REMOVAL OF A LARGE FRAGMENT OF RHODOPSIN WITHOUT CHANGES IN ITS SPECTRAL PROPERTIES, BY PROTEOLYSIS OF RETINAL ROD OUTER SEGMENTS

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1. Introduction

The membranes of the outer segments from the rod cells of the retina contain 60% protein and 40% lipid [1, 2]. Approximately 80% of the protein is the visual pigment, rhodopsin [3, 4], which consists of an apoprotein, opsin, with a mol. wt. of about 39000 [3–6], and a chromophore, 11 *cis* retinaldehyde. The position of rhodopsin in the membranes has been in doubt. X-ray analyses and studies using various labelling compounds for rhodopsin seem now to agree, however, that a portion of the visual pigment is probably exposed on the surface of the disk membranes [7–12]. We have been using proteolytic enzymes to study the topography of the outer segment membranes, and report here that papain rapidly removes one third of the polypeptide chain of rhodopsin, leaving a residue whose spectral properties are unchanged.

2. Materials and methods

Rod outer segments (ROS) were prepared from fresh dark-adapted calf eyes [13] and kept overnight at 0°C. They were then suspended in 67 mM phosphate, pH 7.0, containing 5 mM cysteine, and 2 mM EDTA, to a concentration of about 1.2 mg protein/ml. Samples of the suspension were pre-incubated at 37°C for 10 min before the addition of papain (2X crystallised: obtained from Worthington) to a ratio of 1 part of enzyme to 20 parts of ROS protein (w/w). The ROS were re-incubated for various times and the reaction terminated by the addition of iodoacetamide to a concentration of 10 mM. The incubation tubes were immediately plunged on ice, and the ROS collected by centrifugation at 100000 *g* for 30 min. The pellets were then suspended in phosphate buffer and samples of the suspension taken to measure spectra. The bulk of the suspension was re-centrifuged as above.

For spectras, the samples of ROS were dissolved in an equal volume of a detergent solution (2.0% emulphogen in 67 mM phosphate, pH 7.0) and scanned from 650 to 250 nm with a Cary 14 recording spectrophotometer. The proteins of the ROS were extracted by homogenisation in 10 mM Tris, pH 7.0, followed by a sequential extraction with 0.04, 0.06, 0.08, 0.10, 0.15 and 0.30% sodium dodecyl sulphate (SDS) in 10 mM Tris [13]. After each homogenisation the material was centrifuged at 100000 *g* for 60 min. This procedure separates rhodopsin from most of the minor proteins of the ROS: with dark kept material the visual pigment is only extracted by 0.15 and 0.30% SDS. Each extract was analysed by polyacrylamide gel electrophoresis in the presence of SDS [13,14]. Up to this stage all procedures were performed under dim red light. The polyacrylamide gels were stained with amido black, destained with 10% acetic acid, and scanned with a Vernon densitometer.

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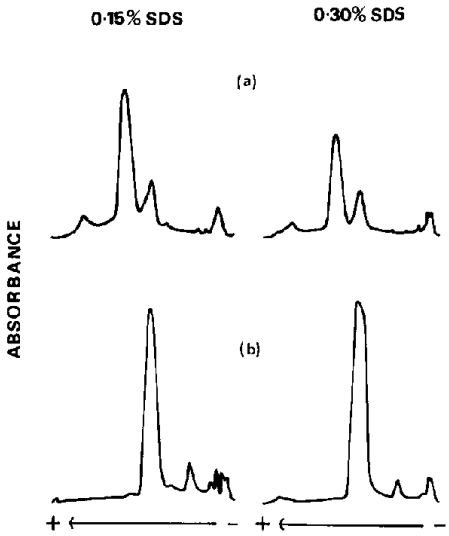

Fig. 1. Densitometric recordings of proteins separated by polyacrylamide gel electrophoresis. After incubation of ROS for 60 min the proteins were extracted by increasing concentrations of SDS, and separated by polyacrylamide gel electrophoresis. The extracts up to 0.10% SDS contained the minor proteins of the ROS: a) shows the proteins in the 0.15 and 0.30% SDS extracts after incubation with papain; b) the control incubated without enzyme.

