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FORMATION OF ORIENTED MEMBRANE MULTILAYERS OF Na/K-ATPase

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Low angle diffraction information from oriented membrane multilayers has been used to determine the organization, conformation and structure of protein and lipid components for a number of membrane systems, including photosynthetic reaction centers (1), acetylcholine receptors (2), rhodopsin (3), and gap junctions (4). By exploiting the complimentary scattering characteristics of both neutrons and X-rays, detailed information on ligand binding sites (5), sites of activity (6), and protein conformation changes (7) have been obtained, despite the fact that diffraction from most oriented membrane multilayers is low resolution. In neutron diffraction, the large difference between the scattering lengths of hydrogen and deuterium is a characteristic which can be used to study the structure of membrane protein systems. For example, the scattering length of the solvent can be adjusted over a wide range by varying mixtures of H_2O and 2H_2O , allowing a determination of the water distribution in a membrane system (8), or, by selectively deuterating membrane components (lipids, proteins, or portions of either), the distribution of any one component can be determined (1,5).

Na/K-ATPase is a membrane bound enzyme vital to mammalian cells; in addition to regulating Na^+ and K^+ concentrations and energy metabolism, it has also been implicated in regulation of the transport of H^+ , Ca^{++} , glucose, and amino acids, as well as being the receptor for the cardiac glycosides. The isolated membrane-bound enzyme retains its ouabain-sensitive ATP hydrolysis activity, and produces ATP-dependent Na^+ and K^+ fluxes when incorporated into phospholipid vesicles (9). The ultimate goal of this work is to determine the low resolution structure of this important enzyme, using both X-ray and neutron diffraction. The first step towards deriving significant structural information via diffraction experiments on membrane proteins in lipid bilayers is to develop a method for preparing oriented membrane

multilayers which will diffract to at least $1/10 \text{ \AA}^{-1}$.

A number of methods were used to impart one-dimensional stacking (lamellar) order to highly purified pig Na/K-ATPase membranes. Ultracentrifugation ($120,000 \times g$) concentrated, but did not impart significant orientation to these membranes. X-ray diffraction from such fully hydrated systems produced continuous diffraction to approximately $1/25 \text{ \AA}^{-1}$. Upon partial dehydration, X-ray diffraction from Na/K-ATPase membrane multilayers at 98% relative humidity yielded discrete reflections of $118 \text{ \AA}$ periodicity, diffracting to $1/14.8 \text{ \AA}^{-1}$, additionally, continuous diffraction to $1/10 \text{ \AA}^{-1}$ was obtained (Figure 1). The amount of lattice disorder present could be estimated from the increase in width of the lamellar reflections as a function of increasing diffraction angle (10). The oriented Na/K-ATPase membrane multilayers have less lattice disorder than oriented sarcoplasmic reticulum membranes, which have a mosaic spread of $\sim 30^\circ$ (11). In parallel neutron diffraction experiments, it was estimated from $W-2\theta$ scans that the mosaic spread of these partially dehydrated membrane multilayers was approximately 15° full width at half maximum. It was crucial that the pellet was initially dried at a relative humidity of $\geq 98\%$ at $\sim 5^\circ\text{C}$. After initial partial dehydration, the pellet, could then be subjected to relative humidities in the range of 92% to 100%; humidities lower than 92% produced an irreversible phase transition. The membrane multilayers produced lamellar diffraction patterns which indexed on a single lattice as long as the temperature during the experiment was maintained between 2°C and 10°C .

Subjecting the membrane multilayers to high magnetic fields improved the quality of the lamellar diffraction dramatically. Neutron diffraction studies of the partially dehydrated Na/K-ATPase membrane multilayers detected a mosaic spread of 2° when the samples were subjected to a magnetic field of 5 Tesla perpendicular to the

membrane surface; the reflections were narrower than the camera line width; hence, the lattice disorder has also decreased significantly, although only four orders were measured. This orientation is comparable to the best orientation thus far achieved for any membrane multilayer system (1) of which we are aware.

Previous electron microscopy studies showed that the Na/K-ATPase membranes are cup-shaped sheets approximate 100 Å thick and consist of a single bilayer (12). Therefore, the 120 Å periodicity of the lamellar diffraction must arise from a single membrane. It is not clear whether the membranes are symmetrically or asymmetrically oriented in the unit cell; electron microscopy studies of the oriented membrane multilayers are underway to help determine the symmetry, so that phasing of the lamellar diffraction patterns can proceed.

In summary, very well ordered multilayers of Na/K-ATPase membrane sheets were prepared by partial dehydration, followed by orientation in a magnetic field. The samples are suitable for low resolution structural studies of the enzyme.

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Figure 1: X-ray diffraction pattern of highly purified pig Na/K-ATPase at 95% relative humidity, 5°C, obtained using a specimen to detector distance of 13.0 cm. The exposure time was ~15 minutes.

INTENSITY

RECIPROCAL ANGSTROMS

TABLE I

EFFECT OF MAGNETIC FIELDS ON THE
ORIENTATION OF MEMBRANE MULTILAYERS

Sample Conditions ¹	Reflection	Intensity ²	Full width at Half Maximum in degrees
Magnetic field: 0 tesla	1/D	3.8×10^3	0.32°
Periodicity: $165 \text{ \AA}$	2/D	1.0×10^4	0.44
2θ at 1/D: 0.85°	3/D	6.4×10^2	0.50
mosaic spread: $\sim 15^\circ$	4/D	5.3×10^2	0.59
Magnetic field: 5 tesla	1/D	4.4×10^3	0.30
Periodicity: $162 \text{ \AA}$	2/D	1.3×10^4	0.33
2θ at 1/D: 0.81°	3/D	7.5×10^2	0.40
mosaic spread: $\sim 2^\circ$	4/D	5.6×10^2	0.51
Magnetic field: 0 tesla	1/D	4.0×10^3	0.33
Periodicity: $165 \text{ \AA}$	2/D	1.1×10^4	0.33
2θ at 1/D: 0.85°	3/D	7.0×10^2	0.44
mosaic spread: $\sim 15^\circ$	4/D	5.2×10^2	0.49

1. The sample was made from 2 mg of purified membraneous Na/K-ATPase, which was formed into a 0.5 cm diameter pellet. The pellet was partially dehydrated at 98% relative humidity for 24 h. at 5°C to impart an initial degree of orientation. During the neutron diffraction experiment, the sample remained at 5°C, under sealed conditions. The monochromatic neutron beam had a flux of 10^8 - 10^7 neutrons/cm²/s at $\lambda = 2.36 \text{ \AA}$; the incident beam dimensions were fixed at 1 mm x 3 mm, and the beam divergence was 0.16° .

2. Intensity is a measure of the sample lamellar diffraction and background scattering due to incoherent neutron scattering and scatter from disoriented sections of the sample.