Biophys. Struct. Mechanism 3, 117–120 (1977)

Biophysics of Structure and Mechanism

Transient Light-Induced Conformational Changes in Rhodopsin*

F. J. M. Daemen and S. L. Bonting

Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

Abstract. Arguments are presented which support the possibility that the unfolding of the rhodopsin molecule during photolysis up to the stage of metarhodopsin II is followed by a spontaneous refolding of the protein, once the isomerized retinaldehyde has left its original binding site. Such a transient conformational change might imply a very similar conformation for rhodopsin and opsin, apart from the presence of the chromophore.

Key words: Rhodopsin – Metarhodopsin II – Opsin – Transient conformational changes.

The visual pigment rhodopsin consists of a single protein chain of about 37,000 Dalton and a chromophoric group, 11-cis retinaldehyde (Wald, 1968). A functionally intact rhodopsin molecule requires a specific three-dimensional arrangement (secondary and tertiary structure, conformation) of its protein chain and the chromophore. Illumination of rhodopsin is known to cause isomerization of 11-cis retinaldehyde to the all-trans form, accompanied and/or followed by a sequence of spontaneous (thermal) reactions, which are identified by absorbance spectroscopy (Wald, 1968). The cis-trans isomerization of the chromophore must involve molecular motion. The spectral changes during photolysis indicate, that this light-induced molecular motion of the chromophore results in molecular motion (conformational changes) in the protein part of rhodopsin as well. The spontaneity of the dark reactions, following the primary photon capture, has led to the idea that the photolysis of rhodopsin is accompanied by an unfolding of the protein.

This idea is supported by a number of observations, in which rhodopsin and the endproduct of its photolysis, opsin, are compared with respect to thermal stability, pH stability, stability towards detergents and accessibility/reactivity of functional groups. Recent evidence suggests that in the absence of detergent, however, the differences between rhodopsin and opsin are less pronounced than hitherto generally

* Presented at the EMBO-Workshop on Transduction Mechanism of Photoreceptors, Jülich, Germany, October 4–8, 1976

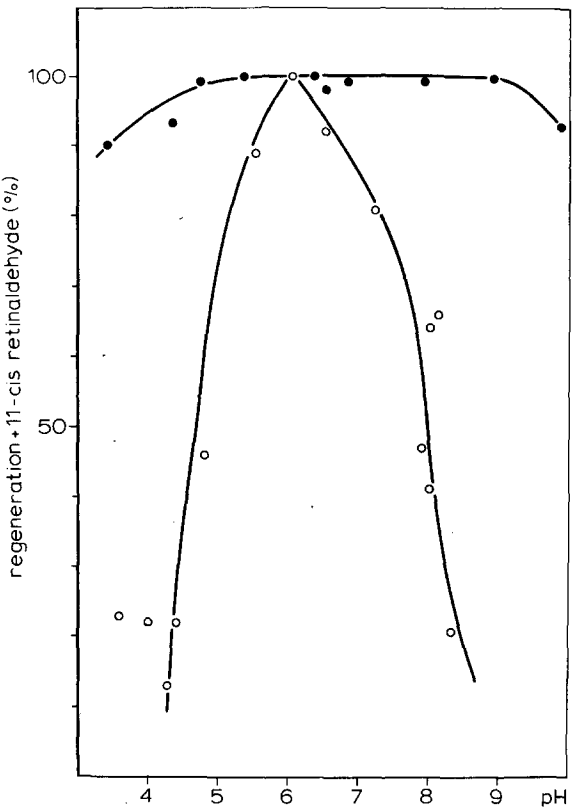

Fig. 1. Regeneration capacity of opsin at pH 6.5 following a 1 h incubation (25° C) at various pH values. Closed circles: membrane suspension; open circles: 2% digitonin solution. The latter data are recalculated from Radding and Wald, 1956

accepted. Thus, whereas in 2% digitonin rhodopsin (as measured by its 500 nm absorbance) is stable between pH 4 and 10, and opsin (as measured by its regeneration capacity) is only stable between pH 5.5 and 7 (Radding and Wald, 1956), in membrane suspension opsin can be incubated for 1 h at 25° C between pH 4 and 10 without appreciable damage to its regeneration capacity (Fig. 1). Similarly, the exposure of 2 additional sulfhydrylgroups in opsin as compared to rhodopsin in digitonin solution (Wald and Brown, 1952), is not observed in suspension (de Grip et al., 1973a).

