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AN ISOMERIZATION MODEL FOR THE PHOTOCYCLE OF BACTERIORHODOPSIN

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ABSTRACT

On the basis of a recent study [2] of the isomerisation potentials of the protonated and unprotonated Schiff base of retinal and of the observation of a 13-cis intermediate [3], a complete conformational assignment of the intermediates in the light-adapted and the dark-adapted photocycle of bacteriorhodopsin is presented.

INTRODUCTION

If one accepts that the chromophore retinal is directly engaged in each step of the proton pump cycle of bacteriorhodopsin in the purple membrane of Halobacterium halobium, the question arises how the known photobehaviour of retinal (in particular its Schiff base moiety) may give rise to the pump action, i.e. the irreversible translocation of a proton across the purple membrane. Stoeckenius (private communication) and Kozlov and Skulachev [1] suggested that a high pK hydrogen bridge chain funnels protons from the cytoplasmic side to the chromophore and a second low pK chain ejects the protons into the extracellular space. With such an arrangement the participation of the chromophore to the proton translocation across the whole membrane needs to involve only minute motions. These motions must entail two steps in principle, a light-induced step

rise of the pK value of the C=N group and motion (of $C=N^{+}_{H}$) through a space which does not conduct protons thermally (1)

and a subsequent dark step

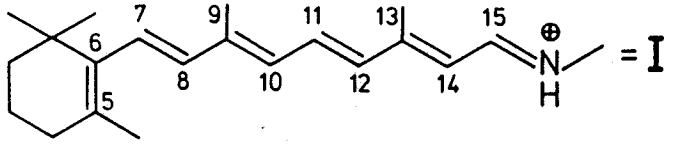
decrease of the pK value of the C=N group and thermal return of the C=N group to the old position only after deprotonation. (2)

The important feature is that the proton transfer must be close to irreversible in the normal pH range, i.e. step 2 must have a low activation energy barrier in the unprotonated form and a high

barrier in the protonated ($C = \overset{H}{N^+}$) form. We have recently suggested[2] that the photoinduced cis-trans isomerisation of the 14-15 single bond of retinal exhibits exactly these features. Together with the observation of Pettei et.al. [3] of an all-trans $\rightleftarrows$ 13-cis isomerisation during the pump cycle, one can actually attribute chromophore conformations to all intermediates of the photocycle of bacteriorhodopsin. The conformations are presented in Fig. 1 together with a schematic potential energy surface in Fig. 2 and will be discussed below.

THE MODEL

The first effect of light excitation of the retinal Schiff base chromophore is a strong increase of the nitrogen pK value by about 14 units suggested by the large bathochromic shift from 410 nm to 570 nm between the protonated and the unprotonated pigment. In case of a hydrogen bridge between a basic group, e.g. histidine or lysine, and the Schiff base nitrogen, optical excitation would induce the proton to move towards the chromophore and break the hydrogen bridge. Figures 1,2 suggest that the concomitant photoreaction I → III involves an all-trans → 13,14s -cis isomerisation. The conventional structural formula corresponding to the all-trans chromophore I is



= I

It is noteworthy that the transition state II of this photoprocess entails no geometrical change of the chromophore except the motion of a hydrogen to the top of carbon 14 if one accepts that this carbon has undergone a $sp_2 \rightarrow sp_3$ rehybridisation in the transition state. The resulting lack of sterical hinderance may explain why the initial photoprocess in bacteriorhodopsin proceeds with such extraordinary rapidity ($\sim$ 6ps). Subsequent crossing to the ground state (II → III) generates a strong force (see Fig. 2) which leads to the distorted 13,14s -cis conformation (III) pulling the covalently bound lysine residue and the proton backbone along.

