What lies behind the tendency of the Haller-Bauer process to preserve configuration at the reaction center? Expectedly, the appreciable stereoselectivity parallels that noted when secondary and tertiary alcohols of related structure are subjected to base-promoted cleavage.[17] Closely related phenomena are certainly at play. Specific details will be presented in the full paper. Suffice it to say that the capacity for generating benzylic carbanions in chiral condition can now be conveniently accomplished.[18]

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FTIR Difference Studies on Apoproteins. Protonation States of Aspartic and Glutamic Acid Residues during the Photocycle of Bacteriorhodopsin

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Received June 1, 1987

Bacteriorhodopsin (bR) contained in the purple membrane of *Halobacterium halobium* is a light-driven proton pump coupled to ATP synthesis.[1] The chromophore of the functionally active light-adapted form (LA) is *all-trans*-retinal bound to the apoprotein Lys-216 via a protonated Schiff base (SBH^+), while that of the inactive dark-adapted form (DA) is a 6:4 mixture of 13-*cis*- and *all-trans*-retinal. Light isomerizes the chromophore to 13-cis and triggers the proton-translocation photocycle: $LA \rightarrow K_{625} \rightarrow L_{550} \rightarrow M_{412} \rightarrow O_{640} \rightarrow LA$. Clarification of protonation states of amino acid residues in this cycle is essential for understanding the proton-pumping mechanism. Fourier transform infrared (FTIR) difference spectroscopy is especially suited for this since it can detect changes occurring in single amino acid residues as well as the chromophore during the photocycle.[2-4]

Table I. Summary of Changes in Asp Groups in Various Bacteriorhodopsins[a]

	DA	LA	K	L	M
Asp-1	COO^- 1392	COO^-	COO^- 1395	COO^-	COOH 1761
Asp-2	COO^-	COO^-	COO^-	COO^-	COOH 1747
Asp-3	COOH 1734	COOH 1741	COOH 1734	COO^- 1399	COOH 1737
Asp-4	COOH	COOH 1734	COOH	COOH 1739	COO^- 1393

[a] Asp residues are numbered 1-4 simply for the ease of following the changes (also see text); thus FTIR difference bands tabulated in the same line may not necessarily represent the same Asp group. The Asp-3 1741 cm^{-1} value for LA is the average of 1738 (Figure 1a), 1742 (1b), 1741 (1c), and 1742 (1d). The Asp-4 1734 cm^{-1} value for LA is the average of 1736 (Figure 1c) and 1732 cm^{-1} (1d). The time-resolved FTIR results[3a,d] for L and M intermediates have been taken into account.

The studies have been performed on LA, DA, K, L, and M species of native bR and pigments enriched with [4-^{13}C]Asp and [5-^{13}C]Glu; bR regenerated from [9,13-CD_3]retinal[5] has also been used. The studies show that four Asp's undergo protonation/deprotonation or environmental changes and that Glu is not involved. Some earlier results[3d,4e,6] have been clarified or modified.

Syntheses of ^{13}C-labeled amino acids, incorporation into bR by growing the bacteria in a synthetic medium[7] containing the labeled amino acids, and isolation of purified bR[8] were carried out as described.[4e,6] Spectra were measured with Nicolet 7199 or Mattson SIRIUS 100 spectrometers. Since the Asp ω-carboxyl can be converted to Glu α-COOH (TCA cycle), prolonged incubation leads to a decrease in labeled Asp. Although conditions for achieving maximal incorporation of labeled Asp are not known, the [4-^{13}C]Asp content of the sample used in the present studies was ca. 85% (Figure 1d), this high incorporation being the crucial factor in the current FTIR analysis. Integrity of the sample was checked by electrophoresis[9] since bR grown in the synthetic medium[7] occasionally gave preparations with cleaved chains.

