PROTEINS OF BOVINE ROD OUTER SEGMENTS CHARACTERIZED BY SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

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Summary: Proteins of bovine rod outer segments (ROS) were subjected to SDS-polyacrylamide gel electrophoresis after repeated washing. Three major proteins are found in the molecular weight region of 30,000 to 42,000. The rhodopsin content of ROS disc membranes is estimated to be only 50 % of total protein.

Introduction: Rhodopsin is considered to constitute 80% to 90 % of the membrane bound protein of rod outer segment (ROS) disc membranes(1,2,3,4). Measuring the total protein and rhodopsin content, our disc preparations always show a much lower rhodopsin concentration. This effect cannot be explained by contaminated preparations or partially bleached rhodopsin, since the absorbance ratio 280 nm/498 nm found, from 2.3 to 2.4, is supposed to represent a pure disc preparation. Another consideration also indicates that the rhodopsin content cannot be so high: The absorbance ratio for pure rhodopsin is 1.6 (4), thus, assuming that the 280 nm absorbance is proportional to total protein, the rhodopsin content is only 70 %. Only 65 % of the 280 nm absorbance of rhodopsin, however, is due to aromatic amino acids, the remainder being derived from hyperchromism and/or the chromophore (5). These considerations lead to a rhodopsin content of 55 % or almost half the accepted value.

To clarify this point and to identify any additional protein components in the disc membrane, we tested our preparations by high resolution SDS-polyacrylamide gel electrophoresis.

Materials and Methods: The retinas of about 60 bovine eyes were excised, gently rubbed in a mortar with isotonic Ringer solution and the suspension filtered through a nylon net. Until the protein extraction all manipulations were carried out under dim red light. The crude ROS-preparation was obtain-

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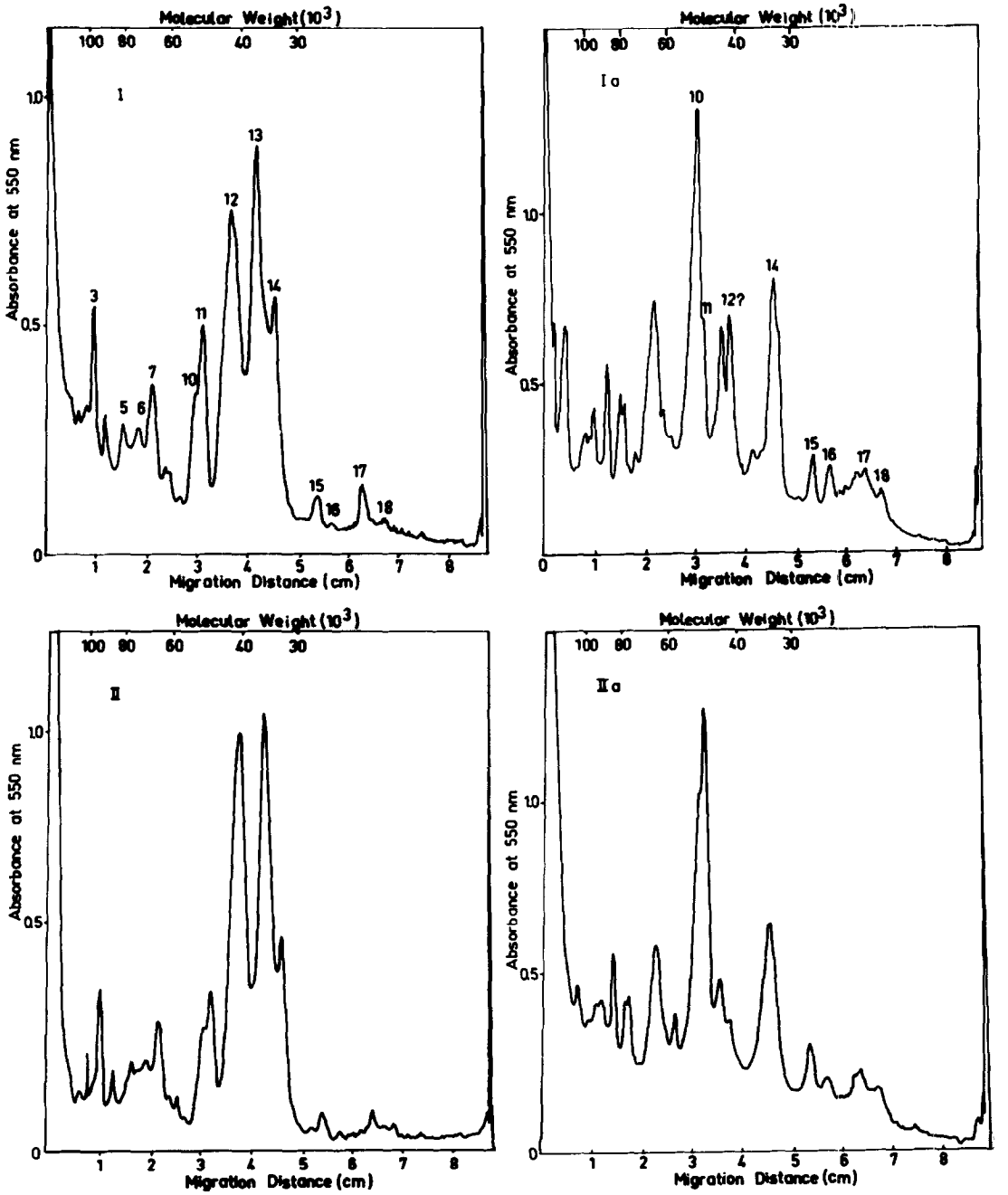

