THE ROLE OF TYROSINE IN THE PROTON PUMP OF BACTERIORHODOPSIN

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1. Introduction

Light-induced proton pump activity of bacteriorhodopsin is a consequence of a photoreaction cycle composed of a number of distinct spectroscopically distinguishable transient species [1,2]. The 412-nm absorbing intermediate is a key component because it is a measure of the deprotonation and reprotonation of the retinal Schiff base [3–5]. The origin of the proton donated to the Schiff base and the specific amino acids which accept the released proton have not yet been identified. Previous studies in our laboratory have demonstrated that chemical modification of tryptophan groups of the molecule causes loss of the 570-nm absorption of the chromophore and a marked inhibition in the decay of the 412-nm species, particularly when half or more of the tryptophan residues have been modified [6]. The total number of tryptophan residues is between 4–7 [7]. This suggests that 2–3 residues are in close proximity to the retinal chromophore. In the present study we have investigated the role of tyrosine to determine whether this aromatic amino acid is also important in determining chromophore environment, since tyrosine is a potential donor or acceptor of protons for the Schiff base of the lysine where retinal is attached to the protein.

The pH dependence of the decay of the 412-nm species indicated that the groups involved had a $pK > 11$, i.e. it was in the range where tyrosine residues are ionized. Furthermore, modification of bacteriorhodopsin by iodination showed that the loss of 1–2 of the 11 tyrosine residues caused marked inhibition of photocycling. These results indicate that tyrosine residues are involved in reprotonation of the chromophore Schiff base and are essential for proton pumping.

2. Materials and methods

Purple membrane preparations in distilled water or 0.2 M NaCl were titrated by adding small volumes of alkali solution (0.2 M–5 M NaOH). After measurement of the pH 1 min after the alkali addition, the formation and decay of the 412-nm intermediate was determined by flash photolysis [6]. Spectrophotometric titration of tyrosine groups of bacteriorhodopsin were made in 0.2 M NaCl solution in order to minimize salt effects due to NaOH addition. The base line absorbance increased gradually as the wavelength fell below 300 nm due to light scattering by the sample. hence absorbance at 295 nm was corrected by extraporating the base line between 350–380 nm to the 295-nm region. The apparent pK_a was determined from the slope of the linear plots $[H^+]\Delta\epsilon$ against the molar absorptivity difference ($\Delta\epsilon$) at a given pH according to the following equation [8]:

$$[H^+]\Delta\epsilon = K\Delta\epsilon_{max} - K\Delta\epsilon$$

where $\Delta\epsilon_{max}$ is the difference in molar absorptivity at 295 nm after complete ionization of all tyrosines, and K is the apparent ionization constant.

For iodination of tyrosine, 0.2 μM bacteriorhodopsin suspensions in 0.2 M borate–Na pH 8.5, were treated at 22°C for 1 h with aliquots of a 1 M iodine solution to give different final concentrations then centrifuged and resuspended in buffer to remove excess iodine. Formation of mono- and di-iodinated tyrosines were determined spectrophotometrically [9].

3. Results

The kinetic properties of the 412 nm intermediate (fig.1) in purple membrane fragments undergo a marked change, relative to pH 7, in the pH region near the pK_a of tyrosine. From a linear plot of spectrophotometric titration data [8] two populations of tyrosine residues, one with an apparent pK at 11.2, and another with a p$K > 12$ are indicated (fig.2). About 1.2 of the 11 tyrosine residues have a pK_a value of 11.2, and for this subpopulation it was possible to calculate the ratio of deprotonated to protonated state (fig.2B). The 412-nm decay was linearly dependent on the ionization of these tyrosine residues, at a pK_a of 11.2; the correlation deviated only above pH 11.5 (fig.2B).

In order to determine more precisely the role of tyrosine, chemical modification by iodination was undertaken. This treatment is known to decrease the pK of tyrosine due to formation of mono- and di-iodinated tyrosines [9]. Figure 3A shows that as the molar ratio of iodine to bacteriorhodopsin is increased, marked bleaching of the 570-nm chromophore occurs after only 1 or 2 of the 11 tyrosine residues have been modified. Concomitant with this modification is a marked inhibition of the initial and secondary phases of the decay of the 412-nm species (fig.3B). The rise time for the formation of the 412-nm species is slightly pH dependent. Since formation of the 412-nm species is associated with deprotonation and the decay of this species with reprotonation, the results implicate a tyrosine residue implicated in the reprotonation of the retinal Schiff base.

