Spectroscopic indication for a trans-cis isomerization of the retinal chromophore of bacteriorhodopsin during the photocycle

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Two mechanisms are postulated for bacteriorhodopsin (bR) to prevent the system to fall back into the ground state after light excitation: charge stabilization (1) and trans-cis isomerization (2). Recently 13-cis retinal was extracted chemically from the L- and M-state intermediate of bR (3) and correlated with the photocycle (4). -

Investigation of the UV-spectral range of light adapted trans-retinal bR during the photocycle revealed a two-phasic "on" reaction. The slow phase spectrum indicates the deprotonation of the tyrosine and proton displacement around two tryptophan amino acids (5) similar in time to the formation of M-412. The fast phase spectrum is composed of absorption decrease below 290 nm with maximum around 280 nm, isosbestic point at 290 nm, absorption increase above 290 nm with absorption peak at 338 nm and shoulders 297, 312, 328, 358 nm. The fast spectrum appears in $<0.2\,\mu$sec at 280 nm and in 0.6-3.5 μsec above 290 nm after a ns-laser flash (1.5°C). -

bR exhibits in the dark an equilibrium of 1:1 of cis- and trans-retinal (dark adapted bR). Upon illumination the equilibrium is shifted to almost 100% trans-retinal (light adapted bR). The difference spectrum between dark and light adapted bR (cis-trans bR) displays in the UV range a similar absorption difference spectrum as found for the fast formation reaction after flash excitation of trans-bR with the isosbestic point given above and maxima at 297, 329, 340, 357 nm.

While the trans-cis transition in the light-dark adaptation is a slow process (t/2 ~20 min at 22°C) the time course of the formation of the fast difference spectrum after laser flash suggests that the trans-cis isomerisation during the photocycle occurs prior to/or concomitant with the formation of the L-550 intermediate. The reverse cis-trans isomerization follows after a lag time approximately the decay of M-412 at pH below neutrality, however, at alkaline pH being slower than the M-412 decay. The data confirm a cis-configuration as part of the photocycle, being consistent with our earlier observation of the photochemical action spectrum in the region between 390-450 nm (6,7).

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