Fig. 1 Scans of SDS-Gels from the Various ROS Preparations. I = sediment of the first wash, Ia = supernatant of the first wash, II = sediment of the second wash, IIa = supernatant of the second wash. Electrophoresis was performed as described in materials and methods. Gel length 9 cm, gel diameter 0.5 cm, 8 mA per gel. All gels were stained with Coomassie blue, 50 µg protein.

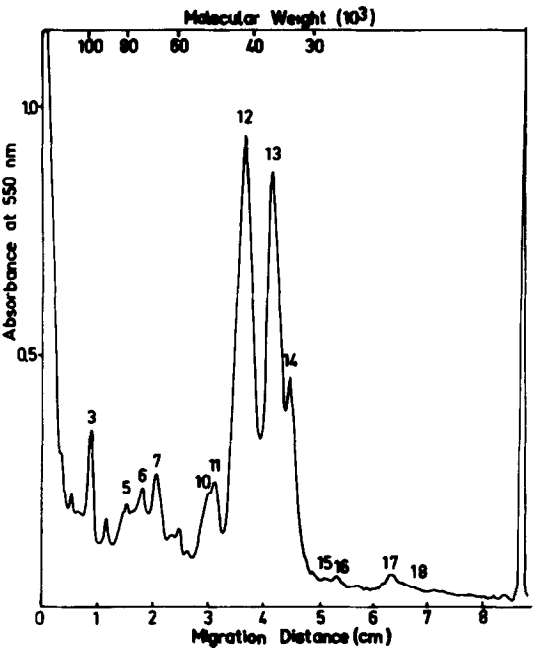

Fig. 2 Scan of an SDS-Gel from a Disc Preparation. Discs were prepared from the sediment II in Fig. 1. Electrophoresis was as in Fig. 1.

ed by a sucrose flotation. It was further purified by two washings. Discs were prepared from the washed ROS-pellet by osmotic shock with destilled water and the subsequent sedimentation of the membraneous material. Protein was determined by the method of Lowry (6). Appr. 30 mg ROS-protein were obtained.

