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Short communication

The shape of a membrane protein derived from rotational diffusion

F. Jähnig

Max-Planck-Institut für Biologie, Corrensstrasse 38, D-7400 Tübingen, Federal Republic of Germany

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Abstract. Membrane proteins are modelled as cylinders with an elliptic cross-section in the plane of the membrane. The coefficient for rotational diffusion about the cylinder axis is calculated as a function of the axial ratio of the elliptic cross-section.

Key words: Theory of rotational diffusion, elliptic cylinder, bacteriorhodopsin

It is well known that the size of a membrane protein can be determined by measuring its rotational diffusion. Since a protein in a membrane can rotate only about an axis parallel to the membrane normal and not perpendicular to it, the rotational diffusion is simply characterized by the diffusion coefficient $D_\parallel$, for which the following relation has been derived (Saffman and Delbrück 1976)

$$D_\parallel = \frac{kT}{4\pi\eta a^2 h}. \tag{1}$$

Here, the protein is described as a cylinder of radius a and height h rotating in a membrane of thickness h and viscosity η. Evidently, only the membrane-incorporated part of the protein is important for rotational diffusion so that only the size of that part can be determined. As a prerequisite, the membrane thickness and viscosity must be known. The thickness can be measured by X-ray diffraction and is of the order of 50 Å; the membrane viscosity is obtained most accurately by measuring the rotational diffusion of a protein of known size, such as bacteriorhodopsin, in the given membrane (Cherry and Godfrey 1981). Knowing h and η, the measured diffusion coefficient $D_\parallel$ can be evaluated according to Eq. (1) for the radius, a, of the membrane-incorporated part of the protein.

It is intuitively clear that the rotational diffusion of a membrane protein is affected by the shape of the protein. The more elliptical the cross-section is in the plane of the membrane, at constant volume, the slower it should rotate. This effect can be calculated analytically by describing the protein as an elliptic cylinder. Perrin (1934) has shown that the diffusion coefficients, D_i, for rotation of an arbitrary ellipsoid in an isotropic medium can be expressed as

$$D_i = \frac{kT}{C_i} \quad (i = 1, 2, 3) \tag{2}$$

with the friction coefficients, C_i, derived by Edwardes (1893)

$$C_1 = \frac{16\pi\eta}{3} \cdot \frac{a_2^2 + a_3^2}{a_2^2 P_2 + a_3^2 P_3}, \text{ etc.} \tag{3}$$

The quantities a_i denote the half axes of the ellipsoid and the P_i are the elliptic integrals

$$P_i = \int_0^\infty \frac{ds}{(a_i^2+s)\sqrt{(a_1^2+s)(a_2^2+s)(a_3^2+s)}}. \tag{4}$$

For the case of a symmetric ellipsoid with $a_1 = a_2$, analytical solutions for $D_1 = D_2 = D_\perp$ and $D_3 = D_\parallel$ have been derived by Perrin (1934)[1]. To treat the case of an elliptic cylinder, as a first step, the assumption $a_{1,2} \ll a_3$ is introduced, this specifies an extreme prolate ellipsoid. Then the integrals of Eq. (4) can be calculated as

$$P_1 = \frac{1}{a_3}\int_0^\infty \frac{ds}{(a_1^2+s)^{3/2}(a_2^2+s)^{1/2}} = \frac{2}{a_1 a_3 (a_1+a_2)},$$

$$P_2 = \frac{1}{a_3}\int_0^\infty \frac{ds}{(a_1^2+s)^{1/2}(a_2^2+s)^{3/2}} = \frac{2}{a_2 a_3 (a_1+a_2)}.$$

From Eqs. (2) and (3) one obtains for the diffusion coefficient $D_\parallel = D_3$ for rotation about the long axis

$$D_\parallel = \frac{kT}{4\eta V} v \tag{5}$$

1 Perrin (1934) corrected an error by Edwardes (1893), whereas an error by Perrin was discovered independently and corrected by Wyman and Ingalls (1943), Memming (1961), and Koenig (1975)