FTIR difference spectra between LA and other species are shown in Figure 1 (parts a-d) for regions 1780-1620 cm^{-1} and ca. 1390 cm^{-1}; negative and positive peaks belong, respectively, to LA and the other species. The following two characteristic bands were used: (a) The COOH bands at 1770-1720 cm^{-1} (solid curves), which shift to lower frequencies by $\sim$10 and $\sim$40 cm^{-1}, respectively, upon conversion to $COOD$[2-4] and ^{13}COOH.[3d,4e] (b) The ν_s COO^- bands at 1390 cm^{-1} (insets);[10] since δ CH_3 bands overlap in this region, [9,13-CD_3]retinal-bR spectra were also taken to corroborate results.

Conclusions derived from Figures 1a-1d (see also Table I) are as follows.

† Deceased August 14th, 1985. This paper is dedicated to the memory of L.E.

‡ Leave of absence from the Faculty of Pharmaceutical Sciences, University of Tokyo.

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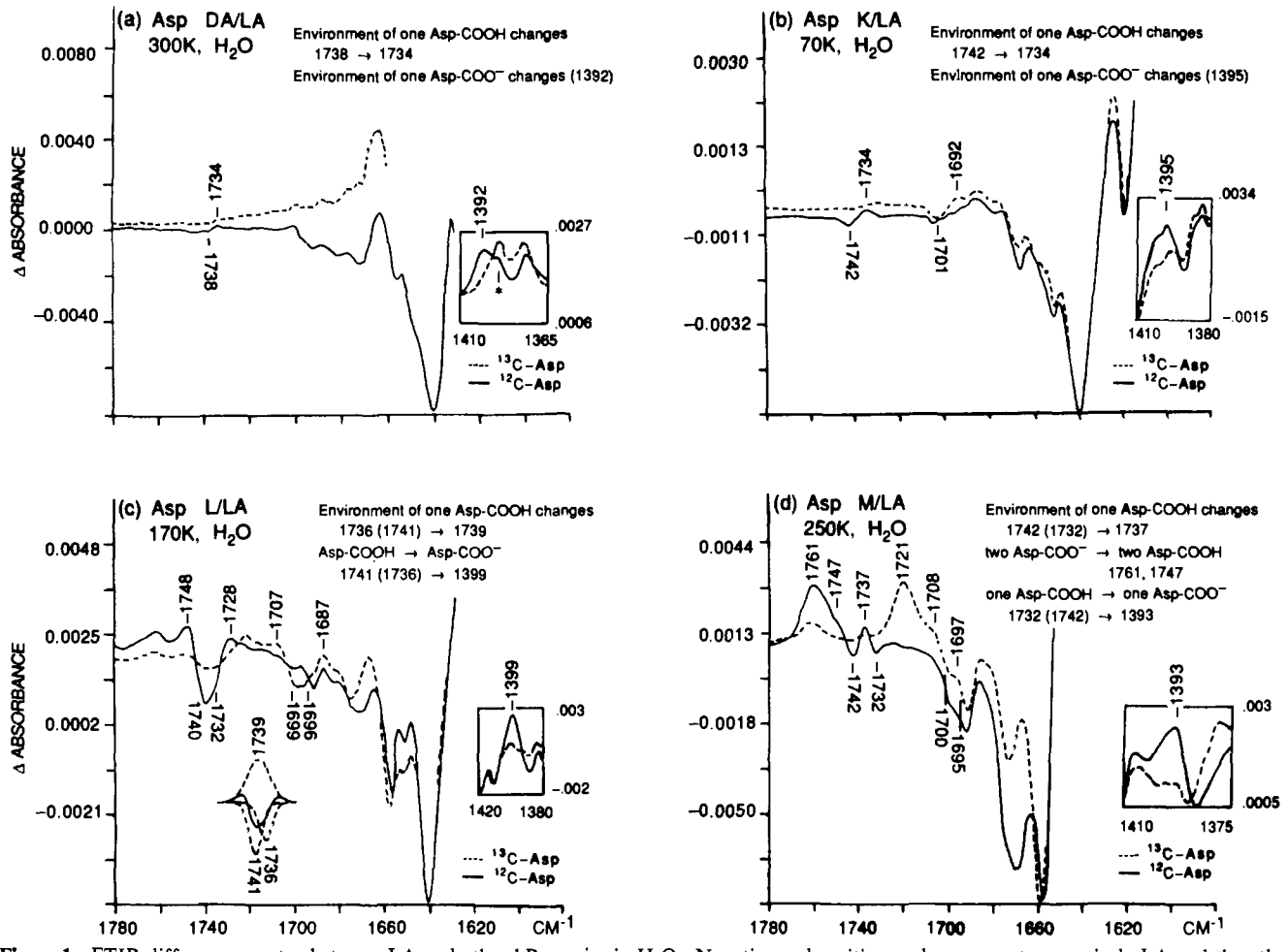

