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Spectral Studies on the Conformation of Rhodopsin*

Charles N. Rafferty

Institut für Neurobiologie der Kernforschungsanlage Jülich GmbH,
P.O.B. 1913, D-5170 Jülich 1, Federal Republic of Germany

Abstract. A summary is given of visible and ultraviolet spectral studies which deal with the conformation of rhodopsin in situ and solubilized with detergent. Emphasis is placed on studies which give specific information about the macromolecular structure and which set quantitative limits on the magnitude of light-induced conformational changes.

Key words: Rhodopsin – Membranes – Conformation – Optical activity – Absorption.

In recent years, considerable information has been obtained from absorption, circular dichroism (CD), and optical rotatory dispersion (ORD) studies about the conformation of the rhodopsin protein as well as the nature of the interaction between the prosthetic group, 11-cis-retinal, and the apoprotein, opsin. Each spectral technique provides complementary rather than identical information about conformation, particularly when applied throughout the entire experimentally accessible wavelength interval in which different electronic transitions associated with structural subunits of the rhodopsin molecule are located. The figure shows a convenient division of the absorption spectrum of detergent solubilized bovine rod outer segment (ROS) membranes in which rhodopsin is the predominant component.

The far ultraviolet, 185–250 nm, is characterized by an intense absorption band at 191 nm, a marked shoulder near 210 nm, and a weaker incompletely resolved band near 227 nm. The shorter wavelength band and shoulder probably arise by exciton splitting of the lowest energy π–π^* peptide bond transition and the weaker band is the n–π^* peptide bond transition. Some background absorption, not easily determined, is due to higher energy π–π^* transitions of the aromatic residues. CD is most successfully used to quantitatively analyze protein secondary structure. How-

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ever, absorption offers some advantages in detecting small conformational changes which do not fall into the helix-β-structure category, due in part to better signal to noise ratios obtainable with present instrumentation. ORD can provide additional structural information since it is a dispersive rather than an absorptive phenomenon and can reflect the presence of optically active transitions with bands outside the experimentally measured spectrum. Since CD and ORD of discrete electronic transitions are transformed by the Kronig-Kramers equations, bands situated below 185 nm, for example, associated with membrane lipid, can be revealed by comparison of experimental far ultraviolet ORD to ORD calculated from experimental CD data obtained in the same far ultraviolet wavelength interval.

The near ultraviolet, 250–300 nm, shows an absorption maximum at 279 nm. Absorption and CD in this region are primarily attributed to the lowest energy π–π^* transitions of the aromatic residues and to n–σ^* transitions of cystine. The usefulness of CD lies in the ability to detect delocalized conformational changes, involving protein secondary or tertiary structure, which should perturb the environment of many of these residues. Absorption offers potential sensitivity (because of high signal to noise ratios) to small localized conformational changes which should perturb only a few of these residues.

The visible, 300–650 nm, shows absorption maxima at 498 nm and 340 nm. Absorption and CD in this region are attributed to retinal π–π^* transitions. Retinal shows optical activity only when bound to opsin. In combination with ultraviolet spectra, visible absorption and CD are particularly valuable probes of retinal-opsin interaction.

Some important results concerning the structure of bovine rhodopsin are now given.

In regard to the structure of the protein moiety before bleaching: 1) Estimates of helix content range from 30–62%. The best value is probably between 30% and 40%. 2) Little β-structure is indicated. 3) Solubilization of the ROS membrane with detergents (excepting digitonin) results in loss of helical structure and perturbation of aromatic residues.

In regard to the structure of the protein moiety during bleaching: 1) Delocalized conformational changes of rhodopsin in situ involving secondary and/or tertiary structure are very unlikely. 2) Localized conformational changes of rhodopsin in situ involving secondary structure must be limited to the involvement of no more than three amino acid residues and localized conformational changes involving tertiary structure must be limited to very short segments of the protein chain containing, at the most, only a few aromatic residues. 3) Large changes in the interaction of lipid and the protein moiety in situ are not indicated by Kronig-Kramer transform calculations. 4) Solubilization by detergents and delipidation significantly decrease the conformational stability of rhodopsin as compared to the in situ conditions. The effect is present but smaller with digitonin.

