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FLEXIBILITY OF MEMBRANE PROTEINS BY BROAD-LINE PROTON MAGNETIC RESONANCE

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Broad-line ^{1}H-NMR studies of reconstituted membranes of bovine rhodopsin and dimyristoylphosphatidylcholine have demonstrated that the rhodopsin undergoes a considerable increase in motion when the proportion of phospholipid is increased from a small amount (12:1 molar ratio) insufficient to surround the protein completely to a large amount (150:1) which suspends the protein in a bilayer.

Several recent studies [1–3] have examined the effect of membrane proteins on the dynamic structure of phospholipids but little is known about the effect of phospholipids on the dynamics of membrane proteins. Here we demonstrate that broad-line proton magnetic resonance (^{1}H-NMR), a technique often considered of little value for systems undergoing anisotropic motion such as biological membranes, is a powerful tool for the quantitative study of protein flexibility. As an example, we investigate the physical state of rhodopsin in reconstituted membranes of bovine retinal rod outer segment rhodopsin and dimyristoylphosphatidylcholine (DMPC).

The use of ^{1}H-NMR in biomembrane research is hampered because the spectra are rather broad and featureless. The spectra of pure phospholipid dispersions have been studied extensively [4] and great care must be exercised in obtaining information from parameters such as linewidth. In biomembranes the dipolar interactions are not averaged to zero by the molecular motions and it is necessary to use NMR techniques normally employed for the study of solids [5]. We shall demonstrate with our example that the second moment of the broad NMR line is a most useful tool.

In a recent deuteron magnetic resonance (^{2}H-NMR) study of rhodopsin DMPC recombinant membranes [3] it was found that the orientational order of the phospholipid chains varied considerably with rhodopsin concentration and temperature. For lipid to protein molar ratios (L:P) less than 30:1 at all temperatures the ^{2}H-NMR spectra had a distinctive form indicating that the hydrocarbon chains of 'rhodopsin associated' phospholipid molecules underwent anisotropic motions considerably different from those of a pure phospholipid system. For larger lipid to protein ratios such a contribution was only present at temperatures below the liquid-crystalline to gel-phase transition indicating a phase separation into free lipid and rhodopsin-associated lipid components at low temperatures. Since the phospholipid chain motions are affected by the presence of rhodopsin, it is important to determine whether the rhodopsin dynamic behavior is affected by a change in phospholipid concentration. Therefore we have carried out a ^{1}H-NMR study on the same samples with particular emphasis on the contribution to the NMR spectra from the rhodopsin.

The simplest way to get quantitative information from the broad ^{1}H-NMR spectra obtained from the rhodopsin/phospholipid recombinants is the method of moments. The second moments (M_2) for each of the NMR spectra were measured using techniques described elsewhere [4]. For all samples the measured second moments, M_2, are the sum of the contributions from the different species of proton present weighted according to the fraction of the ^{1}H nuclei associated with each species. That is:

$$M_2 = \frac{N_L M_{2_L}}{(N_L + N_P)} + \frac{N_P M_{2_P}}{(N_L + N_P)} \quad (1)$$

where N_L and N_P refer to the number of protons and M_{2_L} and M_{2_P} to the ^{1}H second moments of the lipid and the protein parts of the samples, respectively. If one assumes that all protons bonded to oxygen and nitrogen (approx. 500) have exchanged with deuterons in the ^2H_2O, there are about 2100 non-exchangeable protons per rhodopsin molecule [6]. There are 18 protons in each DMPC-d_{54} molecule. Thus the proton NMR signals arising from the lipid and the protein should be in the ratio 1 : 10 for the 12 : 1 sample and 9 : 7 for the 150 : 1 sample.

The ^{1}H-NMR moments for the two rhodopsin/DMPC samples are plotted as a function of temperature in Fig. 1. The gel to liquid-crystalline phase transition for pure DMPC-d_{54} is 20°C [3]. For the 12 : 1 sample for which 90% of the signal is from rhodopsin, the observed M_2 is large ($5 \cdot 10^9$ s^{-2}) and close to the value which one would measure for crystalline proteins (For powdered myoglobin from equine skeletal muscle, obtained from Sigma Chemical Co., $M_2 = 6.4 \cdot 10^9$ s^{-2} at 21°C). Hence, it seems that the rhodopsin molecules in the 12 : 1 sample do not have any molecular motion capable of appreciably narrowing the ^{1}H-NMR line other than those such as methyl group rotation already present in the solid protein sample.