Rhodopsin was determined spectrophotometrically after the membranes were dissolved with sodiumdesoxycholate. The purity of the various preparations was estimated from the absorbance ratio 280 nm/498 nm. Succinate-dependent, NADH and NADPH cytochrome-c reductases served as a test for mitochondrial or micorsomal contamination (7). The crude ROS-suspension and the wash supernatants showed a high NADH-dependent reducatase activity, but the twice washed ROS-preparation did not exhibit any such activity. No succinate or NADPH-dependent activity was observed.

Protein extraction was by the method of Holtzman (8). The organic phase was collected for measuring the contribution of the lipids to the absorption spectrum of dissolved disc membranes. A seasonable dependence of the 280 nm absorbance by lipids was observed. It reached a maximum during summer and increased the absorbance ratio up to 2.8. This high value could be attributed, at least in part, to an increase in lipid peroxidase activity (9). After extraction, the proteins were dissolved and subjected to SDS-polyacrylamide gel electrophoresis according to the method of Weber (10). Molecular weight calibration was made using known liver microsomal proteins (11). After staining with Coomassie

blue and destaining by the method of Fairbanks (12), the optical density of the bands was measured with a Gilford scanning photometer at 550 nm. To identify glycoproteins on the gel, the gels were first stained with periodic acid Schiff-reagent stain (PAS) (12) and, after being scanned at 560 nm, superstained with Coomassie blue and rescanned as above.

Results and Discussion: Fig. 1 and Fig. 2 show scans of gels from different purification steps of a ROS-preparation and from the wash supernatants. The ratio of the absorbance at 280 nm and 498 nm was 3.0 for sample I (after the first wash), 2.5 for sample II (after the second wash) and 2.3 for the disc preparation. No rhodopsin could be spectrophotometrically detected in the supernatant. The bands of the different ROS-peptides were numbered in the order of their decreasing molecular weight, i.e. increasing migration distance.

In the following presentation only the major bands will be discussed. The protein pattern of the flotation supernatant was almost identical to that of the wash supernatant. Bands 10, 11, 14, 15, 16, 17, 18 seem to be only loosely associated, if at all, with the membrane, because their intensity decreases with each wash and they appear in comparable quantities in the wash supernatant. Band 12, although it seems to be present in the wash supernatant, does not decrease after washing; therefore, we believe that the corresponding protein in the sediment is different from that in the supernatant. Bands 12, 13 and 14 are the major membrane proteins and constitute more than 80 % of the total protein. They are situated in the molecular weight region of 30,000 to 42,000 and one of these proteins must be opsin.

In an attempt to identify the opsin, we stained some gels for glycoproteins with PAS-reagent stain, as it has been reported that rhodopsin is a glycoprotein (13, 14, 15), although the exact carbohydrate composition is still not known (14, 15). Fig. 3 shows scans of such a gel: (A) stained for glycoprotein, (B) the same gel superstained with Coomassie blue. In the superstained gel the three large bands appear as one single band at 6.3 cm. This scan resembles the poorer resolution seen in other published gels (1,4). We believe that the poor resolution of bands is due to the high protein concentration for this gel, the high concentration being necessary because of the weak PAS-stain. It always

