Distribution of Lens Sodium-Potassium-Adenosine Triphosphatase

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**Purpose.** The specific activity of sodium-potassium-adenosine triphosphatase (Na-K-ATPase) in lens fiber cells is lower than the specific activity in lens epithelium. To test whether there is a reduction in the expression of Na-K-ATPase molecules in lens fibers, a Western blot technique was used.

**Methods.** Membrane material was isolated from different regions of the rabbit lens. Na-K-ATPase (adenosine triphosphate hydrolysis) activity was measured in each membrane sample and Western blots were performed using an antibody to rabbit kidney Na-K-ATPase.

**Results.** By immunoblotting, Na-K-ATPase polypeptide was detected in all lens cells. In contrast, adenosine triphosphate hydrolysis by the Na-K-ATPase (Na-K-ATPase activity) was not detectable or was detectable only at very low levels in fiber membranes from the lens nucleus and cortex.

**Conclusion.** These findings suggest that plasma membrane adenosine triphosphatase enzyme responsible for sodium-potassium transport is expressed in newly formed lens fibers and the transport molecules are retained as the fibers age and are compressed toward the center of the lens. However, with fiber aging there is a loss of functional ability of the Na-K-ATPase to hydrolyze adenosine triphosphate.

Electrolyte balance is vital to lens transparency. Transparent mammalian lenses (including human lenses) contain approximately 17 mmol/l sodium and 130 mmol/l potassium, whereas most senile cataracts with opacification of the cortex have a dramatically elevated sodium level (up to 100 mmol/l) and a potassium level that is well below 80 mmol/l (see Patmore and Duncan for review). When lens sodium levels become abnormally high and lens potassium levels fall, water accumulation causes cell disruption and impairment of transparency.

It has long been established that the lens contains sodium-potassium-adenosine triphosphatase (Na-K-ATPase), and that this sodium pump mechanism is responsible for maintaining the electrolyte balance in the organ. Initially, Bonting and his coworkers and Palva and Palkama reported that the Na-K-ATPase activity is localized almost entirely in the lens epithelium. However, subsequent investigators using more sensitive enzyme assay methods and cytochemical techniques have shown that there is measurable Na-K-ATPase activity in the lens fiber mass even though the specific activity of Na-K-ATPase in the epithelium is considerably higher than the specific activity in fiber cells. Nevertheless, it should be remembered that although the fibers have a low specific activity of Na-K-ATPase, these cells account for most of the lens bulk and it turns out that the fiber mass contains a significant fraction of the total Na-K-ATPase activity detectable in the lens.
The low specific activity of Na-K-ATPase in lens fiber cells may signify that when the epithelium differentiates to become a fiber, there is a significant reduction in the relative expression of Na-K-ATPase molecules in the elongating cells, or that the Na-K-ATPase in lens fibers becomes inactivated. In the current study, we used immunoblotting to detect Na-K-ATPase polypeptides in different regions of the lens. Na-K-ATPase was detected in all lens cells, including cells in the nucleus where ATP hydrolysis by the Na-K-ATPase could not be measured.

MATERIAL AND METHODS

Lens Dissection

Lenses were obtained from 2-3 kg adult New Zealand white rabbits immediately after they were killed with an overdose of sodium pentobarbital administered by a marginal ear vein. The procedures used in these studies were approved by the University of Louisville Institutional Animal Care and Use Committee and conformed to the ARVO Resolution on the Use of Animals in Research. Lenses were removed quickly from the eyes by opening the posterior of the globe and gently cutting the zonules. Using fine forceps, the capsule/epithelium was removed from the fiber mass. Epithelium membranes and fiber membranes from different sections of the lens were examined separately in these studies. After removal of the capsule/epithelium, the lens fiber mass was frozen rapidly in liquid nitrogen. Using a motor-driven trephine, a 6 mm diameter cylindrical pole-pole core was removed from the frozen lens. The equatorial cortex region surrounding the core was collected. The frozen pole-pole core was then divided into six anterior-posterior sections as shown in Figure 1a.

