Biochimica et Biophysica Acta 849 (1986) 172–174
Elsevier

BBA 40074

BBA Report

On the electrical conductivity of rhodopsin solutions

Vasile Vasilescu, Alexandru Dinu, Ioana Aricescu and Eugenia Chirieri-Kovács

Department of Biophysics, Medical Faculty, 8 P. Groza Blvd., 76241 Bucharest (Romania)

(Received November 4th, 1985)
(Revised manuscript received January 13th, 1986)

Key words: Rhodopsin; Digitonin; Conductivity; Visual pigment; (Frog)

Measurements were made on the conductivity of digitonin extracts of frog rhodopsin with and without previous light exposure. The light-dark difference in conductivity is observed at low concentrations of rhodopsin and detergent.

Rhodopsin is the main protein in the disk membrane of photoreceptor cell. It comprises at least 95% of the total protein of the disk membrane [1]. The structural transitions of rhodopsin induced by light trigger enzymatic and ionic changes which end by hyperpolarizing the cell membrane [2,3]. There is yet no clear picture of the processes induced by rhodopsin structural transitions which are directly involved in cell excitation.

We report here experiments in which electrical conductivity measurements of rhodopsin-digitonin suspensions have been used to investigate the structural transitions of rhodopsin induced by light.

The principle of the method was (i) to extract rhodopsin, (ii) to devide each extract into two equal parts and to bleach one of them, and (iii) to measure the conductivity of the dark-adapted and bleached extracts diluting them successively.

Rhodopsin was extracted from frog (*Rana pipiens*) retinas by using the technique described by Wald et al. [4]. The purpose of using this technique was to extract rhodopsin by a gentle method, preserving, as much as possible, its native structural properties. Dissected retinas were stirred up in 4% potassium alum solution (1 ml/retina) and left to tan for 4 h; then centrifuged at 3500 rot/min and supernatant descarded. Residue was washed twice in distilled H_2O and twice in neutral phosphate buffer in the centrifuge. The final residue was leached for 4 h in 1% digitonin (Merck) to extract rhodopsin.

Digitonin (Merck) was treated according to Bridges [5]. Its soluble fraction varied between 37 and 52%. Solution of 1% (w/v) concentration prepared with the digitonin processed as indicated by Bridges was stable even when kept more than a month at 4°C. pH of the solution was adjusted to 7 by sodium phosphate.

The same digitonin solution was used for all rhodopsin extractions.

Equivalent conductivity of control digitonin solution at different dilutions (4°C) is given in Table I. The critical micellar concentration of digitonin is somewhere between 0.25 and 0.125%. Solvent used for dilution of digitonin and rhodopsin-digitonin stock solutions was distilled water.

Rhodopsin concentration in the extracts, determined by the absorption at 500 nm ranged between $3 \cdot 10^{-5}$ and $3.5 \cdot 10^{-5}$ M. The purity of the extract was estimated by ratios K_{400}/K_{500} and K_{280}/K_{500} which had mean values $K_{400}/K_{500} = 0.23$ and $K_{280}/K_{500} = 2.4$ [6]. All extractions were

TABLE I

Λ WAS CALCULATED USING DIGITONIN CONCENTRATION EVALUATED IN %.

% Digitonin	Λ ($S \cdot cm^2$)
1	$9.5 \cdot 10^{-3}$
0.5	$1.5 \cdot 10^{-2}$
0.25	$2.36 \cdot 10^{-2}$
0.125	$4.0 \cdot 10^{-2}$
0.0625	$7.52 \cdot 10^{-2}$
0.0312	$1.472 \cdot 10^{-1}$
0.0156	$2.816 \cdot 10^{-1}$
0.0078	$5.12 \cdot 10^{-1}$
0.0039	$9.472 \cdot 10^{-1}$

performed at 4°C in dim red light. The extracts were divided into two equal parts, one of which was exposed to daylight for 30 min with the temperature maintained constant at 4°C. The specific conductance (γ) of both extracts was subsequently determined at 4°C in red light. Both samples were then diluted successively by distilled water. Following each dilution the conductivity of the two sets of solutions was measured under the same

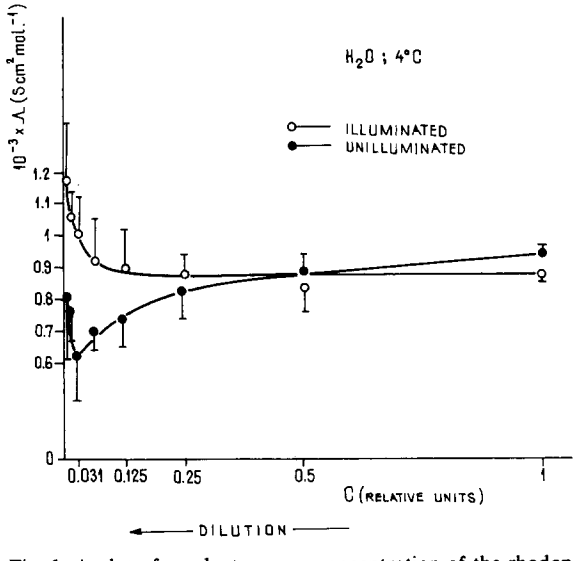

Fig. 1. A plot of conductance vs. concentration of the rhodopsin extract: ●, dark-adapted rhodopsin; ○, bleached rhodopsin. Solvent: H_2O. A concentration of $3.2 \cdot 10^{-5}$ M rhodopsin is taken as unity on the abscissa. Values are presented as the mean ± S.D. for seven sets of measurements obtained on different extracts.

