$ M in 20 mM acetic acid (pH ~3.5). The excitation wavelength was 280 nm. In the upper part of the figure the decay curve is shown together with the laser light (denoted *L*). The vertical scale is light intensity in logarithmic units. The calculated fitted decay curve is almost completely hidden below the experimental one. The residual (in non-logarithmic vertical scale) between observed and fitted curves is shown at the bottom of the figure

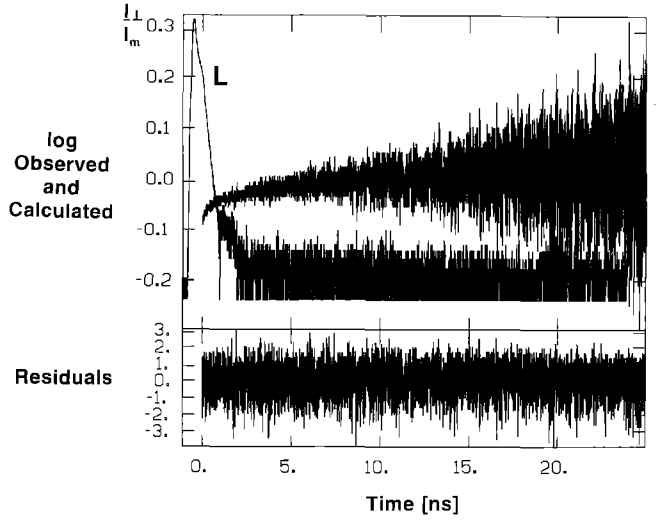


**Fig. 2.** Fluorescence polarization anisotropy decay curve of motilin measured as ($I_\perp/I_m$) at 5 °C. The experimental conditions were as in Fig. 1. In the upper part the anisotropy decay curve is shown together with the laser light (denoted *L*). The calculated fitted curve is partly hidden below the experimental one. The residual between observed and fitted curves is shown at the bottom of the figure

the fluorescence polarization anistropy decay curves for motilin in 20 mM acetic acid and in the same aqueous solution with 30% HFP at 5 °C. Though evaluation with 3 lifetime components gave similar components (data not shown) as those measured at the MAX synchrotron laboratory at Lund, Sweden (Backlund and Gräslund 1992) we now used 4 lifetime components to get better $\chi^2$ values in the fitting of the fluorescence decay curves. The fluorescence decay process was described by the following 4 lifetime components: $\alpha_1$=0.23, $\tau_1$=0.21 ns, $\alpha_2$=0.26, $\tau_2$=0.81 ns, $\alpha_3$=0.49, $\tau_3$=1.80 ns, $\alpha_4$=0.01, $\tau_4$=5.35 ns and $\chi^2$= 1.20 for motilin in water (20 mM HAc, pH 3.5) at 20 °C.

**Table 1.** Fluorescence polarization anisotropy decay components (amplitudes $\beta_i$ and rotational correlation times $\rho_i$, ns) for motilin in 20 mM acetic acid with and without 30% hexafluoroisopropanol (HFP) at different temperatures. Excitation wavelength 280 nm. Filters UG 11 and WG 345 were used on the emission side

| | 5 °C 30% HFP | 20 °C 30% HFP | 40 °C 30%HFP | 56 °C 30% HFP | 20 °C 0% HFP |
|---|---|---|---|---|---|
| $\rho_1$ | 0.10±0.05 | 0.08±0.03 | 0.03±0.12 | 0.04±0.07 | 0.03±0.03 |
| $\rho_2$ | 3.5±0.36 | 2.2±0.22 | 0.83±0.13 | 0.69±0.17 | 0.76±0.07 |
| $\beta_1$ | 0.15±0.08 | 0.18±0.07 | 0.19±0.74 | 0.18±0.24 | 0.24±0.27 |
| $\beta_2$ | 0.12±0.004 | 0.11±0.004 | 0.10±0.01 | 0.08±0.01 | 0.12±0.01 |
| $\chi^2$ | 1.00 | 1.00 | 1.00 | 1.03 | 1.00 |