Formation of mono- and di-iodinated forms of tyrosine shifts the pK of free tyrosine from 10.13 to lower pH, to 8.2 and 6.2 respectively [8]. In bacterio-

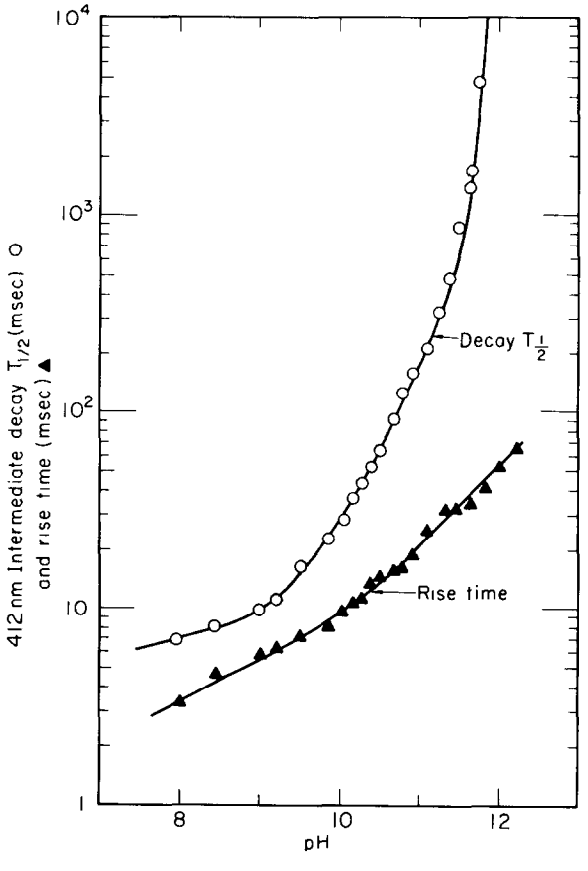

Fig.1. pH dependence of the formation and decay of the 412-nm intermediate of bacteriorhodopsin.

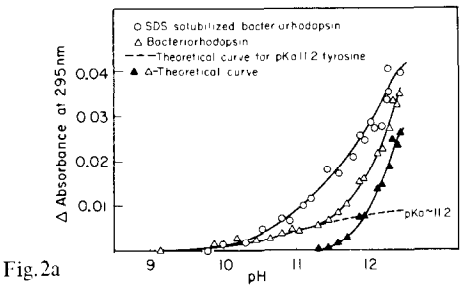

Fig.2a

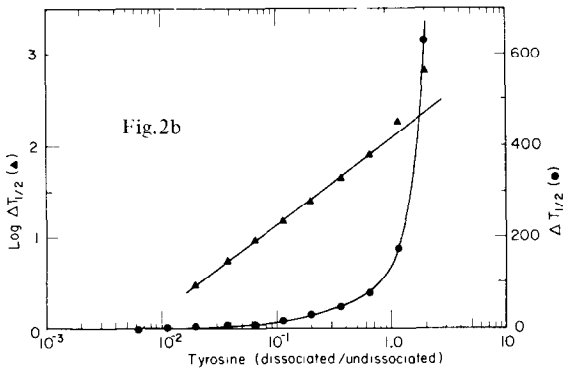

Fig.2. Spectrophotometric titration of tyrosine residues and activity of bacteriorhodopsin. (a) Spectrophotometric titration; (b) correlation of tyrosine dissociation with photoreaction cycle activity.

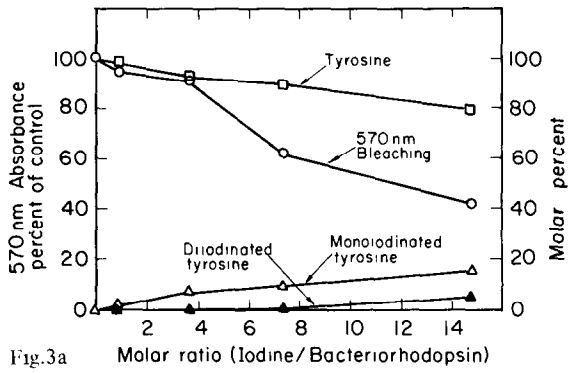

Fig.3a

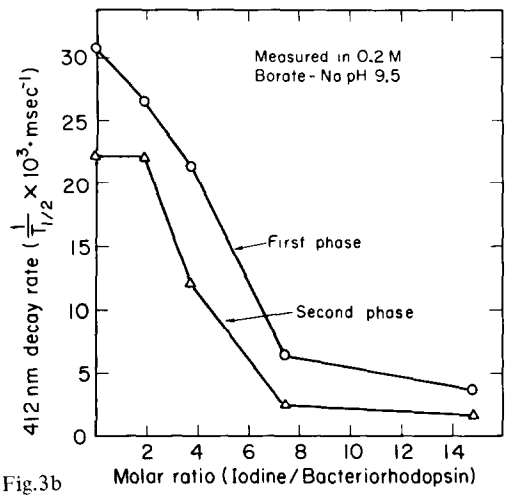

Fig.3b

Fig.3. Chemical modification of bacteriorhodopsin by iodination. (a) Iodination of tyrosine and 570-nm chromophore bleaching; (b) effect of iodination on decay of the 412-nm intermediate.

