A light-induced modification of a 165 kDa polypeptide in crayfish photoreceptors shown by monoclonal antibodies

Kristin M. Baer and Hennig Stieve

Institut für Neurobiologie der Kernforschungsanlage Jülich GmbH, Postfach 1913, 5170 Jülich, FRG

Received 23 May 1985; revised version received 15 July 1985

In the compound eye of invertebrate animals phototransduction takes place in a specialized structure built by the visual cells – the photoreceptor. We describe monoclonal antibody binding to a photoreceptor polypeptide of about 165 kDa. As can be concluded from its solubilization properties this polypeptide might be a component of the cytoskeleton of the photoreceptor. Two of the monoclonal antibodies were shown to bind differently to illuminated or non-illuminated photoreceptor preparations. As a result of the differential binding of these two antibodies, we conclude that the 165 kDa polypeptide is modified by illumination. One of the monoclonal antibodies cross-reacts with an unidentified antigen in preparations of bovine rod outer segments.

Invertebrate photoreceptor *Cytoskeleton* *Monoclonal antibody* *Light-induced protein modification*

1. INTRODUCTION

Visual cells of invertebrate animals contain an area which is specialized for light absorption, the photoreceptor. In the crayfish *Astacus*, the highly ordered photoreceptor is built by infoldings of the plasma membrane, the so-called microvilli, of 7 visual cells [1]. The major integral membrane protein of the *Astacus* photoreceptor is the visual pigment rhodopsin [2]. Light absorption by rhodopsin triggers the generation of the electrical photoresponse, a depolarization of the plasma membrane. Moreover, several further physiological processes in the visual cells of invertebrate animals are controlled by light: the migration of screening pigment granules [3], the metabolic regeneration of visual pigment [4,5] and photosensory membrane turnover (review [6]).

To investigate the molecular mechanisms underlying these light-induced processes, we began to generate antibodies against the proteins of isolated and purified *Astacus* photoreceptors.

2. MATERIALS AND METHODS

Crayfish (*Astacus leptodactylus*) photosensory membrane was prepared essentially as in [2], but using 100 mM Tris-HCl, pH 7, with 10 mM EGTA as preparation buffer in most experiments. To obtain monoclonal antibodies, mice (female BALB/c) were injected several times with 50–100 μg of a photoreceptor preparation in Freund's adjuvant. 3 days after the last immunization, spleen cells of an immunized mouse and cells of the myeloma line X63Ag8.653 were fused using a standard protocol [7]. Hybridomas were tested for production of specific antibody in radioimmunoassay (RIA) and positive hybridomas were cloned. For RIA, microtiter plates (PVC, dynatech) were coated with sonicated preparations of photoreceptors (protein concentration 10 μg/ml). The plates were then successively incubated with 0.1% bovine serum albumin (BSA) to saturate free binding sites of the PVC plates, cell culture supernatant of hybridoma clones, and ^{125}I-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

labelled second antibody(sheep or rabbit anti-mouse Ig). Radioactivity of individual wells was counted in a γ-counter.

Immunoblotting was performed essentially as in [8]. For detergent extraction, photoreceptors were suspended in 1.5% detergent (Triton X-100 or digitonin, in 100 mM Tris-HCl, 10 mM EGTA, pH 7). After incubation on ice (30 min–2 h) the solution was centrifuged for 20 min at approx. 100000 × *g* (Beckman airfuge).

A competition assay was used to examine the binding of a monoclonal antibody to its antigen in illuminated and non-illuminated photoreceptor preparations (for experimental procedure see fig.3 legend). For details of most methods see [9].

3. RESULTS

3.1. *Antigen specificity of monoclonal antibodies*

Thus far we have characterized 5 monoclonal antibodies, all of which bind to the same polypeptide (fig.1). This polypeptide has an apparent molecular mass of 160–165 kDa on SDS-polyacrylamide gels (fig.1, lane 1). It amounts to about 10% of the total photoreceptor protein under our standard preparation conditions and is present in large amounts only in preparations of crayfish photoreceptor in contrast to other crayfish tissues (nerve, muscle, tissue of the eye).