To describe the fluorescence polarization anisotropy decay for motilin 2 rotational correlation times were needed. At 20 °C and 30% HFP the long and short rotational correlation times were found to be 2.2 ns and 0.08 ns, respectively. Table 1 shows the evaluated rotational correlation times at different temperatures. The standard errors of some of the the parameters are high, particularly for those characterising the rapid motion towards the higher temperatures. However, both the long and short rotational correlation times decrease with increasing temperature, which is reasonable. The amplitude parameters $\beta$ (Table 1) contain information about an orientational order parameter (Lipari and Szabo 1982), which can be approximated as $S^2=\beta_2/(\beta_1+\beta_2)$. For the measurements at 20 °C and below, and in 30% HFP, where the amplitude $\beta_1$ has a relatively low standard error, $S^2$ is around 0.4.

Comparing motilin with and without 30% HFP at 20 °C, Table 1, shows that the longer rotational correlation time is 2–3 times as large in a motilin solution with 30% HFP as without HFP. This scales very well with the viscosity which is twice as large in the mixed 30% HFP solvent as in pure water in the temperature range 5–56 °C. Figure 3 shows the evaluated rotational correlation times and the viscosity of the 30% HFP solution as functions of the inverse absolute temperature. The experimental data were fitted to straight lines by linear regression. From the temperature dependence of the rotational correlation times for motilin in 30% HFP we could evaluate activation energies of 6.1 kcal/mol for the slow motion and 4.0 kcal/mol for the rapid motion. The corresponding activation energy for viscosity was found to be 4.6 kcal/mol. These results show that both rotational correlation times and the viscosity vary to a similar extent with temperature. Free tyrosine in solution has the rotational correlation time of 0.13 ns in 20 mM HAc at 20 °C (data not shown), to be compared with the shorter correlation time for motilin which is 0.03–0.04 ns in 20 mM HAc at 20 °C.

Experiments to study the dynamics of motilin and motilin (Y7F) –23W in the presence or absence of negatively charged phospholipid vesicles (DOPG) or negatively charged detergent micelles (SDS) were performed. The re-

**Table 2.** Fluorescence polarization anisotropy decay components (amplitudes $\beta_i$ and rotational correlation times $\rho_i$, ns) for motilin (conc. 8.7 $10^{-5}$ M) in water, SDS and DOPG (conc. 3.2 $10^{-3}$ M) at 22 °C. Excitation wavelength 280 nm. Filters UG 11 and WG 345 were used on the emission side

| | Water | 50 mM SDS | DOPG |
|---|---|---|---|
| $\rho_1$ | 0.04±0.01 | 0.07±0.06 | 0.06±0.01 |
| $\rho_2$ | 0.68±0.07 | 1.5±0.18 | 2.1±0.31 |
| $\beta_1$ | 0.22±0.06 | 0.14±0.16 | 0.30±0.06 |
| $\beta_2$ | 0.12±0.008 | 0.14±0.007 | 0.10±0.005 |
| $\chi^2$ | 1.10 | 1.08 | 1.02 |

**Table 3.** Fluorescence polarization anisotropy decay components (amplitudes $\beta_i$ and rotational correlation times $\rho_i$, ns) for motilin (Y7F) –23W (conc. 3.6 $10^{-5}$ M) in water, SDS and DOPG (conc. 3.2 $10^{-3}$ M) at 22 °C. Excitation wavelength 300 nm. Filters UG 11 and WG 345 were used on the emission side

| | Water | 50 mM SDS | DOPG |
|---|---|---|---|
| $\rho_1$ | 0.06±0.02 | 0.08±0.03 | 0.08±0.03 |
| $\rho_2$ | 0.56±0.08 | 1.0±0.15 | 3.4±0.34 |
| $\beta_1$ | 0.31±0.06 | 0.24±0.06 | 0.21±0.10 |
| $\beta_2$ | 0.14±0.02 | 0.16±0.02 | 0.16±0.006 |
| $\chi^2$ | 1.06 | 1.08 | 1.01 |