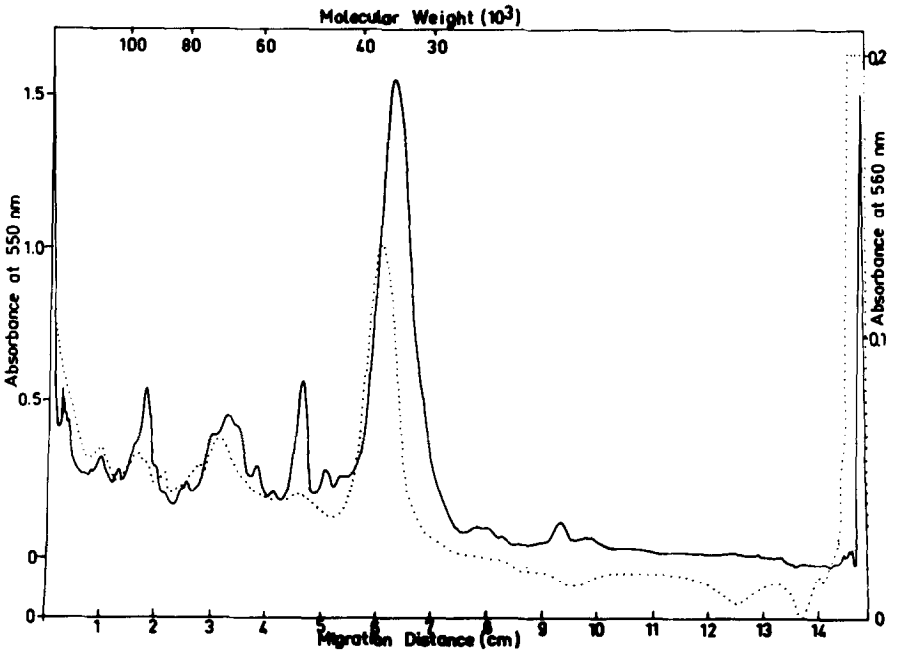

Fig. 3 Scans of an SDS-Gel from the Disc Preparation of Fig. 2.
(.....): Stained for glycoproteins, (———): Superstained with Coomassie blue. Electrophoresis was performed as described in materials and methods. Gel length 14 cm, gel diameter 0.5 cm, 10 mA per gel, 100 µg protein.

appears, however, that the main peak in the superstained gel is shifted to lower molecular weights as compared to the PAS-reagent maximum (peak at 6.05 cm). Using mixed gels of supernatant and disc preparations as well as $(NH_4)_2SO_4$ fractionation of these components, we have an indication, that band 12 is the major glycoprotein in discs, while covalent binding of retinal to opsin by the method of Bownds (16) indicates that band 13 is the opsin. Aggregation of proteins and the resultant poorer resolution in gels for these studies, however, do not allow an unequivocal interpretation of these results as of yet.

From the molecular weight calibration and the fact that in the region of bands 5, 6, and 7 in Fig. 2 a PAS-reagent positive stain has been obtained (Fig.3), we believe that bands 5, 6 and 7 constitute dimers of bands 12, 13 and 14. This is supprted by the fact that the intensity of bands 5, 6 and 7 varies appreciably from gel to gel at the expense of bands 12, 13 and 14.

In all preparations - a total of seven - we observed the three bands 12, 13 and 14. Under unfavourable conditions, especially if too much protein was applied to the gel (Fig. 3), the three bands appeared as one single band. Band 12 varies strongly from preparation to preparation (and from gel to gel), the ratio of the intensity of bands 12 and 13 ranging from 0.3 to 1.0. This may partially be due to differences in aggregation and partially to different protein ratios in ROS. The amount of band 14 is reproducible under identical preparative conditions. This protein, however, can be almost completely washed out with water of low ionic strength and with 10^{-3} M EDTA.

In previous reports by others (1,17), where by manipulations the protein concentration in the region of the opsin was low, two bands can be discerned. A ROS-peptide with a molecular weight slightly higher than opsin has also been reported by Zorn (18).

On the basis of

1. the calculation presented in the introduction,
2. gels published by other groups, where under special conditions - sometimes thought to be an artefact - two bands can be discerned,
3. a report of an additional protein with a molecular weight slightly higher than rhodopsin,
4. our own results of high resolution SDS-polyacrylamide gel electrophoresis,

we conclude that in the molecular weight region of 30,000 to 42,000 daltons three major proteins are present in the disc membrane, none of these representing more than 50 % of total protein. At the moment, the assignment of one band to the glycoprotein and/or to rhodopsin cannot be made with certainty. Preliminary experiments with $(NH_4)_2SO_4$-fractionation of the disc proteins tend to support the view presented above.

The existence of two additional major components of similar molecular weight raise the possibility that the signal transduction in disc membranesis dependent on the coupling of rhodopsin to other proteins.

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