Membrane Preparation

Using capsule/epithelium or sections of cortex, membranes were isolated by homogenization in ice-cold buffer A (150 mmol/l sucrose, 10 mmol/l N-hydroxyethylpiperazine-\(\text{N}^\text{\(\cdot\)}\)-2-ethanesulfonic acid, 1 mmol/l dithiothreitol) containing the protease inhibitors leupeptin (10 \(\mu\)g/ml), PMSF (40 \(\mu\)M), aprotinin (0.03 trypsin inhibitor units/ml), antipain (10 \(\mu\)g/ml), and pepstatin A (10 \(\mu\)g/ml). The homogenate was centrifuged at 600 \(\times\) g; this low-speed pellet containing debris and nuclei was discarded. The supernatant was then placed in a centrifuge for 45 minutes at 100,000 \(\times\) g. The pellet was resuspended in buffer A containing 0.6 M KCl to remove extrinsic proteins and then placed in a centrifuge again at 100,000 \(\times\) g for 45 minutes. The pellet was then resuspended in buffer A, placed in a centrifuge once more at 100,000 \(\times\) g for 45 minutes to give a final pellet of partially purified membrane material that was resuspended in a small amount of buffer A and stored in liquid nitrogen. The protein content of the partially purified membrane material was determined using the Peterson\(^{10}\) modification of the Lowry assay.\(^{11}\)

![FIGURE 1.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933173/) (A) After removal of the capsule/epithelium, a 6 mm diameter cylindrical core was removed from the lens. The core was divided into six anterior-posterior slices. The lens equator material surrounding the core was also collected. (B) Immunoblot of rabbit lens Na-K-ATPase polypeptides in membrane material isolated from epithelium (ep+), equatorial fibers (equ) and fibers in six pole-pole sections (1 = Anterior, . . . , 6 = Posterior). Na-K-ATPase \(\alpha\) subunit shows densely at \(\approx 100\) kDa; beta less clearly at \(\approx 40\) kDa. In epithelial membranes prepared without protease inhibitors (ep−), loss of \(\alpha\) subunit and appearance of a Na-K-ATPase proteolysis product at \(\approx 30\) kDa was observed. To obtain these results, proteins were blotted to immobilon P membranes and probed with goat anti-(rabbit kidney Na-K-ATPase). Immunopositive polypeptides were seen with alkaline phosphatase-coupled antigoat gamma globulin. The molecular weight indications (100 k and 40 k) were determined from molecular weight standards in parallel lanes. (C) The polypeptide composition of lens membrane material in these samples is shown in a Coomassie Blue-stained sodium dodecyl sulfate gel (C), which was run in parallel with the immunoblot.
Measurements of Na-K-ATPase Activity

Na-K-ATPase activity was determined by adding membrane material to a buffer containing 100 mmol/l sodium chloride, 10 mmol/l potassium chloride, 40 mmol/l histidine, 5 mmol/l magnesium chloride, 10^{-8} M calcium-ethyleneglycol-bis-(aminoethyl ether)N,N,N',

tetraacetic acid buffer and 11 μg/ml of alamethicin, at pH 7.0 and 37°C. The alamethicin was added to make all membrane vesicles accessible to the buffer constituents as described by Xie and coworkers. After 5 min of preincubation, the ATPase reaction was started by the addition of 1 mmol/l ATP containing a tracer amount of ATP labeled with 32P at the gamma position (Amersham, Arlington Heights, IL). ATP hydrolysis, which is linear up to 40 min, was quantified by determining the 32P liberated from labeled ATP using the methods described by Socci and Delamere. To specify the ouabain-inhibitable Na-K-ATPase activity, the reaction was run in the presence and absence of 10^{-3} M ouabain. Na-K-ATPase activity was expressed in terms of protein (nmoles ATP hydrolyzed/hr/ug protein).

Immunoblotting

Membrane proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Laemmli on a 7.5% gel. Equivalent amounts of membrane material (based on protein content) were applied to each lane of the gel. The separated proteins were transferred to immobilon P membrane in 3-[cyclohexamino]-2-hydroxy-1-propanesulfonic acid buffer, pH 11.0 at 0.4 amps for 30 min. The immobilon membrane was then blocked with 3% gelatin in TTBS (0.02 M Tris buffer at pH 7.5, 0.5 M sodium chloride, and 0.05% Tween 20) and exposed overnight to the primary Na-K-ATPase antibody (goat anti-(rabbit kidney Na-K-ATPase), Calbiochem, San Diego, CA). After washing the immobilon membrane with TTBS, second antibody coupled to alkaline phosphatase was added for 1 hr. The immobilon membrane was then washed again with TTBS before visualization of the Na-K-ATPase polyepitide immunoblots with alkaliine phosphatase substrates (Bio-Rad, Richmond, CA).