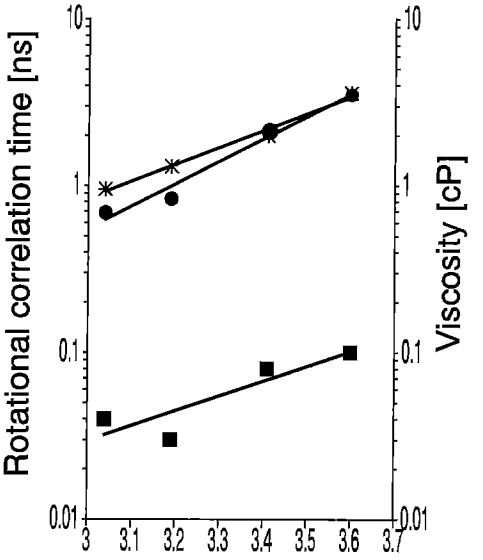


**Fig. 3.** Rotational correlation times of motilin evaluated from fluorescence polarization anitotropy decay measurements, as functions of the inverse absolute temperature (1000/$T$). The temperature dependence of the viscosity of an aqueous solution of 30% HFP is included. The motilin concentration was 1 $10^{-4}$ M in 20 mM acetic acid (pH ~3.5) and 30% HFP. (✳), viscosity; (●), the long rotational correlation time; (■), the short rotational correlation time

sults showed major changes in the fluorescence anisotropy decay for motilin and the motilin analogue depending on the presence of DOPG vesicles or SDS micelles (data not shown). The rotational correlation times evaluated in water, SDS and DOPG are presented in Tables 2 and 3. The shorter rotational correlation time did not change significantly in solvents containing DOPG or SDS compared to pure water. However, the results in Tables 2 and 3 show that the longer rotational correlation times for motilin and the motilin analogue are significantly increased in a solvent containing SDS micelles or particularly DOPG vesicles.

## Discussion

The time resolved fluorescence measurements show that 2 rotational correlation times are needed to describe the fluorescence polarization anisotropy decay data. The shorter rotational correlation time is of the same order of magnitude as that of tyrosine alone in solution. It should be related to the internal movement of the tyrosine side chain in the peptide, which seems to be about as mobile as in free tyrosine. The long rotational correlation time (2.2 ns at 20 °C in 30% HFP, Table 1) should describe the motion of the whole peptide. The presence of a longer rotational correlation time component in the peptide shows that the rapid motion is restricted in space by the presence of the neighboring residues.

The longer rotational correlation time varies approximately like viscosity with temperature and solvent. In a 30% HFP solution the viscosity and the rotational correlation time are about twice as large as in a solution without HFP. The viscosity dependence of the short rotational correlation time shows that also the rapid motion is coupled to the viscosity of the colvent.

Calculations of the overall rotational correlation time (Eq. (5)) with a model of a cylinder with length 33 Å, the approximate length of the peptide, and diameter 17.5 Å give an estimated rotational correlation time of 3.9 ns at 20 °C in 30% HFP. The dimensions were estimated from the three dimensional structure determined previously for motilin (Edmondson et al. 1991). If we use an ellipsoid with axes 33 Å, 17.5 Å as a model the rotational correlation time becomes 2.6 ns in 30% HFP. Previous estimations based on synchrotron excited tyrosine fluorescence of motilin at 20 °C gave as a result 0.5 ns and 6 ns for the two rotational correlation times in 30% HFP (Backlund et al. 1992). It was considered in the previous study that these values were probably correct only within a factor of two owing to the low fluorescence intensity in that study. The present parameter values for rotational correlation times show a reasonably temperature and viscosity dependence (Table 1, Fig. 3) as well as agreement with calculations. The accuracy of the measured rotational correlation times should be better than ±20%. The shorter correlation time, around 30 ps in aqueous solution and 80 ps in 30% HFP at 20 °C (Table 1), is of the same order of magnitude as that observed (44 ps) in [Leu$^5$]enkephalin for the local motion of a single tyrosine fluorophore under similar conditions (Lakowicz et al. 1993).