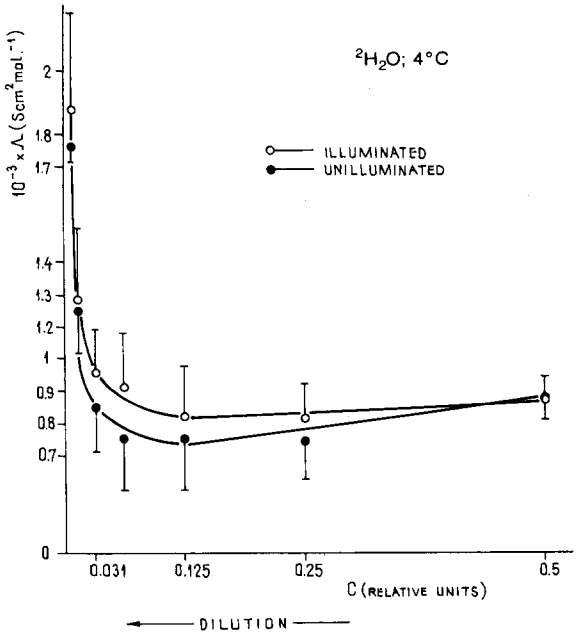

Fig. 2. A plot of rhodopsin conductance vs. concentration of the extract diluted by ^2H_2O. The unit concentration is $3.2 \cdot 10^{-5}$ M. The values indicated are means of five results obtained on different extracts ± S.D.

conditions. Two sets of conductivity values were thus obtained.

Water used for the extraction solutions and dilution of rhodopsin samples was triply distilled and deionized ($\gamma = 1.1 \cdot 10^{-6}$ S/cm at 4°C); the pH was adjusted to 7 by 100 mM sodium phosphate for extracts kept in the dark as well as for those exposed to light.

In another series of experiments, ^2H_2O was used for succesive dilution of rhodopsin, all other conditions being kept unchanged. Heavy water (99.9%) was supplied by Carlo Erba ($\gamma = 5 \cdot 10^{-6}$ S/cm at 4°C).

Conductivity of the extracts was measured by a conductivity meter (L. Seibold, Vienna). Before dilution the conductivity of rhodopsin extracts was of the order of 10^{-3} $S \cdot cm^2 \cdot mol^{-1}$. The equivalent conductance for rhodopsin is formally defined as $\Lambda = 1000\gamma/c$, where c is the concentration of rhodopsin in mol/l. This was plotted vs. concentration for seven sets of dilutions in H_2O and five sets of dilutions in ^2H_2O (Figs. 1 and 2).

A marked difference is observed between the solutions in H_2O exposed to light and those kept

in the dark (Fig. 1). As a rule the conductivity of bleached rhodopsin is higher than that of the rhodopsin kept in the dark.

In all conductivity curves of dark-adapted rhodopsin the systematic presence of a 'pocket' was observed at a concentration of about $0.18 \cdot 10^{-5}$ M; the pocket was only barely present or absent in the curves of the bleached samples. In most experiments in the 'pocket' area the presence of isolated points may be observed, situated more or less outside the curve, suggesting the instability of the micellar system in that region of concentration.

In ^2H_2O the difference between the conductivities of bleached and dark-adapted samples is very slight, the 'pocket' of the latter being vaguely outlined (Fig. 2).

A possible explanation of the observed phenomena might be that at concentration of the extract around 0.25% at which the sudden drop of conductivity usually occurs, digitonin micelles are desrupted (attaining their critical micellar concentration) liberating rhodopsin into the aqueous suspension. Being unstable, rhodopsin molecules aggregate [6] causing the observed decrease of conductivity. Aggregates are then dissociated at lower concentration. Aggregation might be absent or masked by other phenomena in the bleached extracts.

^2H_2O seems to prevent somehow the formation of aggregates in the dark. It is possible that ^2H_2O hinders the structural dark-light transitions of rhodopsin as have been earlier suggested by electrophysiological experiments on deuterated retinas [7,8].

However, the micellar system of digitonin, protein, phospholipids and salt extracts from retina is too complex to draw any speculative conclusion in molecular terms from the data available. Further refinements of the described experiments are necessary to assign the observed 'pocket' to the behaviour of a certain molecule within the micellar system.

It seems appropriate to recall here the earlier suggestion of McCaslin and Tanford [9] that rhodopsin is found in an oligomeric state if extracted by detergents which keep intact the ability of opsin to recombine with 11-*cis* retinal (digitonin is such a detergent). More recently Downer and Cone [10,11] have shown that rhodopsin is monomeric within the membrane; they admit, however, that in environments other than the native membrane, rhodopsin may be stabilized by protein–protein interactions, forming oligomers.

References

1 Papermaster, D.S. and Dreyer, W.J. (1974) Biochemistry 13, 2438–2444
2 Hagins, W.A. (1972) Annu. Rev. Biophys. Bioenerg. 2, 131–158
3 Kühn, H. (1984) Progress in Retinal Res. 3, 123–157
4 Wald, G. and Brown, P.K. (1952) J. Gen. Physiol. 35, 797–821
5 Bridges, C.D.B. (1977) Vision Res. 17, 301–302
6 Hubbard, R. (1954) J. Gen. Physiol. 37, 381–399
7 Chirieri, E., Aricescu, I. Ganea, C. and Vasilescu, V. (1977) Naturwissenschaften 64, 149
8 Chirieri-Kovács, E. and Vasilescu, V. (1982) J. Chem. Phys. 14, 281–289
9 McCaslin, D.R. and Tanford (1981) Biochemistry 20, 5212–5221
10 Downer, N.W. and Cone, R.A. (1985) Biophys. J. 47, 277–284
11 Downer, N.W. (1985) Biophys. J. 45, 285–293