RESULTS

Immunoblotting of Lens Na-K-ATPase

Membrane proteins were isolated from rabbit lens epithelium and from fiber membranes collected from different regions of the lens cortex and nucleus as described in Methods (Fig. 1a). Membrane proteins were separated on 7.5% sodium dodecyl sulfate-polyacrylamide gels, transferred to immobilon membranes and probed with an antibody raised against rabbit kidney Na-K-ATPase. Na-K-ATPase polyepitides were detected in membrane material from all sections of the lens; highest in the epithelium and lowest in fiber membranes from the nucleus (Fig. 1b). The Na-K-ATPase subunit was clearly visible at around 100 kDa and the subunit less clearly at approximately 40 kDa. The difference in detectability of the subunit may be a property of this particular antisera. However, the subunit was seen more clearly on the wet immobilon membrane directly after color development of immunoblot, but the color faded during the drying period.

In parallel with the immunoblot, another sodium dodecyl sulfate gel was run and then stained with Coomassie Blue to reveal the polypeptide composition of each lens membrane preparation (Fig. 1c). Although there was not a major polyepitide at 100 kDa, the position of the Na-K-ATPase subunit in the immunoblot, a higher molecular weight (116 kDa) major protein band appeared in samples from lens fiber, but not in the epithelium. It is therefore unlikely that spurious, nonspecific binding of Na-K-ATPase antibodies to major lens proteins contributed to our findings. In addition, the stained gel illustrates that there was little contamination of the epithelial membrane preparation with fiber material since the major 116 kDa fiber protein band is nearly absent from the epithelial preparation.

To obtain the membrane preparations used in these studies, lens material was homogenized and centrifugated at cold temperatures in the presence of protease inhibitors. To test whether the Na-K-ATPase is sensitive to proteolytic breakdown, a sample of epithelium was prepared in the absence of protease inhibitors and the sample (sample ep-, Fig. 1b) was allowed to come briefly to room temperature. In an immunoblot of this membrane preparation there was evidence of considerable conversion of the Na-K-ATPase subunit to a small immunoreactive polyepitide appearing significantly below 40 kDa.

Na-K-ATPase Activity

The Na-K-ATPase activity (rate of ouabain-sensitive ATP hydrolysis) was measured in each of the membrane samples isolated from lens epithelium or various regions of the lens fiber mass. The highest Na-K-ATPase activity was observed in the epithelium, which contained 50% of the total lens Na-K-ATPase (Table 1). However, it should be stressed that detectable levels of Na-K-ATPase activity were observed in fiber membranes isolated from the deeper cortex (Sections 2 and 5). Na-K-ATPase activity was not detectible in material from the nucleus (Sections 3 and 4). These same sections contained substantial amounts of Na-K-ATPase subunit as indicated by immunoblotting (Fig. 1b).

It is important to note that the equatorial section of the fiber mass contained approximately 32% of the
TABLE 1. Na,K-ATPase Activity Determined in Sections of the Rabbit Lens

<table>
<thead>
<tr>
<th>Lens Section</th>
<th>Specific Activity of Na,K-ATPase (nmol ATP/μg protein/hr)</th>
<th>% of Total Lens Na,K-ATPase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>6420 (373)</td>
<td>50.9</td>
</tr>
<tr>
<td>Anterior 1</td>
<td>242 (27)</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>59 (10)</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>11 (24)</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>20 (11)</td>
<td>0.7</td>
</tr>
<tr>
<td>5</td>
<td>118 (26)</td>
<td>4.1</td>
</tr>
<tr>
<td>Posterior 6</td>
<td>278 (37)</td>
<td>5.4</td>
</tr>
<tr>
<td>Equatorial</td>
<td>1226 (92)</td>
<td>32.3</td>
</tr>
</tbody>
</table>

Data are the mean ± SE of six measurements. Lenses were dissected into sections as shown in Figure 1A. Na,K-ATPase activity was determined in membrane material isolated from each section.

total lens Na-K-ATPase activity. This section of the lens is made up mostly of fiber “middles”; the tips of each lens fiber extend toward the sutures at the poles of the lens.