The present motional model assumes two independent motions, operating on different timescales if they are to be resolved with good precision. The model is analogous to the so called model-free approach (Lipari and Szabo

1982), often used to interpret dynamic results from NMR measurements. Certain discrepancies between the results form the two common methods to study molecular dynamics experimentally, $^{13}C$ NMR relaxation and fluorescence polarisation anisotropy decay, have been reported recently. For a 25 amino acid long zinc finger peptide (Palmer et al. 1993) an about 25% longer rotational correlation time for the overall motion was evaluated from the $^{13}C$ NMR relaxation data, compared to the fluorescence results. Even larger differences were reported from $^{13}C$ NMR relaxation and fluorescence measurements on the small protein thioredoxin (Kemple et al. 1994), where the NMR results gave about a factor of two longer rotational correlation times compared to fluorescence.

For motilin, $^{13}C$ relaxation measurements on a synthetic peptide with a specific $^{13}C$ label on the $\alpha$ carbon of Leu 10 yielded about a factor of two longer rotational correlation times for the global motion of the molecule under similar conditions (Allard et al. 1994), compared to the present fluorescence study. In the motilin comparison the probes of the dynamics are differently located in the $^{13}C$ NMR and fluorescence studies. A complete solution of the diffusion equation for the motion of a side chain coupled to a large body motion yields three relaxation times, the weighting of which depends on the relative location of the spectroscopic absorption and emission vectors (Rigler and Ehrenberg 1973). This solution can be approximated by motional models (Kinosita et al. 1977; Lipari and Szabo 1982) that introduces an order parameter and correlation times for local and global motions. Both correlation times are however weighted averages which will depend on the geometrical arrangement of the vectors actually observed in an NMR or fluorescence experiment. When these vectors are different, precise measurements should in the general case not give identical results. The two types of measurements on motilin dynamics, when evaluated in the framework of the model free approach (Lipari and Szabo 1982), are characterized by order parameters of different magnitude. For the fluorescent tyrosyl ring probe we estimated $S^2=0.4$ at 20 °C and in 30% HFP, whereas the order parameter for the motion of the $^{13}C^{\alpha}$–$H^{\alpha}$ vector was found to be 0.9 in a similar solvent (Allard et al. 1994). The fluorescence results should therefore report quite accurately on the rapid motion of the tyrosyl side chain, but the slow motion may be influenced by motions on a shorter time scale. A more general consideration is that the fluorescence and NMR studies are normally made at widely different peptide/protein concentrations. Hence at least part of the observed differences might be explained by a higher viscosity in the more concentrated NMR samples (Allard et al. 1994).

The time-resolved fluorescence studies on the interaction between motilin and negatively charged micelles (SDS) or phospholipid vesicles (DOPG) show that the fluorescence polarization anisotropy decay processes are clearly slowed down by the interaction. Earlier CD measurements have shown that more $\alpha$-helical secondary structure is induced by SDS or DOPG in the peptide (Backlund et al. 1994). According to the present results the interaction with SDS or DOPG also makes the overall motion of the peptide, whether monitored by Tyr-7 or Trp-23, significantly slower. However, the phospholipid vesicles should have radius of about 110 Å (Backlund and Gräslund 1992). Their rotational correlation time according to Eq. (5) should therefore be on the order of 1 μs, much larger than what could be observed for the associated between peptide and vesicle, leaving considerable freedom of independent motion for the peptide. There was no significant effect on the shorter correlation time in either solvent system.

In conclusion, the present results on motilin describe the dynamics on the ns and sub-ns time scale of the motion of the whole peptide and of the tyrosine side chain, respectively. The whole peptide has a rotational correlation time correlated with viscosity at various temperatures. The motion of the tyrosine side chain, on a timescale below 100 ps at room temperature, is about as fast as free tyrosine in solution and is also dependent on viscosity. Interaction with SDS micelles or DOPG vesicles influences the overall motion of the peptide, which is considerably slowed down.