3. Results and discussion

When the ROS were incubated with papain for 60 min the most striking change was the disappearance of the band on the gels (from the 0.15 and 0.30% SDS extracts) corresponding to rhodopsin (fig. 1). This change was accompanied by the quantitative appearance of a new band which migrated further. A second, but very minor, new band of a mol. wt. closed to 12 000 also appeared. It might be a fraction of the fragment detached from rhodopsin. The apparent absence of intermediates between rhodopsin and the major new band may be an indication that most, if not all, of the fragment is removed by the cleavage of one bond. The two new bands like rhodopsin itself, were only present in the 0.15% and 0.3% extracts due probably to the presence of a high amount of non polar amino acids and interaction with membrane lipids. The conversion of rhodopsin to the major new protein band took place without any alteration in the spectral properties of the membranes ; the λ_{max} (498 nm), the absorption at the λ_{max}, and all other aspects of the

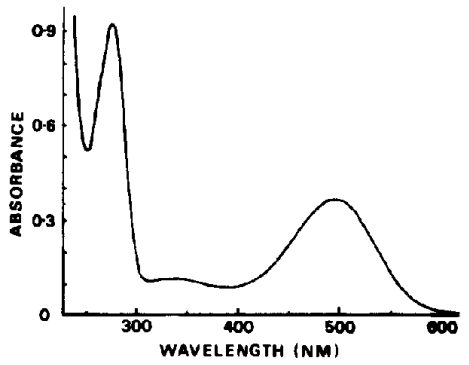

Fig. 2. Spectra of ROS after incubation with papain for 60 min. After centrifugation ROS were suspended in phosphate buffer and samples taken and dissolved in emulphogen.

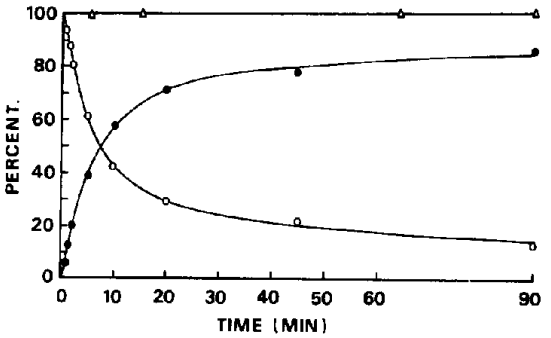

Fig. 3. Kinetic study of the conversion of rhodopsin to the 'partially digested rhodopsin'. ROS were incubated for various times with papain and the proteins extracted and separated (fig. 1). The densitometric recordings on the gels of the 0.15 and 0.30% SDS extracts were quantitated for rhodopsin and partially digested rhodopsin, and each expressed as a percentage of the total of the two: (●–●–●) % partially digested rhodopsin after incubation with papain; (○–○–○) % rhodopsin after incubation with papain; (△–△–△) % rhodopsin in the control.

spectrum were identical to the control (fig. 2).

A kinetic study of the formation of the rhodopsin digestion product from rhodopsin was performed. This revealed that a 50% conversion occurred after 7 to 8 min incubation of the ROS with papain (fig. 3). The spectral properties of the membranes were constant throughout the incubation period. The partially digested rhodopsin in the ROS was resistant to further attack by papain, as demonstrated by the constancy of the gel patterns and the spectra follow-

ing addition of more enzyme after 45 min incubation and reincubation for another 30 min (unpublished work).

The molecular weight of the partially digested rhodopsin was determined by polyacrylamide gel electrophoresis in the presence of SDS [15] using the following marker proteins: bovine serum albumin (mol. wt. 67 000), ovalbumin (mol.wt. 45 000), rhodopsin (mol.wt. 39 000) and cytochrome *c* (mol. wt. 12 000). A value of 24 800 ±200 (mean ±standard deviation for 4 determinations) was obtained. Since the molecular weight of rhodopsin is about 39 000 [3–6], papain removed in the region of 36% of the polypeptide chain of the visual pigment without affecting its spectral properties. Thus a substantial part of the rhodopsin molecule is not involved in the maintenance of the characteristic spectrum. It is possible, however, that the part removed by papain is necessary for the regeneration of the visual pigment, following its bleaching by light.

The very rapid removal of a distinct fragment of rhodopsin from ROS by papain shows that in the disk membranes a particular region of the visual pigment is readily accessible to the enzyme. This adds further evidence to the idea that a part of rhodopsin is exposed to the aqueous phase on the surface of the membranes [7–12].

It is of interest that the spectra of the ROS after incubation with papain (obtained after removal of the material released from the membranes by the enzyme) showed the same absorption at 278 nm as the control (fig. 2). In the case of incubation for 60 min, about 36% of the polypeptide chain was removed by the enzyme from 85% of rhodopsin molecules (fig. 3). Since practically all of the absorption of the ROS at 278 nm is due to protein, the data indicate that very little, or no, tryptophan and tyrosine are present in the rhodopsin fragment removed by papain. As these two amino acids are hydrophobic, this points to the probable rather hydrophilic nature of the region of the visual pigment exposed on the membrane surface.

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