Revised assignment of energy storage in the primary photochemical event in bacteriorhodopsin

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angular dependence for electron transfer. If so, a quite different pattern of reactivity might be anticipated for systems with different nodal structures. For example, porphyrin radicals have different nodal planes than do the corresponding excited-state systems. We are currently designing experiments to test the effects of such electron redistribution in an intramolecular electron transfer rate.

In summary, we have prepared a series of bis-porphyrin molecules in which the angle between the donor and acceptor porphyrins is systematically varied. In these molecules, the intramolecular electron transfer rate varies by over 1 order of magnitude as the angle changes. This variance is anticipated by symmetry arguments which focus on the nodal properties of the molecules.[3]

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Revised Assignment of Energy Storage in the Primary Photochemical Event in Bacteriorhodopsin

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Bacteriorhodopsin is the light-transducing protein in the purple membrane of *Halobacterium halobium*.[1-5] Irradiation of the light-adapted form (bR) initiates a photocycle that pumps protons across the membrane. An accurate assignment of the energy storage associated with the primary event of bR is important to an understanding of the molecular mechanism and the stoichiometry of proton pumping.[4-6] Previous photocalorimetric studies have concluded that ~16 kcal mol^{-1} is stored in the K photoproduct, an energy sufficient to pump two protons per photocycle.[6-8] However, this enthalpy (ΔH_{12}) was assigned assuming that the forward (Φ_1) and reverse (Φ_2) quantum yields associated with the bR $\rightleftarrows$ K photoreaction are $\Phi_1 = 0.33$ and $\Phi_2 = 0.67$.[9] More recent investigations indicate that the above quantum yield values are significantly underestimated.[10-15] Recently Govindjee et al.[13] and Balashov et al.[14] reported temperature-independent quantum yields of $\Phi_1 = 0.65 \pm 0.05$ and $\Phi_2 = 0.95 \pm 0.05$. The significant revisions in Φ_1 and Φ_2 have significant implications with respect to the energy storage, and this recognition prompted our reevaluation of the photocalorimetry data. On the basis of the revised quantum yield assignments, the K photoproduct stores only 11.6 ± 3.4 kcal mol^{-1}. As noted below, the revised value of ΔH_{12} precludes a proton/photocycle stoichiometry larger than 1.

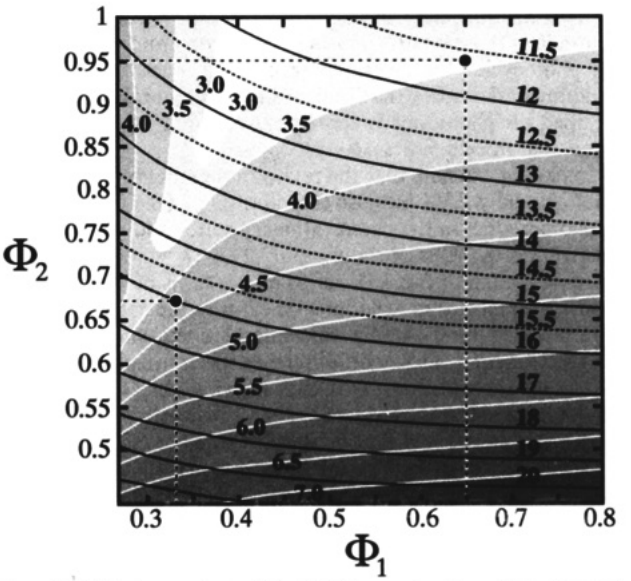

Figure 1. Enthalpy contour plot of ΔH_{12} as a function of the forward (Φ_1) and reverse (Φ_2) quantum yields associated with the photochemical interconversion of bR and K at 77 K based on experimental data from refs 6 and 7. The black contours indicate the ΔH_{12} values (enthalpies above contours on right). The white lines represent the error contours (ΔH_{12} standard deviations in gray on contours). The black dot on the left indicates the previously assigned value of energy storage ($\Delta H_{12} \cong 16$ kcal mol^{-1}; $\Phi_1 = 0.33$, $\Phi_2 = 0.67$). The black dot at upper right indicates the revised value of energy storage ($\Delta H_{12} = 11.6 \pm 3.4$ kcal mol^{-1}; $\Phi_1 = 0.65$, $\Phi_2 = 0.95$) (see text).