In regard to the nature of retinal-opsin interaction: 1) One or two tryptophan and/or tyrosine residues are located near the binding site of 11-cis-retinal and very likely influence the electronic properties of the protonated Schiff base. 2) The optical activity of the prosthetic group probably arises by means of preferential binding by opsin of one intrinsically optically active atropisomer of 11-cis-retinal from a pool of thermally interconvertible atropisomers in solution. 3) The conformation of bound

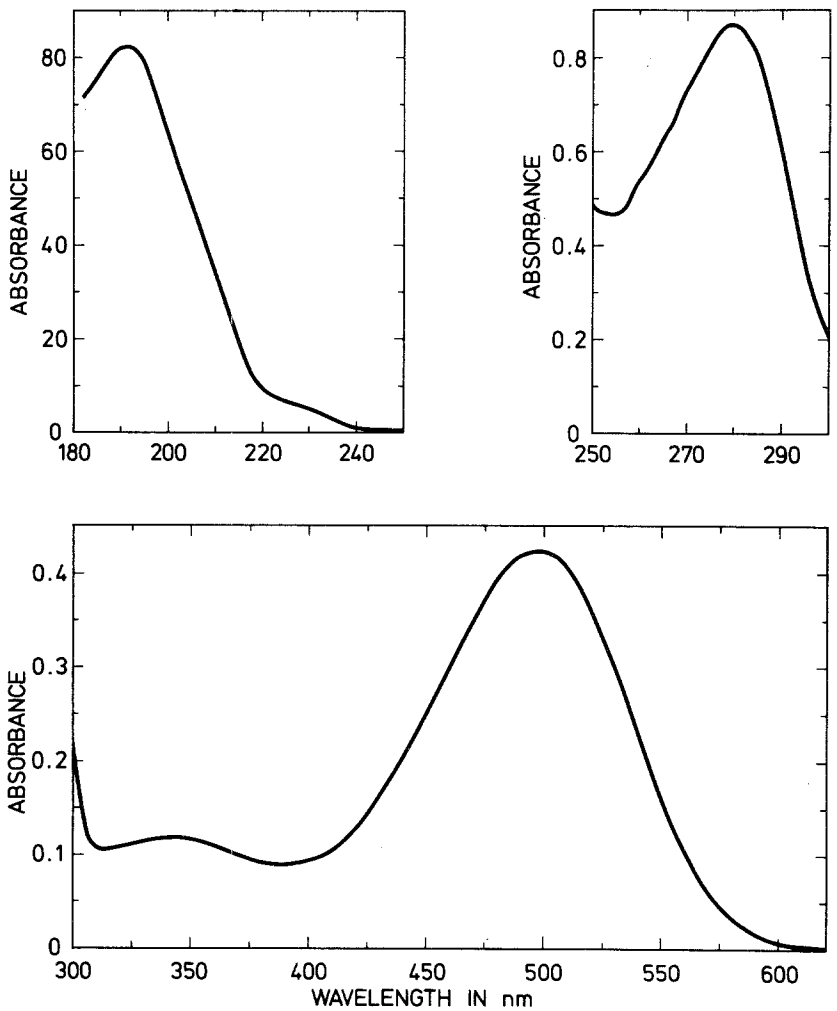

Fig. 1. Absorption of ROS membranes in 1% Emulphogene BC-720. All experimental absorbance data were transformed to correspond to absorbance measured with a spectrophotometer cell of 1.000 cm pathlength. Rhodopsin concentration – 10.44 nmoles/ml. Protein concentration – 0.632 mg/ml. pH – 7.00. Temperature – 25° C

11-cis-retinal, as defined by specific angles of rotation around individual single bonds, is probably changed upon solubilization of the ROS membrane by detergent. This change in conformation of the prosthetic group is probably coupled to protein conformational changes induced by solubilization.

Current models of how absorption of visible light by photoreceptor pigments is coupled to the production of neural signals invariably assume a conformational change of the rhodopsin molecule. The emerging picture for rhodopsin as a major component of the ROS membrane is that light-induced conformational changes must be restricted to only small regions of the protein in which only a few residues

participate, ruling out the possibilities of a unique delocalized conformational change. It is suggested that models requiring delocalized conformational change as an essential part of the mechanism for light induction of neural signals be reexamined. However, changes in conformation of the prosthetic group and changes in orientation of the prosthetic group relative to the protein moiety undoubtedly occur during bleaching.

The observed large values of entropy and enthalpy of activation associated with the decay of thermal intermediates of rhodopsin in situ have previously been taken as evidence for large delocalized light-induced conformational changes in the protein moiety. However, in view of the results from ultraviolet spectral measurements, this interpretation is obviously incorrect. Great caution must be exercised before equilibrium thermodynamic parameters can be equated to parameters for the thermal transition states of chromoproteins, particularly when the measurement technique is sensitive only to one portion of the chromoprotein. In the rhodopsin case, visible spectral changes probe only the visible chromophore, which is defined as the prosthetic group plus neighboring amino acid residues.

Finally, the probability is high that chemical and spectroscopic experiments on detergent solubilized ROS membranes will yield results which are unrelated to the physiological function of the intact membrane system.

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