Although we only found monoclonal antibodies against the 165 kDa polypeptide, it is not very immunogenic: in immunoblots with antiserum from an immunized mouse it is only weakly labelled whereas most serum antibodies bind to rhodopsin (fig.1, lane 2). The unexpected binding of all the monoclonal antibodies to only the 165 kDa polypeptide might be caused by selective binding of the photoreceptor polypeptides to the plates

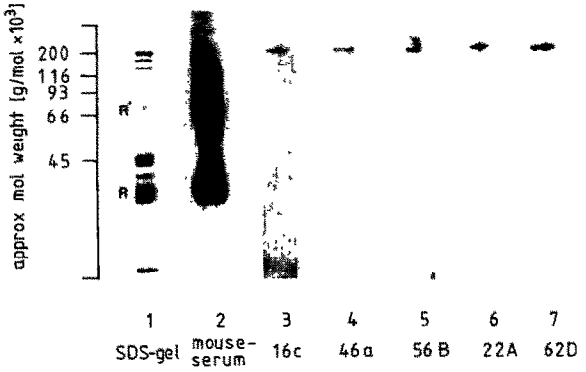

Fig.1. Identification of antigen reactive with different monoclonal antibodies. An immunoblotting method was used according to [8]. Lane 1: 10% polyacrylamide SDS gel with separated *Astacus* photoreceptor proteins, stained with Coomassie brilliant blue. The *Astacus* visual pigment and its dimer are marked by R and R'. Lane 2: immunoblot with the serum of a BALB/c mouse which had been immunized with *Astacus* photoreceptor preparation. The antiserum contains antibodies against almost all photoreceptor proteins, in particular to the visual pigment, rhodopsin. Lanes 3–7: immunoblots with different monoclonal antibodies as indicated. Within the precision of the method used, all monoclonal antibodies bind to a polypeptide with an apparent molecular mass of approx. 165 kDa.

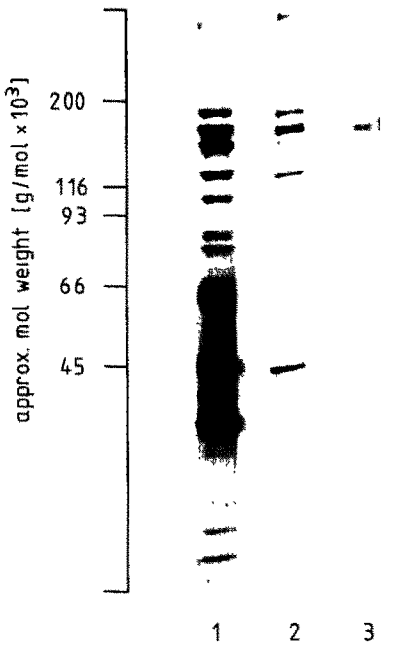

Fig.2. Protein composition of *Astacus* photoreceptors. SDS-polyacrylamide gel electrophoresis according to [11] on a 5–15% polyacrylamide gradient gel. Lane 1: *Astacus* photoreceptors purified by sucrose density centrifugation. Lane 2: photoreceptor proteins insoluble in 1.5% detergent (see section 2). The polypeptide with an apparent molecular mass of about 42 kDa co-migrates with rabbit skeletal muscle actin. Lane 3: immunoblot with detergent-insoluble fraction of photoreceptor proteins. The 165 kDa polypeptide is marked by one of the monoclonal antibodies (16c).

used in the RIA screening procedure. The 165 kDa polypeptide is highly insoluble in buffer solutions. When the photoreceptor preparation is treated with detergent to solubilize the membranes, the 165 kDa polypeptide is one of the major components of the detergent-insoluble fraction (fig.2, lane 2 and immunoblot lane 3).

There is a strong cross-reactivity of one monoclonal antibody (22A) with preparations of bovine rod outer segments [10] when tested in the RIA. We were unable to identify the cross-reacting antigen in the bovine photoreceptor system. In addition, antibody 22A cross-reacts in RIA with preparations of *Astacus* nerve tissue.

3.2. *Effects of illumination on antibody binding*

To determine whether the monoclonal antibodies were able to recognize a light-induced modification of their antigen, we used a competition assay to study the binding of antibodies to illuminated and non-illuminated photoreceptor preparations (fig.3).

