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Lanthanide Modulation of the Orientation of Macromolecules Induced by Purple Membrane

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The purple membrane (PM) from *Halobacterium salinarum* has been suggested as a tool to provide partial orientation of biomolecules in solution.[1,2] The mechanism of action of PM is believed to be mostly electrostatic, as opposed to the dominant steric orientation caused by the commonly used bicelles.[3] The induced orientation will be related to the charge distribution that, in general, is unique even for structurally related domains in multidomain proteins. PM can be viewed as a source of an oriented local electric field and may constitute a good model system to study the interaction of soluble charged proteins with charged membrane proteins (e.g. ion-channels).[4] By manipulating the orientation of PM, the local electric field can be forced to adopt different orientations with respect to the magnetic field.

PM is constituted by two-dimensional aggregates of acidic lipids and bacteriorhodopsin (BR), a protein with an α-helical fold. In these planar aggregates, the protein molecules are arranged in trimers with pseudohexagonal symmetry, with the α-helices oriented roughly perpendicular to the plane.[5] BR molecules constitute about 75% in weight of the whole PM. The calculated magnetic anisotropy of each BR molecule is about 2.5×10^{-32} m^3, mostly due to the helices perpendicular to the PM plane. The macroscopic shape of PM fragments is that of disks with about 7500 Å diameter and a thickness of about 50 Å. It is estimated that there is an average of about 40 000 BR molecules per PM fragment, so the global magnetic susceptibility anisotropy of an individual fragment is about 10^{-27} m^3.[6] At magnetic fields higher than 10 T, PM is nearly 100% oriented, with its normal parallel to the magnetic field. This behavior is opposite to that of lipid bicelles, whose normal is perpendicular to the magnetic field.

The magnetic susceptibility anisotropy of paramagnetic metal ions may be high enough to provide partial orientation when embedded in a protein. Typically, the magnetic anisotropy per metal ion ranges from 2×10^{-32} m^3 (i.e. the same as that of a BR molecule) to 10−20 times higher.[7] It has already been shown that paramagnetic iron-containing proteins or lanthanide-substituted calcium binding proteins self-orient in solution allowing the measurement of residual dipolar couplings (rdc) and their use as structural constraints.[8] Addition of lanthanide ions has been previously used to revert[9] or reinforce[10] the orientation of lipid bicelles. As PM possesses binding sites for multivalent cations with dissociation constants in the micromolar range, we have decided to study the effect of lanthanides on the orientation of PM.

The orientation of PM was monitored at 14.9 T by measuring the rdc induced by 2.4 mg/mL of PM in 0.8 mM His-tagged ^{15}N-labeled [A20V]protein L (protein L) at pH 7.4 in 20 mM Tris buffer with 50 mM NaCl. Splittings were measured by fitting individual F1 traces of F1-coupled HSQC spectra. This rather tedious approach provides, however, estimates of the line widths of the different peaks.

The cation binding sites in PM are not well established.[11] Therefore, we initially explored the addition of thulium (Tm^{3+}) and terbium (Tb^{3+}) cations that have large magnetic susceptibility anisotropies of different sign in equivalent coordination environments.[7] Addition of increasing amounts of Tb^{3+} causes first a decrease and then a change in sign of the observed dipolar couplings. At 0.8 equiv of Tb^{3+} with respect to BR, all dipolar couplings were scaled by a factor of −0.5 as expected if the normal to the membrane plane changes from parallel ($\theta = 0°$, $^1/_2(3\cos^2(\theta - 1)) = 1$) to perpendicular ($\theta = 90°$, $^1/_2(3\cos^2(\theta - 1)) = -0.5$) to $\mathbf{B_0}$. Addition of Tm^{3+} has nearly no effect, as PM is already close to 100% oriented at 14.9 T.

The orientation tensors of protein L under different conditions, as well as their orientation with respect to the structure, were obtained by nonlinear fitting of the measured dipolar couplings to the equation

$$D_{NH} = D_{ax}[(3\cos^2(\theta - 1)) + {}^3/_2 R(\sin^2\theta\cos 2\phi)] \qquad (1)$$

using the spherical coordinates of the individual NH vectors obtained from the X-ray structure of protein L transformed to the principal axes system using the Euler angles α, β, γ. The errors were estimated by using a Monte Carlo approach. The results are shown in Table 1. A plot of experimental versus calculated rdc values is provided as Supporting Information.

The orientation tensors in the presence of different amounts of PM are nearly identical, except for D_{ax} which includes the orientation of the membrane fragments. The normalized scalar product[2] of the alignment tensors obtained with 0.8 equiv of Tb^{3+} and PM alone is −0.96. The similarity of the tensors indicates that the addition of Tb^{3+} has little effect on the interaction of protein L with PM and rules out any spurious direct interaction between protein L and Tb^{3+}.

It has been previously reported that the addition of PM causes a general line broadening and a corresponding intensity loss in ubiquitin that is partially reversed by the addition of NaCl.[2] We also observe broadening and cross-correlation effects that result in substantial differential broadening between the upfield and downfield components of the NH doublets in the spectra of protein L after the addition of PM. Plots of the fitted line widths of both lines for protein L alone and in the presence of 2.4 mg/mL of PM are shown in Figure 1a,b.