The experimental second moment for the 150 : 1 sample is about 4-times smaller than that for the 12 : 1 sample throught the temperature range studied. To obtain the rhodopsin second moment, M_{2_P}, one must use Equation 1 along with a reasonable value for the phospholipid headgroup second moment, M_{2_L}. The upper limit for M_{2_P}, which one would obtain in the unlikely event that the contribution of the phospholipid headgroup to M_2 were decreased to zero by the presence of the protein, is about $3 \cdot 10^9$ s^{-2}. A larger value for M_{2_L} or an overestimate in the number of exchangeable rhodopsin protons which have exchanged with deuterons would lead to a smaller value for the rhodopsin second moment.

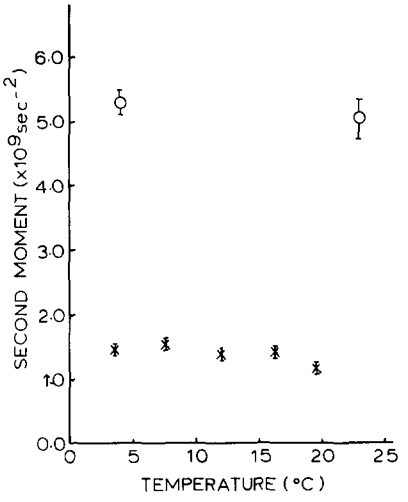

Fig. 1. Second moments measured from NMR spectra of recombinant membranes of rhodopsin and dimyristoylphosphatidylcholine with perdeuterated chains (DMPC-d_{54}) with L : P = 12 : 1 (○) and 150 : 1 (×). After ^{2}H-NMR measurement, the samples used in Ref. 3 were suspended with 20 ml ^2H_2O buffered with phosphate at p^{2}H = 7.8 and centrifuged at 150000 × g for 30 min. This was repeated several times after which the pellet was placed inside the NMR tube. Dark conditions were maintained throughout to keep the rhodopsin in its unbleached state. ^{1}H-NMR measurements were performed at 90 MHz using a Bruker SXP 4-100 pulsed spectrometer.

This reduction by a factor of about two or more of the rhodopsin second moment on increasing the lipid to protein molar ratio from 12 to 150 implies the onset of some protein molecular motion capable of partially averaging the dipolar interactions on a timescale of about $(M_2)^{-1/2} \sim 15$ μs [7]. This reduction is not due to overall rotation of the rhodopsin since the correlation time for this motion for the 150 : 1 sample has been found by EPR techniques [8,9] to be 50 μs at 23°C and about an order of magnitude longer at 4°C. However, it could be due to the onset of small angle librational

motions. Other types of motion capable of providing this reduction are increased side chain conformational motions and/or undulations of the protein surface. As shown by ^{2}H-NMR on the same samples [3], the 150 : 1 sample is aggregated at 4°C into two phases, a gel phase and a phase consisting of 30 lipids associated with each protein. Our result indicates unambiguously that the aggregated protein phase in the presence of excess phospholipid (L : P > 30 : 1) has internal motions which are not present when the phospholipid concentration is less than 30 phospholipid molecules per protein molecule.

In this report we have demonstrated that the method of moments used with ^{1}H-NMR studies provides important information on protein dynamics as manifested in the average behavior of all the protons in our samples. This kind of information, which would be difficult to obtain with other techniques, is quite different from that one obtains from specific probe magnetic resonance experiments such as spin-label electron paramagnetic resonance and spin-label ^{2}H-NMR which give detailed results about a limited number of atomic positions and may require complex chemical preparation. The type of information obtained in this study may be more aptly compared to that obtained from other bulk measurements such as calorimetry, circular dichroism and light scattering.

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