Antibody 22A binds about 100-times better to its antigen from illuminated than from non-illuminated crayfish photoreceptor preparations. In the experiments shown in fig.3 photoreceptors were illuminated for 15 min with orange light. Weak pre-illumination (for 1 s, of the order of 1 photon per rhodopsin), however, was already suf-

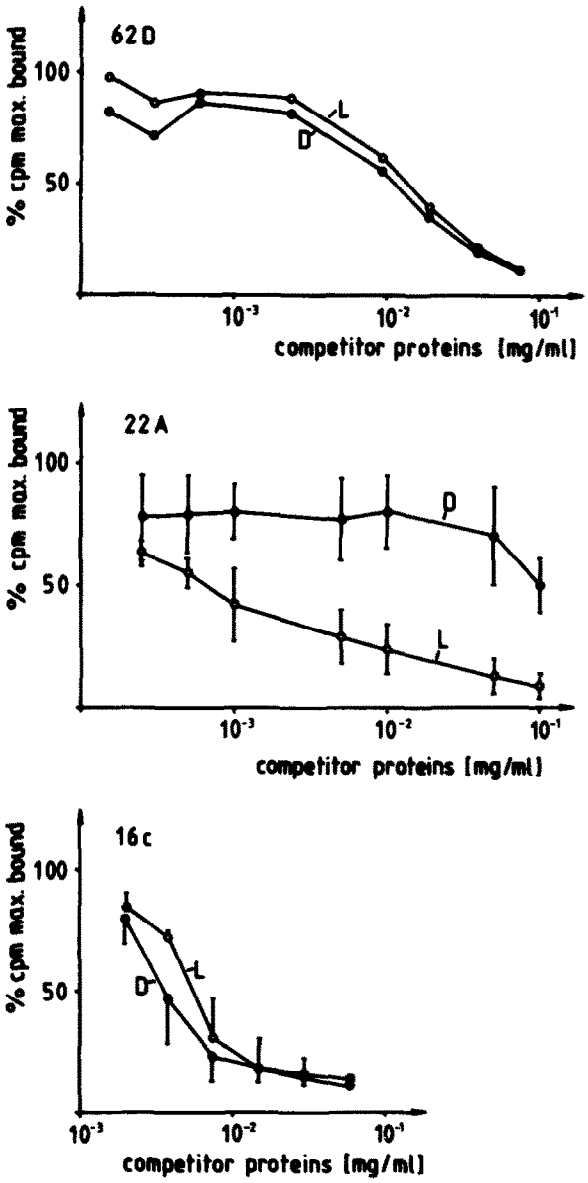

Fig.3. Binding of monoclonal antibodies to illuminated and non-illuminated photoreceptor preparations as measured in a competition assay. Crayfish photoreceptors were prepared and sonicated in dim red light ($\lambda > 690$ nm). The preparation was divided and one half was kept in the dark (D), while the other half was illuminated for 15 min with orange light ($\lambda > 540$ nm). Both samples (D, L) were serially diluted and incubated with hybridoma culture supernatant containing a limiting amount of specific antibody. The concentration of photoreceptor protein in these samples is plotted on the abscissa (competitor proteins). After 2 h incubation in the dark (D samples) and in room light (L samples), aliquots of each sample were tested for free antibody in the RIA. The maximum binding capacity of free antibody was detected in a control sample, where buffer was added instead of photoreceptor preparation. This value was called 100%. Binding in other samples, where antibody had been preadsorbed with increasing amounts of photoreceptor protein, was expressed as a percentage of the maximum binding and plotted on the ordinate. As a measure for the different antibody binding of the light and dark samples the concentrations of competitor proteins which resulted in 50% max. binding, were compared. Results: antibody 62D binds equally well to illuminated and non-illuminated photoreceptor preparations. The data shown are from a single experiment. Antibody 22A shows an enhanced binding (100-times) to illuminated photoreceptor preparation ($n = 4$), antibody 16c to non-illuminated preparation ($n = 3$).

ficient to cause a distinct increase in the binding of antibody 22A (not shown). Two further monoclonal antibodies were tested for a light-dark difference in binding. One of them (62D) binds equally well to illuminated or non-illuminated photoreceptors, while the other (16c) shows a slightly (about 1.5-times) better binding to non-illuminated photoreceptor preparations (4 different experiments).