The observed line broadening and cross-correlation effects are rather nonuniform and therefore cannot be explained by changes in the isotropic correlation time resulting from changes in sample viscosity. Differential broadening of the two components of the

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Table 1. Fitted Orientation Tensors of His-Tagged [A20V]Protein L in the Presence of PM (2.4 mg/mL) and Increasing Amounts of Tb^{3+}

Tb^{3+}/BR	D_{ax} (Hz)	R	α (deg)	β (deg)	γ (deg)
0.0	3.00 ± 0.07	0.22 ± 0.03	105.6 ± 1.3	63.1 ± 1.0	−44.8 ± 4.8
0.2	2.96 ± 0.06	0.16 ± 0.03	102.7 ± 1.0	57.7 ± 0.7	−47.6 ± 6.2
0.5[a]	0.56 ± 0.05	0.14 ± 0.12	112.3 ± 4.5	59.1 ± 3.8	42.2 ± 30.0
0.8	−1.49 ± 0.05	0.29 ± 0.04	104.9 ± 2.0	56.3 ± 1.2	−74.1 ± 5.6

[a] The increased uncertainty in the parameters results from the very small rdc's observed.

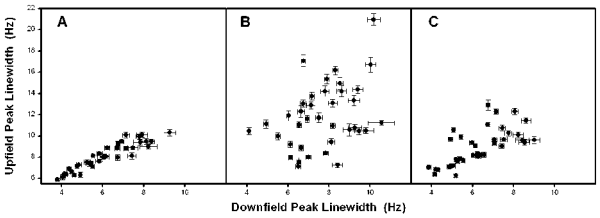

Figure 1. Upfield vs downfield line widths of (A) protein L alone, (B) protein L plus PM, and (C) protein L plus PM and 0.8 equiv of Tb^{3+}.

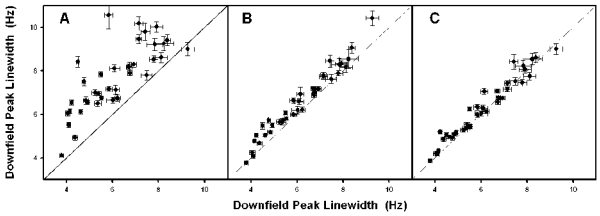

Figure 2. Comparison of the line widths of the downfield components of NH signals of protein L alone (horizontal axis) with those measured in the presence of 2.4 mg/mL of PM and (A) no lanthanide, (B) 0.5 equiv of Tb^{3+}, and (C) 0.8 equiv of Tb^{3+}.

doublet cannot be explained either by chemical exchange between two isotropic states.

Sass et al.[2] have suggested that a possible explanation for the observed line broadening is anisotropically hindered rotational diffusion in the vicinity of the membrane and they report variations of about 20% between the $T_1/T_{1\rho}$ ratios of individual NH groups in the presence of PM. We have observed some correlation between the line broadening and the square of the rdc induced by PM on protein L. A plot of the difference of line widths in the presence and in the absence of PM versus the square of the rdc measured for each NH doublets is given as Supporting Information.

The electrostatic orientation of a protein is probably dominated by its dipole moment, although at very short distances the quadrupolar moment may be important. This provides a unique axis for the interaction with the membrane surface and reorientation on the surface will be restricted to rotations around this particular axis. Since PM is oriented with the normal to the plane parallel to the external magnetic field, this reorientation is expected to be rather ineffective in modulating the dipolar interaction and substantial broadening effects are expected. On the other hand in Tb^{3+} doped PM, with the normal of the plane perpendicular to the magnetic field, rotational diffusion around the same unique axis will effectively modulate both the dipolar and chemical shift anisotropy interactions for most NH groups, leading to narrower line widths and reduced cross-correlation effects.

Figure 1c shows the effect of the addition of Tb^{3+} on the differential line widths of the two lines in the NH doublets of protein L. Figure 2 shows the effect of adding Tb^{3+} on the line width of the downfield component of coupled NH signals. Conversely, addition of Tm^{3+} (see Supporting Information) does not decrease the line widths.

Reversing the orientation of the purple membrane results in substantial line narrowing and reduction of cross-correlation effects.[12] These effects were not observed by adding Tm^{3+}, which does not change the orientation of PM. This rules out effects derived from dilution, changes in ionic strength, or membrane surface charge.

Addition of Tb^{3+} reduces the rdc's of protein L observed in the presence of PM by a factor of $-1/2$ and the line widths become substantially narrower. We suggest that both effects can be explained by a change in the orientation of PM fragments with respect to the magnetic field. The narrower line widths of proteins in the presence of Tb^{3+} doped PM make this system a valuable alternative to induce partial orientation mainly based on the charge distribution of biomolecules.

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Supporting Information Available: Table with experimental rdc during Tb^{3+} and Tm^{3+} addition and PM induced line broadening; plot of calculated versus experimental rdc; comparison of line widths of protein L in the presence of PM and Tb^{3+} and Tm^{3+}; plot of PM induced line broadening versus the square of the induced rdc values (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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