DISCUSSION

Na-K-ATPase is found in the plasma membranes of cells as a heterotetramer of 100 kDa α subunits and 40 kDa β subunits.15,16 By immunoblotting, we were able to detect the Na-K-ATPase α subunit very clearly in lens membranes. The Na-K-ATPase β subunit was less demonstrable, perhaps because of technical difficulties; the β subunit band faded as the immobilon membranes in that region react with the Na-K-ATPase antibody. This finding suggests that as lens epithelium differentiates to give rise to fiber cells, Na-K-ATPase is expressed in the newly formed fibers and the transport molecule is retained by these cells as they age and are compressed toward the center of the lens. However, our data indicate that with fiber differentiation and subsequent aging, there is a loss in the functional ability of the Na-K-ATPase to hydrolyze ATP. For example, membrane samples from lens epithelium had approximately 25 times more Na-K-ATPase activity than superficial cortical fibers at the anterior or posterior pole. Yet all these regions had a relatively similar Na-K-ATPase activity. In the lens nucleus, for example, the Na-K-ATPase activity was nil, although the fiber cell membranes in that region react with the Na-K-ATPase antibody. This finding suggests that as lens epithelium differentiates to give rise to fiber cells, Na-K-ATPase is expressed in the newly formed fibers and the transport molecule is retained by these cells as they age and are compressed toward the center of the lens. However, our data indicate that with fiber differentiation and subsequent aging, there is a loss in the functional ability of the Na-K-ATPase to hydrolyze ATP. For example, membrane samples from lens epithelium had approximately 25 times more Na-K-ATPase activity than superficial cortical fibers at the anterior or posterior pole. Yet all these regions had a relatively similar Na-K-ATPase activity.

The Na-K-ATPase was susceptible to proteolysis. Loss of α and β subunits of Na-K-ATPase was observed in epithelial membranes prepared without protease inhibitors (Fig. 1b); in this membrane sample, a small (less than 40 kDa) immunoreactive polypeptide was observed, perhaps representing Na-K-ATPase breakdown products. There was no evidence of such low-molecular-weight Na-K-ATPase breakdown products in membrane material isolated in the normal manner. The lack of Na-K-ATPase breakdown fragments in fiber membranes from lens nucleus suggests that there may be relatively little proteolysis of lens Na-K-ATPase as fiber cells age.

On the basis of this study, we can only speculate on the possible reasons why lens fiber Na-K-ATPase appears to lose functional activity as it ages. Lens fiber membranes have an unusually high cholesterol/phospholipid ratio.17 The cholesterol/phospholipid ratio, which increases toward the center of the lens, gives lens fiber membranes an unusually high degree of order; the membranes are rigid.18 It is possible that the lack of membrane fluidity impairs the functional ability of fiber cell Na-K-ATPase, particularly in the nucleus where fluidity is lowest. An influence of membrane fluidity on Na-K-ATPase activity has been observed in a number of cell types.19,20 Low membrane fluidity might also inhibit the activity of plasma membrane Ca-ATPase in lens fibers as suggested by the temperature-dependence studies of Delamere and coworkers.21

If lens fiber Na-K-ATPase indeed becomes inactivated as the fiber cells age, lens sodium—potassium balance must rely heavily on a functional Na-K-ATPase in the epithelium and equatorial cortex; together, these two regions account for more than 80% of the ouabain-sensitive ATP hydrolysis activity in the lens (Table 1). The specific activity of Na-K-ATPase in the equatorial region of the fiber mass was considerably higher than that in the fiber cell samples collected from the anterior-posterior core, which we removed from the optical axis of the lens. Each lens fiber stretches from the anterior to the posterior pole; our observations suggest that the tips of lens fibers contain less functional Na-K-ATPase molecules than the middles (equatorial) region of the cells. It is possible that active sodium—potassium transport by fiber cell Na-K-ATPase at the equator plays an important role in lens electrolyte regulation. A role for the equatorial portions of fibers in performing ion transport duties for the whole lens is consistent with reports that extensive gap junctions between adjacent fibers are seen near the lens equator and not close to fiber tips.22,23 Ion pumping by cells close to the surface can perhaps influence the cytoplasmic composition of underlying packed cells in regions where cell-cell coupling by gap junctions is extensive. Additionally, extracellular diffusion path-
ways that could channel sodium out from the lens are best developed at the lens equator\textsuperscript{24} and the lens equator is associated with a marked outward potassium current.\textsuperscript{25}

**Key Words**

Na-K-ATPase, lens, rabbit, immunoblot, Ca-ATPase

**References**