4. DISCUSSION

When a typical photoreceptor preparation was analyzed by light and electron microscopy, the only contamination found was screening pigment granules which were attached to the photoreceptor surface. When we injected this preparation into mice and following fusion with X63Ag8.653 myeloma cells we obtained monoclonal antibodies which bind to a polypeptide with an apparent molecular mass of 165 kDa. This 165 kDa polypeptide is present in large amounts (10% of total photoreceptor protein content) and is, therefore, likely to originate from the photoreceptor. It is different from rhodopsin in its molecular mass as well as in its solubilization properties [2]. The polypeptide is insoluble in non-ionic detergents, which indicates that it might be a component of the cytoskeleton of the microvilli. A cytoskeleton inside the microvilli, consisting of an axial filament in the microvillus lumen which is connected by side arms to the microvillus membrane, has been shown to be present in the photoreceptor of squid [12] and several arthropods [13–15]. In photoreceptor preparations of the squid [12] and the crayfish *Cherax* [15], several proteins have been described which are not solubilized by detergents and which have been assigned to the cytoskeleton. The polypeptide pattern on SDS gels of the putative cytoskeletal fraction from the crayfish *Cherax* is similar to the pattern shown here for *Astacus* (see fig.2).

The cytoskeleton is likely to maintain the highly ordered structure of the photoreceptor even under osmotic stress [16], and might be the reason that diffusion of rhodopsin molecules within the microvillus membrane is largely constrained [17]. The cytoskeleton has been implicated in the breakdown and reassembly of the photosensory membrane [12,18]. Whether it also has a significant role in the metabolic regeneration of the photopigment or in the transduction process has yet to be investigated.

For this purpose antibodies might be very useful. Two of our monoclonal antibodies show a differential binding to illuminated and non-illuminated photoreceptor preparations. This effect already occurs with brief illumination (1 s–1 min) with moderate light intensity (30 mW/cm^2). The differential binding of the antibodies could reflect a covalent modification or a change in structure or accessibility of their antigen, a putative component of the cytoskeleton.

One of these antibodies shows strong cross-reactivity with preparations of bovine rod outer segments. It is known that there is also a cytoskeleton in rod outer segments [19]. However, cross-reactivity of a monoclonal antibody does not necessarily mean that the 2 proteins have a related function: it is known that an antibody recognizes only a few amino acids of its antigen [20]. Microinjection of monoclonal antibody (16c) into *Limulus* photoreceptor cells caused no significant effect on the electrical light response in contrast to the injection of polyclonal antisera against the *Astacus* photoreceptor preparation [21].

Further experiments are necessary to elucidate the function of the cytoskeleton in the visual cell.

ACKNOWLEDGEMENTS

We wish to thank S. Hall for critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft, SFB 160.

REFERENCES

[1] Krebs, W. (1972) Z. Zellforsch. 133, 399–414.
[2] Hamacher, K. and Stieve, H. (1984) Photochem. Photobiol. 39, 379–390.
[3] Frixione, E. and Arechiga, H. (1979) J. Neurobiol. 10, 573–579.
[4] Cronin, T.W. and Goldsmith, T.H. (1984) J. Gen. Physiol. 84, 63–81.
[5] Schwemer, J. (1984) J. Comp. Physiol. 154, 535–547.
[6] Blest, A.D., Stowe, S. and DeCouet, H.G. (1984) Sci. Prog. Oxf. 69, 83–100.
[7] Lemke, H., Hämmerling, G.J., Höhmann, C. and Rajewsky, K. (1978) Nature 271, 249–252.

[8] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[9] Baer, K. (1984) Berichte der Kernforschungsanlage Jülich, Jül-1943.
[10] McDowell, H. and Kühn, H. (1977) Biochemistry 16, 4054–4060.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Saibil, H. (1982) J. Mol. Biol. 158, 435–456.
[13] Blest, A.D., Stowe, S., Eddey, W. and Williams, D.S. (1982) Proc. R. Soc. Lond. B 215, 469–479.
[14] Blest, A.D., Stowe, S. and Eddey, W. (1982) Cell Tissue Res. 223, 553–573.
[15] DeCouet, H.G., Stowe, S. and Blest, A.D. (1984) J. Cell Biol. 98, 834–845.
[16] Winterhager, E. and Stieve, H. (1982) Cell Tissue Res. 223, 267–280.
[17] Goldsmith, T.H. and Wehner, R. (1977) J. Gen. Physiol. 70, 453–490.
[18] Stowe, S. (1980) Cell Tissue Res. 211, 419–440.
[19] Roof, D.J. and Heuser, J.E. (1982) J. Cell Biol. 95, 487–500.
[20] Tzartos, S., Rand, D.E., Einarson, B.L. and Lindstrom, J.M. (1981) J. Biol. Chem. 256, 8635–8645.
[21] Baer, K., Stieve, H. and Bruns, M. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1270.