Figure 1. FTIR difference spectra between LA and other bR species in H_2O. Negative and positive peaks represent respectively LA and the other species. Native bR species are shown by solid curves and [4-^{13}C]Asp-bR species by dashed curves. The asterisked band (1a, inset) disappears in [9,13-CD_3]retinal-bR.

(1) All COOH bands of native bR (solid curves) are shifted by ~40 cm^{-1} in [4-^{13}C]Asp-bR (dashed curves); moreover, only incorporation of [4-^{13}C]Asp led to changes of bands in the 1400 cm^{-1} COO^- region. In contrast, difference spectra between native bR and [5-^{13}C]Glu-bR remained unchanged in all cases. Thus *only Asp COOH's, but no Glu COOH's, are involved in the cycle.*

(2) DA vs LA (Figure 1a). The positive 1392-cm^{-1} band in DA is due to Asp-COO^- as it shifts (disappears) in [4-^{13}C]Asp-bR; in the DA/LA spectrum of [9,13-CD_3]retinal-bR (data not shown), the asterisked 1384-cm^{-1} shoulder disappears but 1392 cm^{-1} is unchanged. Since no clear negative COOH appears, the 1392-cm^{-1} band reflects an *environmental change in one Asp-COO^- residue*; the 1392-cm^{-1} aspartate may represent the SBH^+ counteranion because the DA/LA change is accompanied by a 13-ene isomerization.[1] The weak 1738/1734-cm^{-1} pair (1729/1723 cm^{-1} in D_2O, data not shown) may represent *change in surroundings of one Asp-COOH* since it disappears in [4-^{13}C]-Asp-bR (shifted peak is too weak to be seen). There appears to be no change in protonation states of Asp.

(3) LA vs K (Figure 1b). Shift of the 1742/1734 cm^{-1} pair to 1701/1692 cm^{-1} shows that formation of primary photoproduct K is accompanied by an *environmental change of one Asp(COOH) residue.* Earlier deductions[3d,4e] had ascirbed the pair to Glu because the lower incorporation of [4-^{13}C]Asp had obscured this isotope shift. In agreement with the noninvolvement of Glu residues in the LA → K transition, the K/LA difference curve of [5-^{13}C]Glu-bR is identical with that of native bR. In addition, a positive Asp-COO^- band is seen at 1395 cm^{-1} which is reduced in intensity in [4-^{13}C]Asp-bR but not in [9,13-CD_3]retinal-bR. No corresponding negative COOH peak could be detected implying an *environmental change in one Asp-COO^-*, which again could be the SBH^+ counteranion (Table I).