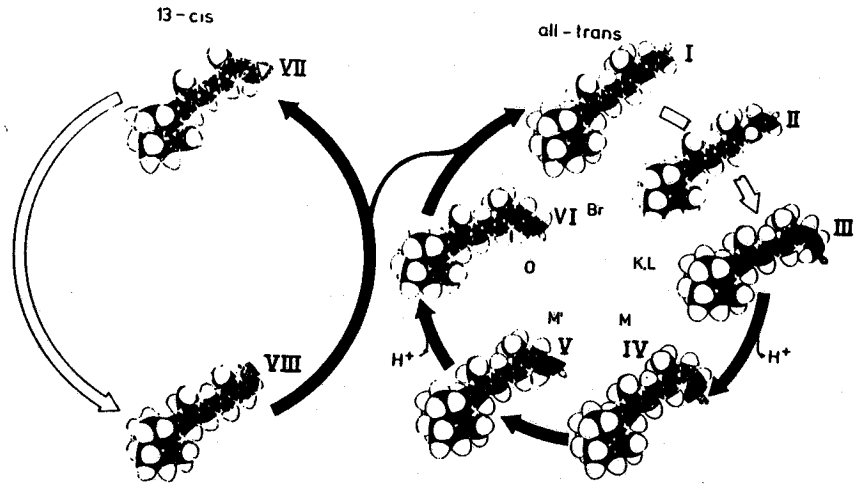


Figure 1

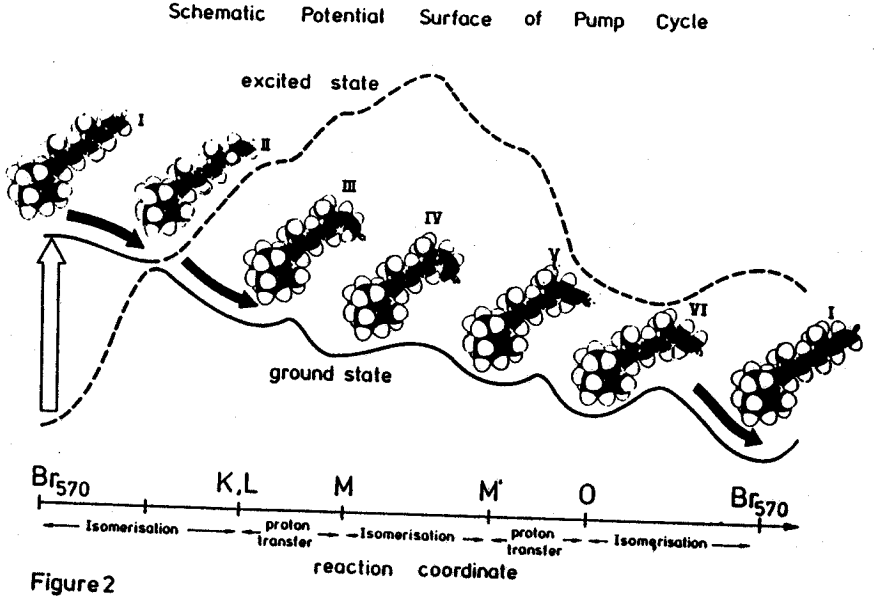


Figure 2

The sterical energy thus stored could provide the forces which restore the original all-trans conformation during the remaining dark part of the pump cycle (III → I).

As argued in Ref. 2 the first dark reaction (III → IV), a rotation about the 14-15 bond, proceeds only after deprotonation of the chromophore. This is the essential step for the irreversible proton translocation. The subsequent 13-cis → trans isomerisation (V → VI) is probably acid catalyzed, i.e. occurs after reprotonation (IV → V). This step may pull a proton out of the high pK hydrogen chain connecting with the intracellular space.

The occurrence of a 13-cis intermediate in the photocycle of the light-adapted pigment raises the question in what respect it differs from the 13-cis intermediate contributing to the dark-adapted pigment. Figure 1 suggests that the dark-adapted pigment contains, in fact, 13,15-cis retinal (VII). This conformer entails only a minimal geometrical difference with respect to the light-adapted all-trans form which is in agreement with the small spectral (10 nm) and small free energy difference (thermal equilibrium 50:50). Light excitation of VII may produce again via an intermediate with a sp_3- hybridized C14, a 14s,15-cis conformer VIII which proceeds to the 15-cis conformation. This latter process involves a rotation around the 14-15 bond in the protonated form which may occur contrary to the light-adapted cycle because of the sterical hindrance of VIII due to the C13 methyl group. The 15-cis conformer reverts then back to the 13,15-cis chromophore VII by 13-14 double bond rotation or (with a small yield) to the light-adapted form I by rotation around the C = N bond.

ACKNOWLEDGMENT

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