Weighted least-squares regression is carried out on the photocalorimetric data measured previously.[6,7] The three experiments described in refs 6 and 7 generate a set of three equations:

$$\{0.907 \pm 0.021\} = \{\alpha_{565}^{>620}(1 - \Phi_1\Delta H_{12}/50.6)\} + \{(1 - \alpha_{565}^{>620})(1 + \Phi_2\Delta H_{12}/50.6)\} \quad (1)$$

$$\{1.294 \pm 0.033\} = \{\alpha_{699}^{500}(1 - \Phi_1\Delta H_{12}/40.9)\} + \{(1 - \alpha_{699}^{500})(1 + \Phi_2\Delta H_{12}/40.9)\} \quad (2)$$

$$\{1.201 \pm 0.024\} = \{\alpha_{643}^{500}(1 - \Phi_1\Delta H_{12}/44.5)\} + \{(1 - \alpha_{643}^{300})(1 + \Phi_2\Delta H_{12}/44.5)\} \quad (3)$$

where numbers in italics are in kcal mol^{-1}. Values for the three photochemical partition functions $\alpha_{565}^{>620}$, α_{699}^{500}, and α_{643}^{300} are dependent upon Φ_1 and Φ_2 and are assigned on the basis of the spectroscopic data of ref 6. The three equations are not truly independent,[6] and a least-squares regression analysis can generate best fit values for only two variables as a function of one of the three variables Φ_1, Φ_2, and ΔH_{12}. In our previous studies, we arbitrarily chose Φ_1 to be the independent variable and assigned Φ_2 and ΔH_{12} as a function of Φ_1. However, when values of Φ_1 exceed ~0.5, the regression analysis incorrectly predicts values of Φ_2 that exceed unity (e.g., Table III of ref 6). This problem suggests that least-squares regression, in the absence of additional constraints, cannot provide an accurate assignment of ΔH_{12} for values of Φ_1 exceeding ~0.5. We conclude further that our photocalorimetry data cannot assign Φ_1/Φ_2 with confidence. The problem can be corrected by treating both Φ_1 and Φ_2 as independent variables and carrying out a weighted least-squares regression to assign ΔH_{12}. The results are presented in Figure 1.

Evaluation of the results shown in Figure 1 indicates that the primary event stores $\Delta H_{12} = 11.6 \pm 3.4$ kcal mol^{-1} ($\Phi_1 = 0.65$; $\Phi_2 = 0.95$), ~4.4 kcal mol^{-1} less than our previous assignment of ~16 kcal mol^{-1}. A minimum of ~6 kcal mol^{-1} of energy storage is required to pump a proton under ambient conditions.[6] When entropic contributions are included, the revised value of

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ΔH_{12} is not sufficient to pump two protons.[6] This observation contradicts those reports indicating that two protons are pumped per photocycle. However, our results are consistent with those experimental studies that indicate that the number of protons pumped per photocycle is approximately equal to $\sim 0.6/\Phi_1$ (see discussion in refs 5 and 16-19).

In closing, we note that the revised assignments for Φ_1 and Φ_2 affect not only ΔH_{12} but also the value of χ_K (the fraction of the K photoproduct in the photostationary state) and the calculated spectrum of K. Our previous value of χ_K (λ = 500 nm) of 0.46 increases to 0.53 (Table II, ref 6) on the basis of $\Phi_1/\Phi_2 = 0.68$, which is equal within experimental error to the 510-nm value of 0.56 reported in ref 14. A recalculated K spectrum based on our bR spectrum and our K - bR difference spectrum assuming $\Phi_1/\Phi_2 = 0.68$ is nearly identical with that shown in ref 14. This means that our raw spectroscopic data are consistent with those presented in ref 14, and this observation supports the use of our raw spectroscopic data to assign the partition functions that appear in eqs 1-3 as a function of Φ_1 and Φ_2. The fact that our photocalorimetry data are more consistent with a ratio of $\Phi_1/\Phi_2 = 0.45$ versus the revised value of $\Phi_1/\Phi_2 = 0.68$ is reflected in our least-squares regression by a $\sim$1.5-fold percentage increase in the standard deviation for our revised enthalpy (11.6 ± 3.4 (29%) kcal mol^{-1}) relative to the standard deviation associated with our previous assignment (15.9 ± 3.2 (20%) kcal mol^{-1}).