Indications for light-induced protein conformational changes are particularly strong for the transition of metarhodopsin I (MR I, λ_{max} 480 nm) to metarhodopsin II (MR II, λ_{max} 380 nm). First, in addition to the large spectral shift, thermodynamic data suggest large positive entropy changes in this step (Matthews et al., 1963). Secondly, photoreversal studies (see Williams, 1975) show that the intermediates through MR I instantaneously revert to photopigment with the photo-reisomerized chromophore, whereas in MR II photoreversal is less efficient and is delayed. This suggests that in the latter case substantial protein rearrangement precedes rhodopsin regeneration. Thirdly, only at the MR II stage does the covalent Schiff base linkage between retinaldehyde and apoprotein become accessible to aqueous reagents like hydroxylamine and sodium borohydride. Finally, photolysis of rhodopsin

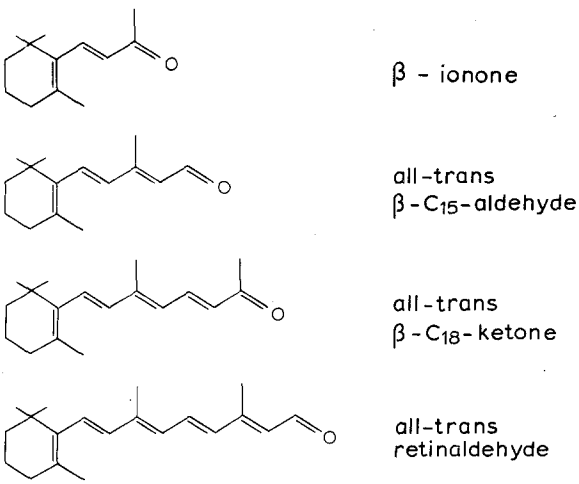

Fig. 2. Retinaldehyde analogues tested for their effect on the rate of rhodopsin regeneration from opsin and 11-cis retinaldehyde

at room temperature is easily blocked at the MR I to MR II transition by a variety of experimental conditions: in the absence of water (Wald et al., 1950), after removal of the membrane lipids, after glutaraldehyde fixation and after exhaustive sulfhydryl group modification (Daemen et al., 1976). We suggest that experimental conformational damage to rhodopsin preferentially blocks the MR I to MR II transition, since it is here that the most extensive (light-induced) conformational changes take place. Most of this evidence is consistent with unfolding of the apoprotein at this stage.

On the other hand, several features of the decay of MR II to opsin seem to suggest a refolding of the protein. The release of all-trans retinaldehyde from its original Schiff base binding site during the decay of MR II, gives rise to molecular species, which are able to react with 11-cis retinaldehyde under formation of photopigment (Rotmans et al., 1974). Protein aminogroups are less reactive in opsin as compared to MR II (de Grip et al., 1973b). Photophosphorylation of rhodopsin by ATP or GTP takes place during photolysis, but before opsin is formed (H. Kühn, personal communication). Photoreversal does occur rather easily from metarhodopsin III (Reuter, 1976).

Finally, the effects of retinaldehyde analogues (see Fig. 2) on the rate of the regeneration reaction between opsin and 11-cis retinaldehyde show the existence in opsin of a rather specific "chromophore binding space", in analogy to the specific "chromophoric space" in rhodopsin. Whereas β-ionone (Matsumoto and Yoshizawa, 1975) and 11-cis retinol, and most effectively all-trans β-C_{15}-aldehyde (and alcohol) are efficient inhibitors, all-trans β-C_{18}-ketone and all-trans retinaldehyde have no effect on the velocity of rhodopsin regeneration. Apparently, upon surpassing a critical length of the side chain, the "analogues" of 11-cis retinaldehyde no longer fit in the "chromophore binding space". This suggests a similar structure for rhodopsin and opsin, apart from the presence of the chromophore.