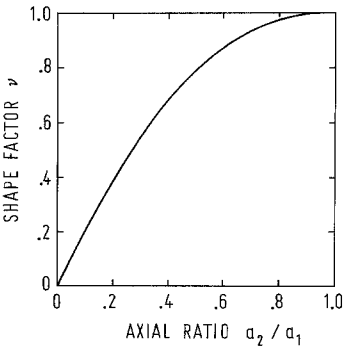

Fig. 1. The dependence of the shape factor v in the diffusion coefficient $D_\parallel$ of a membrane protein on the ratio a_2/a_1 of the half axes of the protein

with V denoting the volume, $V = \frac{4\pi}{3} a_1 a_2 a_3$, and v the shape factor

$$v = \frac{2a_2/a_1}{1+(a_2/a_1)^2}. \tag{6}$$

The dependence of v on the axial ratio a_2/a_1 is shown in Fig. 1. For the other two coefficients D_1 and D_2 the corresponding shape factors become zero.

In a second step, one passes from the extreme prolate ellipsoid to an elliptic cylinder by cutting a segment of length $h \ll a_3$ out of the middle of the ellipsoid and putting it into a sheet of viscous medium. The thickness of the sheet equals h and the cylinder is oriented to span the sheet. The viscosities above and below the sheet are assumed to be vanishingly small so that the upper and lower boundaries of the cylinder are not subject to friction. Then the diffusion coefficient for rotation about the cylinder axis is given by Eq. (5) with V replaced by the volume of the elliptic cylinder, $V = \pi a_1 a_2 h$. Thus, the rotational diffusion coefficient of a membrane protein modelled as an elliptic cylinder results as

$$D_\parallel = \frac{kT}{4\pi\eta a_1 a_2 h} v. \tag{7}$$

In the limit $a_1 = a_2$, Eq. (6) yields $v = 1$ and Eq. (7) becomes identical to Eq. (1). Upon deformation of the protein at constant volume, i.e. for $a_1 > a_2$ with $a_1 a_2 = \text{const}$, the shape factor v becomes smaller (Fig. 1) and $D_\parallel$ decreases. As expected, the more elliptical a protein is in the plane of the membrane, the slower it rotates. However, the shape factor v can only be derived from $D_\parallel$ and Eq. (7), if the volume V of the membrane-incorporated part of the protein is known. This is the case for proteins which are completely incorporated into the membrane and lack any peripheral part. Then V can be calculated from the molecular weight of the protein. Knowing η and V, the measured $D_\parallel$ can be evaluated for v according to Eq. (7), which yields the axial ratio a_2/a_1 according to Eq. (6).

As an example for the determination of the membrane viscosity η, the case of bacteriorhodopsin will be considered. The shape of the monomer can be described approximately by an elliptic cylinder of half axes $a_1 = 18$ Å, $a_2 = 13$ Å, and height $h = 45$ Å (Henderson and Unwin 1975). These values yield $V = 33{,}100$ Å^3 which leads, using a partial specific volume of 0.74 cm^3/g, to the correct molecular weight of 26,800. Furthermore, the axial ratio of $a_2/a_1 = 0.72$ yields the shape factor $v = 0.95$. The rotational diffusion coefficient of bacteriorhodopsin in fluid membranes of dimyristoylphosphatidylcholine at $T = 28$ °C is $D_\parallel = 6.7 \cdot 10^4\,\text{s}^{-1}$ (Cherry and Godfrey 1981). From Eq. (7) one then obtains $\eta = 4.5$ poise.

In the case of bacteriorhodopsin, the magnitude of the shape effect on $D_\parallel$ is of the order of 5%. This value may be considered as typical. Hence, the shape effect is smaller than the present experimental error in $D_\parallel$ which is of the order of 30%. With further progress in sample preparation and instrumentation, however, the experimental error may become smaller so that the shape effect will become observable and may be used to determine the shape of membrane proteins.

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