(4) LA vs L (Figure 1c). Computer simulation shows the 1748–1728-cm^{-1} band envelope, which shifts to 1707–1687 cm^{-1} in [4-^{13}C]Asp-bR, to be best represented by two negative bands at 1741/1736 cm^{-1} and a positive band at 1739 cm^{-1}. Furthermore, a positive 1399 cm^{-1} COO^- peak remains unchanged in [9,13-CD_3]retinal-bR. These results suggest that the environment of one *Asp(COOH) is changed (1736 or 1741 vs 1739 cm^{-1}) while another (1741 or 1736 vs 1399 cm^{-1}) is deprotonated* in L relative to LA. Since no deprotonation is seen in LA → K, the *deprotonation occurs in the K → L transition*, a result in agreement with kinetic FTIR measurements.[3d]

(5) LA vs M (Figure 1d). The entire band envelope 1761–1732 cm^{-1} (solid curve) is shifted to 1721–1695 cm^{-1} (dashed curve) demonstrating that all absorptions are due to Asp; furthermore, relative intensities of the 1761- and 1721-cm^{-1} bands in the dashed curve show that incorporation of the labeled acid is ca. 85%.[11] The simplest interpretation of the difference spectra is as follows. (i) *Environment of one Asp-COOH changes*, 1742 (or 1732) → 1747 cm^{-1}. (ii) *Two Asp-COO^- residues in LA become protonated in M*, 1761 and 1747 cm^{-1}. Kinetic FTIR studies by Siebert et al.[3a,b] have shown these changes to occur around the L → M transition. The unusually high frequency of the 1761 cm^{-1} COOH band indicates that it is not hydrogen-bonded and is likely to be in hydrophobic surroundings; it may originate from the SBH^+ counteranion. (iii) *One Asp(COOH) in LA is deprotonated in M*, 1732 (or 1742) → 1393 cm^{-1} (band is unchanged in [9,13-CD_3]retinal-bR).

These FTIR difference results show that the bR photocycle involves at least four Asp residues and no Glu groups, the latter

(11) The lower [4-^{13}C]Asp content in previous preparations[3d,4e] led to results implying participation of Glu.

conclusion being supported by studies with [5-^{13}C]Glu-bR. Possible changes of the four Asp residues are proposed in Table I. It is interesting to note that the currently favored models[12] for the folding of bR show that of the nine Asp residues, four reside within the interior of the membrane. Groups which change protonation state concomitantly with the proton-pumping process might be involved as a conducting wire.[13] In addition to Asp, we have observed protonation/deprotonation steps in the following changes: DA (Tyr-OH) → LA (Tyr-O$^-$) → (Tyr-OH) → L (Tyr-OH) → M (Tyr-O$^-$),[4f,g] while Rothschild and co-workers have also shown[2f] that a tyrosinate protonates in LA → K. Clearly the technique of difference FTIR provides a powerful method which will contribute to the understanding of subtle dynamic processes exemplified by proton translocation.

Acknowledgment. This work has been supported by PHS GM 32455 (to L.E.), PHS 1S1ORR2298 (to L.E., for purchase of Mattson Sirius 100), and NSF CHE 84-12513 (to K.N).

Supplementary Material Available: The DA/LA, K/LA, L/LA, and M/LA difference spectra of the entire region and of the expanded carboxylate region for native and [4-^{13}C]Asp-bR; the DA/LA, K/LA, L/LA, and M/LA difference spectra for native and [5-^{13}C]Glu-bR (12 pages). Ordering information is given on any current masthead page.

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TiO_2 and CdS Colloids Stabilized by β-Cyclodextrins: Tailored Semiconductor–Receptor Systems as a Means to Control Interfacial Electron-Transfer Processes

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Received April 17, 1987

The photocatalytic activity of semiconductor particles is of substantial interest in synthesis[1] as well as a means of solar energy conversion and storage.[2,3] Electron-transfer reactions such as charge ejection or charge injection at semiconductor–solution interfaces are important factors that control the photocatalytic activity of the semiconductors.[4,5] Electrostatic attraction of solute to the semiconductor surface has improved charge ejection from the excited semiconductor to solute relays.[6] Adsorption of dyes to the semiconductor surface, i.e., by hydrophobic interactions, resulted in effective charge injection to the semiconductor and consequently the photocatalytic activity of semiconductors operative in the UV region could be shifted to the visible absorption spectrum.[5,7] Substantial efforts have also been directed in recent years toward the preparation of semiconductor particles in colloidal forms to improve their light harvesting and interfacial electron-transfer properties.[8-10] Stabilization of semiconductor colloids by polymers, microemulsions, and vesicle encapsulation[10] has been reported. Here we report on a novel method for stabilizing semiconductor colloids by β-cyclodextrin (β-CD).