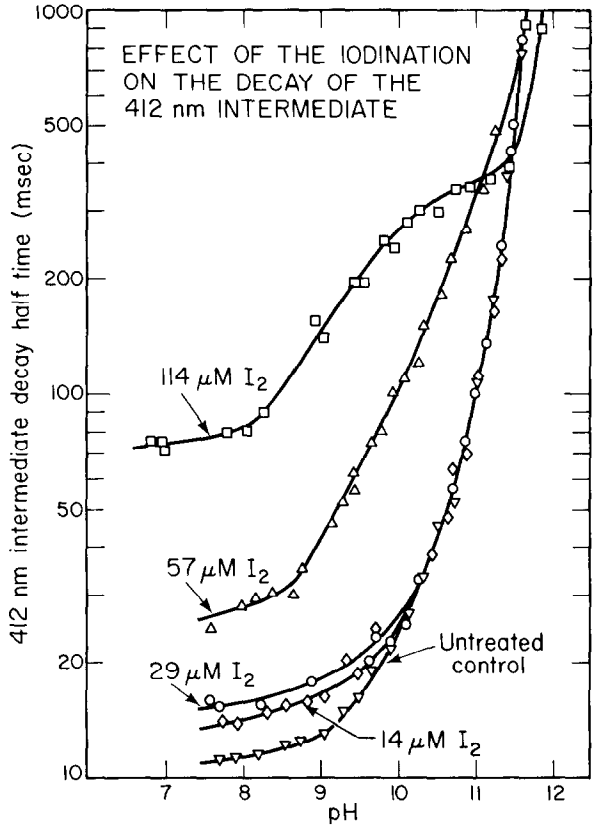

Fig.4. pH dependence of the decay of the 412-nm intermediate by formation of mono- and di-iodinated forms of tyrosine.

rhodopsin the pK of tyrosine is at a higher pH because it is an apolar environment, hence a shift in pK, for the decay of the 412-nm intermediate, by formation of increasingly iodinated tyrosine, yields apparent pKs of about 9.5 and 9.0 (fig.4).

4. Discussion

These and other studies [10–12] from our laboratory suggest that the mechanism for the proton pump of bacteriorhodopsin is as depicted in fig.5.

Step 1: Dark-light-adaptation state. The dark adapted state contains retinal in 1:1 mixture of 13-*cis* and all-*trans* configuration [13,14]. The 13-*cis* form is shown in the diagram; this form of retinal may not be able to interact properly with tryptophan or tyrosine residues.

Step 2: Light adapted state. The retinal chromophore orients and makes a close contact with the tryptophan and tyrosine residues [12].

Step 3: Light excites the retinal chromophore and the electron shifts over the retinal molecule to the tryptophan nucleus. (This explains the initial bathochromic shift of absorbance.) This results in an electron deficiency on the Schiff base nitrogen which drives the proton release from the nitrogen atom. A neighboring negative (low pK_a residue) group

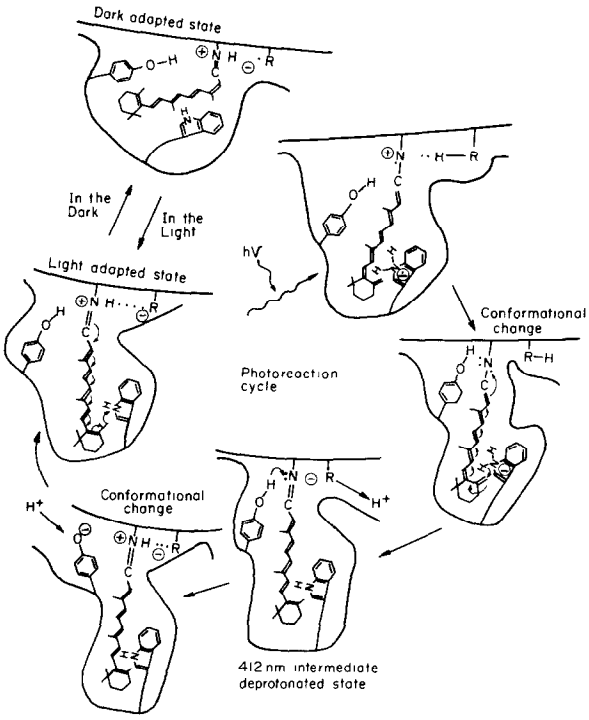

Fig.5. Mechanism of proton translocation by bacteriorhodopsin.

(perhaps Asp 38, designated as R, cf. Ovchinnikov et al. [15]) accepts the proton. This charge neutralization induces a protein conformational change, and separates the transferred proton from the Schiff base nitrogen. This assumption is consistent with the observation that dimethyl adipimidate cross-linking of the protein inhibits the 412-nm intermediate formation [10,11].

Step 4: The electron returns from the tryptophan and increases the electron density on the Schiff base nitrogen sufficiently to pick up a proton from the neighboring tyrosine residue. Since the decay of the 412-nm intermediate is directly dependent on the protonation state of a tyrosine, but the formation of the 412-nm species is unaffected, the proton must come from the neighboring tyrosine residue. This process may be assisted by the negatively charged group, R, which has released the proton to the other side. Bogomolni, Renthal and Lanyi [16] have also concluded recently, from analysis of UV absorbance changes during photocycling, that a tyrosine is associated with a reprotonation of the retinal Schiff base.

Step 5: The phenolate formed accepts a proton from the medium and may induce a conformation change of the protein, yielding the original light-adapted state.

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