THREE INDEPENDENT ASSAYS OF A SPECIFIC MAGNESIUM ATPase IN THE ROD OUTER SEGMENTS OF VERTEBRATE PHOTORECEPTORS

Rainer Uhl, Renate Zellmann,

Max Planck Institut für biophysikalische Chemie
Am Fassberg, D-3400 Göttingen, West Germany

Tom Borys, and E.W. Abrahamson

Dept. of Chemistry, University of Guelph,
Guelph, N1G 2W1, Ontario, Canada

Reports on ATPase activity in rod outer segments (ROS) have been conflicting, mainly because it has been very difficult to establish that the ATPase activity measured from ROS material actually resides in the ROS and not in small amounts of contaminating material. Here we present evidence for the existence of a very specific Mg-ATPase system in ROS of both bovine and frog retinae. Its activity can be monitored in three ways:

1) When using a conventional ATPase assay, in the presence of Mg^{2+} and ATP, our ROS preparation exhibit a rapid initial ATPase activity which decays with a half-time of 1-2min. It is completely inhibited by 20µM vanadate, 30µM DCCD, 100µM quercetin, and, in a competitive way, by the ATP analogue AMP-PNP. The K_M of the reaction is ca. 40µM ATP and a few mM GTP, indicating high specificity. Neither ouabaine, nor azide show any inhibition, ruling out interference from the Na/K-ATPase of the inner segment and the mitochondrial Mg-ATPase.

2) Simultaneously to the Mg-ATPase activity, with the same time course, ROS undergo a dramatic structural change in the presence of Mg^{2+} and ATP, as evident from a ca. 30% decrease in turbidity. This light scattering signal, which we have labelled "A_D", has the same K_M and the same inhibitor sensitivity as the Mg-ATPase.

3) After preincubation with Mg^{2+} and ATP, ROS react to flash-illumination with a rapid (ca. 20msec at 37°C) light scattering transient. The extent of this further decrease in light scattering shows the action spectrum of rhodopsin bleaching, is proportional to the fraction of unbleached rhodopsin present in the ROS and can be regenerated with 11-cis-retinal. It amounts to a light-intensity change of ca. $4\text{-}5 \times 10^{-4}$/%of rhodopsin bleached. All inhibitors of the rapid initial Mg-ATPase activity and of the light scattering increment "A_D" also block the rapid light scattering transient "A_L". Moreover, there is a strong correlation between the extent, to which "A_D" has been completed, and the amplitude of the following light response "A_L".

On the basis of the above findings we propose the existence of a specific Mg-ATPase system in the ROS. It is by this enzyme that the ROS are set up in the dark to react to light with a rapid structural response. Both the "enabling process" and the light response can be readily monitored as a decrease in light scattering from the ROS.