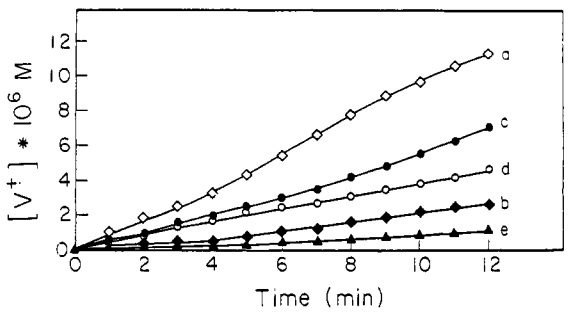

Figure 1. Rate of $C_8V^{\bullet+}$ and $MV^{\bullet+}$ formation at time intervals of illumination in the presence of TiO_2–β-CD colloid. All experiments include $[C_8V^{2+}]$ or $[MV^{2+}] = 5 \times 10^{-5}$ M, 2 mL of TiO_2, 2 g·L^{-1}, stabilized by 1% β-CD: (a) $C_8V^{\bullet+}$ formation, (b) $MV^{\bullet+}$ formation, (c) rate of $C_8V^{\bullet+}$ formation, with [phenol] = 0.018 M, (d) $C_8V^{\bullet+}$ formation with [phenol] = 0.024 M, (e) $C_8V^{\bullet+}$ formation, with [phenol] = 0.05 M.

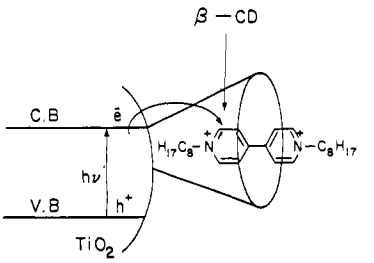

Figure 2. Schematic function of the receptor–semiconductor colloid in improving interfacial electron transfer.

Cyclodextrins have been extensively examined as molecular receptors that bind solutes to their hydrophobic cavities[11-12] Thus, the tailored semiconductor-receptor configuration allows the association of solutes to the β-CD cavity and consequently improves the interfacial electron transfer process at the semiconductor-solution interface.

We have stabilized CdS and TiO_2 colloids in aqueous solutions with β-CD. The CdS colloid has been prepared by slow addition of $Cd(NO_3)_2$ into the solution of 1% β-CD + Na_2S. The TiO_2 colloid was prepared by the slow addition of $TiCl_4$ to the β-CD solution at 0 °C. The mean diameter of the CdS and TiO_2 particles was determined by TEM to be 80 and 100 Å, respectively. In the absence of β-cyclodextrin the semiconductors precipitate out of solution.

N,*N*′-Dioctyl-4,4′-bipyridinium (octyl viologen, C_8V^{2+}) associates with the β-CD cavity[13] (eq 1), $K_{ass} = 5.6 \times 10^3$ M^{-1}. Illumination of the TiO_2–β-CD colloid ($\lambda > 335$ nm), pH 2.5, in the presence of C_8V^{2+}, 5×10^{-5} M, and 2-propanol, 5×10^{-3} M, as electron donor results in the formation of $C_8V^{\bullet+}$ in its monomer form. Figure 1a shows the rate of $C_8V^{\bullet+}$ formation at time intervals of illumination that corresponds to a quantum yield of $\phi = 1.1 \times 10^{-3}$.[14] Similarly, illumination of the CdS–β-CD colloid

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