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## References

Allard P, Jarvet J, Ehrenberg A, Gräslund A (1995) Mapping of the spectral density function of a $C^{\alpha}$–$H^{\alpha}$ bond vector from NMR relaxation rates of a $^{13}C$ labelled $\alpha$-carbon in motilin. J Biomol NMR (in press)

Backlund B-M, Gräslund A (1992) Structure and dynamics of motilin. Time-resolved fluorescence of a peptide hormone with a single tyrosine residue. Biophys Chem 45:17–25

Backlund B-M, Wikander G, Peeters TL, Gräslund A (1994) Induction of secondary structure in the peptide hormone motilin by interaction with phospholipid vesicles. Biochim Biophys Acta 1190:337–344

Claesens F, Rigler R (1986) Conformational dynamics of the anticodon loop in yeast $tRNA^{Phe}$ as sensed by the fluorescence of wybutine. Eur Biophys J 13:331–342

Cantor CR, Schimmel PR (1980) Biophysical Chemistry, part 2. Freeman, New York, p 461

DeLoof H, Nilsson L, Rigler R (1992) Molecular dynamics simulation of galanin in aqueous and nonaqueous solution. J Am Chem Soc 114:4082–4035

Edmondson S, Khan N, Shriver J, Zdunek J, Gräslund A (1991) The Solution Structure of Motilin from NMR Distance Constraints, Distance Geometry, Molecular Dynamics, and an Iterative Full Relaxation Matrix Refinement. Biochemistry 30:11271–11279

Gräslund A, Kim SK, Eriksson S, Nordén B, Jernström B (1992) Dynamics of benzo[a] pyrene diol epoxide adducts in poly(dG-dC) · (dG-dC) studied by synchrotron excited fluorescence polarization anisotropy decay. Biophys Chem 44:21–28

Kemple MD, Yuan P, Nollet K, Fuchs JA, Silva N, Prendergast FG (1994) $^{13}C$ NMR and fluorescence analysis of tryptophan dynamics in wild-type and two single-trp variants of Escherichia coli thioredoxin. Biophys J 66:2111–2126

Kinosita JR K, Kawato S, Ikegami A (1977) A theory of fluorescence polarization decay in membranes. Biophys J 20:289–305

Khan N, Gräslund A, Ehrenberg A, Shriver J (1990) Sequence-Specific $^1H$ NMR Assignments and Secondary Structure of Porcine Motilin. Biochemistry 29:5743–5751

Lakowicz JR, Gryczynski I, Laczko G, Wiczk W (1993) Intensity and anisotropy decays of [$Leu^5$] enkephalin tyrosyl fluorescence

by 10 GHz frequency-domain fluorometry. Biophys Chem 47:33–40

Lipari G, Szabo A (1982) Model-free approach to the interpretation of nuclear magnetic resonance relaxation in macromolecules 1. Theory and range of validity. J Am Chem Soc 104:4546–4559

MacKerell Jr AD, Rigler R, Nilsson L, Hahn U, Saenger W (1987) Protein dynamics: A time-resolved fluorescence, energetic and molecular dynamics study of ribonuclease $T_1$. Biophys Chem 26:247–261

Palmer III AG, Hochstrasser RA, Millar DP, Rance M, Wright PE (1993) Characterization of amino acid side chain dynamics in a zinc finger peptide using $^{13}C$ NMR spectroscopy and time-resolved fluorescence spectroscopy. J Am Chem Soc 115:6333–6345

Rigler R, Ehrenberg M (1973) Molecular interactions and structure as analysed by fluorescence relaxation spectroscopy. Quart Rev Biophys 6:139–199

Rigler R, Kristensen O, Roslund J, Thyberg P, Oba K, Eriksson M (1987) Molecular structures and dynamics: Beamline for time resolved spectroscopy at the MAX synchrotron in Lund. Phys Scr T17:204–208