PROTEINS OF BOVINE RETINAL OUTER SEGMENTS: ELECTROPHORESIS ON POLYACRYLAMIDE GELS IN THE PRESENCE OF SODIUM DODECYL SULFATE

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1. Introduction

Rhodopsin, the pigment for night vision, is present in substantial amounts in bovine retinal outer segments. It is a conjugated protein consisting of the poly-unsaturated aldehyde retinal and the glycoprotein opsin. Due to the work of Wald et al. [1] the nature and the role of the prosthetic group of the visual pigment are well understood. The apoprotein moiety (opsin) is known to be highly insoluble in aqueous solutions. Only the use of detergents permits the solubilization of this protein. Among these have to be mentioned digitonin, alkoxypoly(ethyleneoxy)ethanol (Emulphogene), and cetyltrimethylammonium bromide (CTAB). Bowness [2] used digitonin for extraction and calcium phosphate column for purification. Heller [3] extracted with CTAB and purified by gel filtration chromatography. Shichi et al. [4] applied Emulphogene for solubilization, followed by purification on calcium phosphate. Recently, other proteins have been isolated from bovine retinal rods [5]. These are low molecular weight components.

In this paper we want to describe briefly the extraction of total protein from bovine retinal outer segments (ROS) with sodium dodecyl sulfate (SDS) (and with Triton X-100) and the subsequent electrophoretical analysis of this extract on polyacrylamide gels. This method of detergent extraction and electrophoresis, first developed by Grossfeld and Shooter [6] for the proteins of the aqueous-insoluble portion of total mouse brain, has subsequently been used for the electrophoretical analysis of total protein extracted with SDS from rat brain myelin [7]. We find upon electrophoresis of SDS extracts of ROS on polyacrylamide gels 2 major proteins, one of which is opsin (according to its molecular weight); the other protein, however, is higher in molecular weight than opsin and, to our knowledge, has not been thoroughly characterized.

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2. Materials and methods

Unless stated otherwise all operations were done in the cold and in dim red light. Eyes were kept in the cold and in the dark for up to 4 hr after slaughtering.

Preparation of bovine retinal outer segments (ROS) followed basically procedures described by Shichi [4]. In a typical experiment 35 bovine retinas were ground in a mortar with 35 ml 0.066 M phosphate buffer, pH 7.0, made up to 40% with sucrose, followed by homogenization in a Potter homogenizer (20 up-and-down strokes). The suspension was centrifuged at 2,000 *g* for 15 min, the resulting reddish supernatant was collected and diluted with the same amount of phosphate buffer, and then centrifuged at 25,000 *g* for 20 min. The pellet was homogenized in 7.5 ml 0.066 M phosphate buffer (40% in sucrose) and carefully layered under 7.5 ml of phosphate buffer (containing no sucrose). Centrifugation was done in a swing-out rotor at 45,000 g for 20 min. This centrifugation produced a pellet which is black at the bottom (black layer = BL, cf. fig. 2) and whitish at the top (white layer = WL, cf. fig. 2) and also a red band at the interphase 0.066 M phosphate–40% sucrose/0.066 M phosphate (crude ROS). The red material at the interphase was removed, diluted with phosphate buffer, spun down to a pellet and redistributed in

buffered 40% sucrose as described above. This procedure was repeated 2 or 3 times until no pellet was formed. The purified ROS were washed in phosphate buffer and finally spun down to a pellet at 25,000 *g* for 20 min. All materials were then kept frozen until used. Extraction of proteins from BL, WL, and ROS was done by homogenization in 0.1% SDS–0.01 M tris (pH 7.0); separation of insoluble material by centrifugation at 100,000 *g* for 120 min. Stepwise extraction of ROS was performed by homogenization in 0.04% SDS–0.01 M tris (pH 7.0), followed by centrifugation at 100,000 *g* for 120 min. After careful removal of the supernatant (giving the 0.04% extract) the pellet was rehomogenized in 0.06% SDS–0.01 M tris (pH 7.0) and the suspension centrifuged at the same speed (giving the 0.06% extract). This process was repeated with 0.08%, then with 0.10%, and finally with 0.15% SDS–0.01 M tris (pH 7.0).

Polyacrylamide gel electrophoresis of SDS extracts was performed in daylight and at room temperature as described in a previous short communication [7].

In addition to SDS extractions ROS were solubilized with 1% Triton X-100–0.01 M tris (pH 7.0) and separated from very small traces of whitish material by centrifugation at 100,000 *g* for 120 min. The supernatant was dialyzed against 0.01 M tris (pH 7.0) for 3 days. Thereafter, material was electrophoresed on standard polyacrylamide gels (tris–glycinate system, pH 8.9, 7.5% acrylamide, 0.2% BIS). The preceding operations were done in the dark and in the cold. A sharp orange band which migrates approximately 4–5 mm into the gels was cut out and extracted with 0.1% SDS–0.01 M tris (pH 7.0). This extract was then re-electrophoresed on SDS gels as described before [7].

Preparation of Rhodopsin was carried out according to Shichi [4] by extraction of ROS with the non-ionic detergent Emulphogene (BC-840) and purification on calcium phosphate column. However, the purification on ECTEOLA was omitted.

3. Results and discussion

The purity of the ROS preparation was examined by electron microscopy (fig. 1). The preparation is devoid of mitochondria. The structure of the discs is reasonably well preserved. In contrast, electron microscopy of the black layer (BL) and of the white layer (WL) shows only ill-defined debris (not shown here). Examination of SDS extracts of these three fractions underlines the results of electron microscopy (fig. 2). There are numerous bands in the case of BL and WL but only 2 major bands with ROS. These bands are absent in the gels of BL and WL. It is known that some defined membranes contain only a limited number of proteins. Therefore, we regard this ROS preparation as sufficiently pure for further investigation.