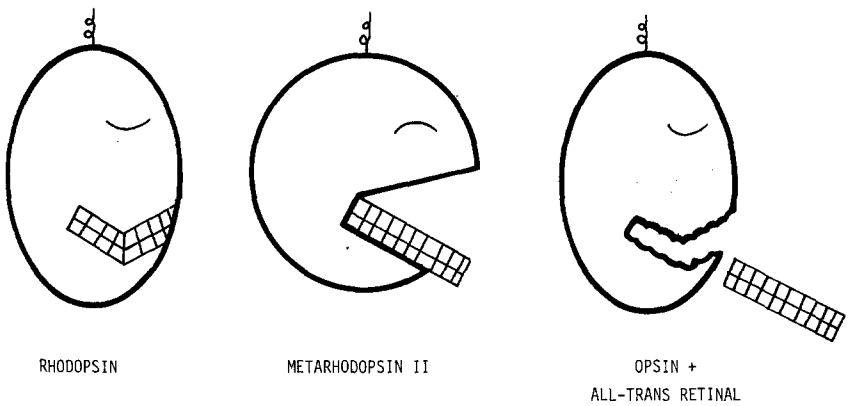

Fig. 3. Summary

Hence, we suggest that during photolysis of rhodopsin transient light-induced conformational changes occur in its protein part with maximal unfolding of the molecule at the metarhodopsin II stage and spontaneous refolding to a rhodopsin like conformation during transition to opsin (Fig. 3).

References

Daemen, F. J. M., van Breugel, P. J. G. M., Jansen, P. A. A., Bonting, S. L.: Relation between sulfhydryl groups and properties of rhodopsin studied by means of methylmercuric iodide. Biochim. biophys. Acta (Amst.) **453**, 374–382 (1976)

de Grip, W. J., van de Laar, G. L. M., Daemen, F. J. M., Bonting, S. L.: Sulfhydryl groups and rhodopsin photolysis. Biochim. biophys. Acta (Amst.) **325**, 315–322 (1973a)

de Grip, W. J., Daemen, F. J. M., Bonting, S. L.: Amino group modification in bovine rod photoreceptor membranes. Biochim. biophys. Acta (Amst.) **323**, 125–142 (1973b)

Matsumoto, H., Yoshizawa, T.: Existence of a β-ionone ring binding site in the rhodopsin molecule. Nature (Lond.) **258**, 523–526 (1975)

Matthews, R. G., Hubbard, R., Brown, P. K., Wald, G.: Tautomeric forms of metarhodopsin. J. gen. Physiol. **47**, 215–240 (1963)

Radding, C. M., Wald, G.: The stability of rhodopsin and opsin. Effects of pH and aging. J. gen. Physiol. **39**, 923–933 (1956)

Reuter, T.: Photoregeneration of rhodopsin and isorhodopsin from metarhodopsin III in the frog retina. Vision Res. **16**, 909–917 (1976)

Rotmans, J. P., Daemen, F. J. M., Bonting, S. L.: Binding site and migration of retinaldehyde during rhodopsin photolysis. Biochim. biophys. Acta (Amst.) **357**, 151–158 (1974)

Wald, G.: Molecular basis of visual excitation. Science **162**, 230–239 (1968)

Wald, G., Brown, P. K.: The role of sulfhydryl groups in the bleaching and synthesis of rhodopsin. J. gen. Physiol. **35**, 797–821 (1952)

Wald, G., Durell, J., St. George, R. C. C.: The light reaction in the bleaching of rhodopsin. Science **111**, 179–181 (1950)

Williams, T. P.: Dynamics of opsin, a visual protein. Acts Chem. Res. **8**, 107–112 (1975)

Received November 5, 1976