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Registry No. Hydrogen ion, 12408-02-5.

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New Strategy for the Synthesis of Oligodeoxynucleotides Bearing Adducts at Exocyclic Amino Sites of Purine Nucleosides

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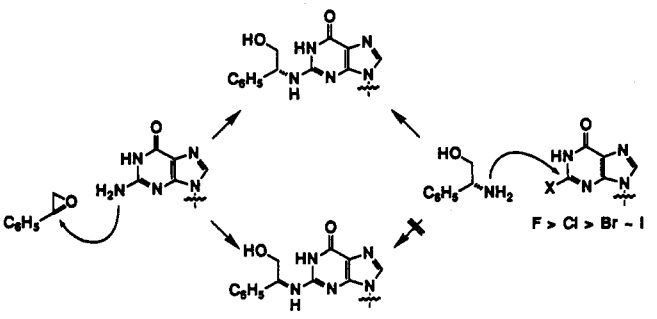
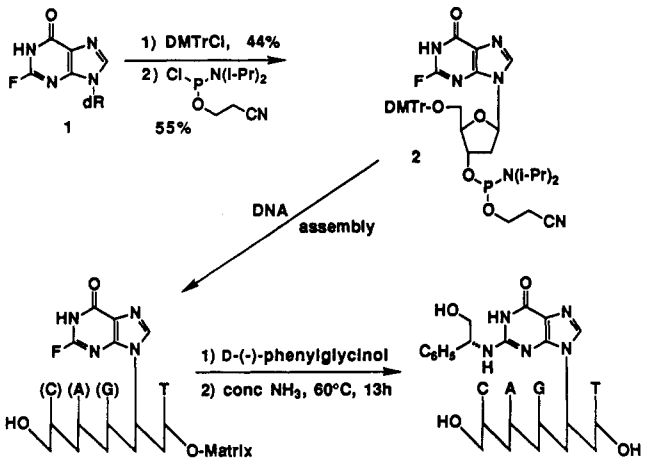
An understanding of the structure and conformation of nucleic acid-mutagen adducts is essential to the elucidation of events involved in chemical carcinogenesis. Conformations can be established by NMR spectroscopy[1] and X-ray crystallography[2] using oligonucleotides containing structurally defined adducts; these oligomers can also be used for site-specific mutagenesis studies.[3] Oligodeoxynucleotides bearing specifically linked carcinogens have been prepared by enzymatic or chemical assembly of the oligomer using an adducted nucleoside,[4] adduction of an oligomer containing only one reactive site,[5] or chromatographic separation of the mixture resulting from reaction of a mutagen with several sites in an oligomer.[6] Herein we report a novel postoligomerization strategy that provides complete regiochemical and stereochemical control of adduction. In this method the natural polarity of reaction, i.e., with the heterocyclic base as the nucleophilic species and the adducting moiety as electrophile, is reversed. Thus, an amino derivative of the mutagen is used to displace halogen from the appropriate halo-substituted heterocyclic species. Modified deoxynucleotides have been prepared previously by such strategies,[7] but their conversion to oligodeoxynucleotides poses formidable problems if the adduct contains reactive functional groups which must be protected during oligomer synthesis.

The key to the present method is that the displacement reaction is carried out while the oligomer is still attached to the solid support.[8] Styrene adducts at guanine N^2 and adenine N^6 were chosen for the initial demonstration of this strategy. Styrene is metabolically oxidized to the epoxide, which is mutagenic and carcinogenic.[9] Reaction of the epoxide with DNA occurs at a variety of sites, involving both ends of the epoxide and with varied stereochemical results.[10] Reaction at guanine N^2 occurs solely at the α carbon of styrene oxide but is not stereospecific; deoxyadenosine adducts have not yet been observed but may be present as minor products.

Scheme I



Scheme II



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