The method of stepwise SDS extraction of proteins from membranes, as studied first with the protein components of rat and guinea pig myelin [8], was applied to the ROS preparation (fig. 3). With 0.04% SDS a large number of different proteins is extracted, giving a pattern which is not representative of the extraction pattern obtained when 0.10% SDS is directly used (cf. fig. 2). Extraction with 0.06% and 0.08% SDS yields very little protein. However, we can see the slow appearance of the major bands 1 and 2 with 0.08% SDS. This trend is very pronounced with the 0.10% SDS extraction. There seems to be a slight preponderance of band 1 over band 2. This is reversed with 0.15% SDS, where band 2 is much stronger than band 1. Further extraction with 0.30% SDS yields only negligible traces of protein bands 1 and 2. Different ratios of bands 1 and 2 in the 0.10% and 0.15% SDS extracts exclude the possibility of band 1 being a dimer of band 2. Furthermore, SDS is known to dissociate aggregated proteins to monomers.

SDS dissociates retinene from opsin: the yellow-orange color migrates with the front of the SDS gels. No color is liberated with SDS concentrations of 0.06% or less, traces are seen with 0.08% SDS, the majority of color is found with the 0,10% and 0.15% SDS extracts.

Bands 1 and 2 (fig. 3) give strong positive PAS (periodic acid Schiff) reaction, as does material migrating with the front.

Approximate molecular weights of proteins can be determined using SDS gels [9]. We compared the migration of bands 1 and 2 with those of bovine serum albumin (MW 69,000), ovalbumin (MW 43,000) and lysozyme (MW 14,400) (fig. 3). Band 1 is material of molecular weight (>70,000) higher than that of serum albumin and ovalbumin, whereas band 2 is of lower molecular weight. On this basis it is assumed that band 2 is SDS-complexed opsin. Heller [3] found during rhodopsin purification by chromatography on

Fig. 1. Electron microscopic examination of retinal outer segment (ROS) preparation. 21,000X.

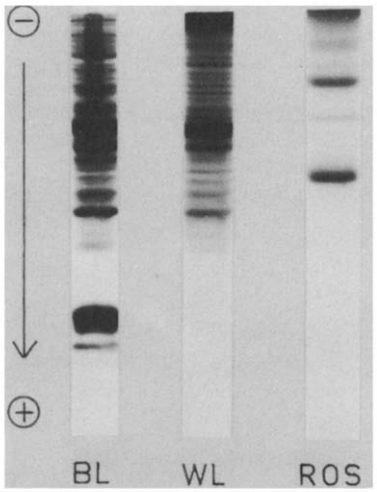

Fig. 2. Polyacrylamide gel electrophoresis of 0.1% SDS extracts of black layer (BL), white layer (WL), and retinal outer segments (ROS). 12% polyacrylamide, tris–glycinate system, pH 8.9, 0.1% SDS in samples, gels and buffers.

Sephadex G-200 a "colorless high molecular weight protein contaminant". It is not unlikely that our band 1 is identical with this protein.

In order to further test the purity of the ROS preparation we also used the non-ionic detergent Triton X-100 for solubilization. Upon electrophoresis in the dark of the dialyzed Triton extract a single sharp band is observed in the gels. The content of this band is extracted with SDS and examined on SDS gels. We find bands 1 and 2. The same result is obtained with rhodopsin material prepared with Emulphogene for extraction and calcium phosphate for purification [4].

In summary: Treatment of purified bovine retinal outer segments with low quantities of SDS (0.04%), thus removing uncharacterized protein material, makes the extraction of rather pure protein components 1 (of high molecular weight) and 2 (opsin) possible. This, in conjunction with the technique of preparative SDS electrophoresis as applied to myelin

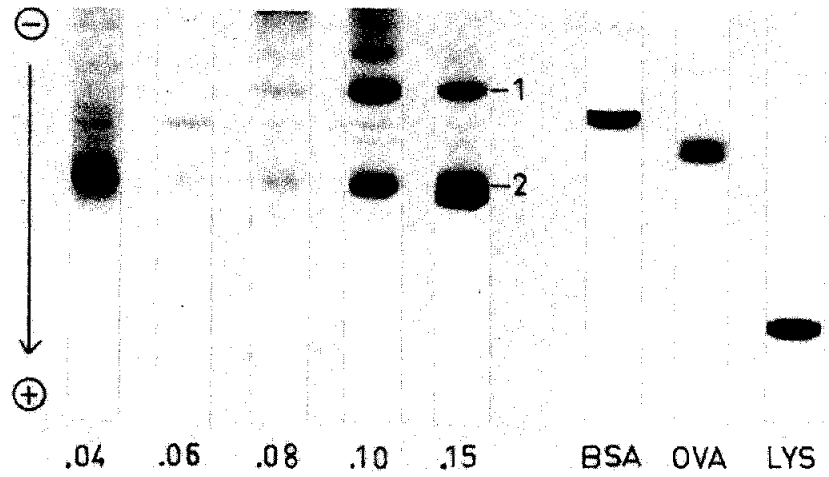

Fig. 3. Polyacrylamide gel electrophoresis of stepwise extraction of retinal outer segments (ROS) with increasing concentrations of SDS (0.04%, 0.06%, 0.08%, 0.10%, 0.15% SDS). 12% polyacrylamide, tris–glycinate system, pH 8.9, 0.1% SDS in gels and buffers. BSA = bovine serum albumin, OVA = ovalbumin, LYS = lysozyme.

[10], permits the isolation of pure opsin and a hitherto not sufficiently characterized high molecular weight protein component. The relatively high amount of the latter protein is indicative of its involvement as a structural component of the ROS discs beside the "miniproteins" recently described [5].

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