Enthalpy−Entropy Compensation in a Photocycle: The K-to-L Transition in Sensory Rhodopsin II from *Natronobacterium pharaonis*

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The *cis*−*trans* double bond chromophore photoisomerization in biological photosensors triggers a cascade of reactions involving conformational changes in chromophore and protein responsible for the specific function of each sensor.[1,2] In archaeal retinal proteins (bacteriorhodopsin, BR, as well as the sensors SRI and SRII) an early, red-shifted intermediate (K in BR and K-like in the sensory rhodopsins) is formed in sub-ns which proceeds to the L intermediate in μs.[3] Although their major conformational changes occur in the ms to s time, all retinal proteins exhibit movements accompanying the early steps of the respective photocycle. These movements, detected as structural volume changes (ΔV) by laser-induced opto-acoustic spectroscopy (LIOAS),[4] reflect the response of the protein upon chromophore photoisomerization and indicate how the sub-ns conformational changes drive the functional process.[5−8] We have reported enthalpy changes, ΔH, and ΔV values for the formation and decay of K_{510} in purified wild-type sensory rhodopsin II from *Natronobacterium pharaonis* (pSRII-WT) and its histidine-tagged analogue (pSRII-His) in a variety of aqueous media (various ionic strengths, detergent, and lipids in different concentrations).[5] The large expansion (10 mL/mol) upon K_{510} formation is restricted to the chromophore−protein cavity displaying no medium influence, although the K_{510} energy level is affected by the medium.[5]

With the study of pSRII-His assembled with its C-terminally truncated transducer, 1−159-HtrII = trHtrII (pSRII-trHtrII, prepared as already published[9]), reported here, we complete the characterization of this sensory rhodopsin. The linear correlation found between ΔH_{KL} and ΔV_{KL} for the $K_{510} \rightarrow$ L decay in all samples (including pSRII-trHtrII) is attributed to an enthalpy−entropy compensation and affords the value of the intrinsic ΔG_{KL} for the μs step, not accessible by other methods.

Experimental conditions were as described.[5] The narrow temperature ranges used with samples **1**, **2**, **4**, and **5** (see caption to Figure 1) allow to consider that ΔH and ΔS during the transition under scrutiny are constant over the range for each of the media. Data obtained with the two-temperatures method (at two close temperatures)[5] were very similar to those from the several-temperatures method. The LIOAS signal for each sample was deconvoluted with that for a calorimetric reference (bromocresol green) in the same medium.[5] The amplitudes, φ_1 and φ_2, of the biexponential pressure evolution [$\varphi_i = q_i/E_\lambda + \Delta V_i\, \Phi_i/E_\lambda$ ($c_p\, \rho/\beta$), q_i: total heat emitted during each step] plotted vs ($c_p\, \rho/\beta$) (c_p: heat capacity, ρ: mass density, β: cubic expansion coefficient) afforded, respectively, the ΔH_K and ΔV_K values for K_{510} formation and ΔH_{KL} and ΔV_{KL} for its decay to L. The $\Delta H_K = E_K$ (the energy level of K_{510}) values were calculated with the equation: ($q_1/E_\lambda = 1 - \Phi_K\, E_K/E_\lambda$), E_λ the molar laser energy, and Φ_K the quantum yield for K_{510} formation.[5] $\Delta H_{KL} = -\, q_2/\Phi_K$, under the assumption that the $K_{510} \rightarrow$ L efficiency is unity. $\Phi_K = 0.4$ for pSRII-trHtrII was determined as described for the transducer-free samples.[5] A detailed analysis of the $K_{510} \rightarrow$ L decay in pSRII under all conditions[5] (including the data for the pSRII-trHtrII sample), shows that ΔH_{KL} linearly correlates with ΔV_{KL} (Figure 1), that is, $\Delta H_{KL} = C + X\, \Delta V_{KL}$.

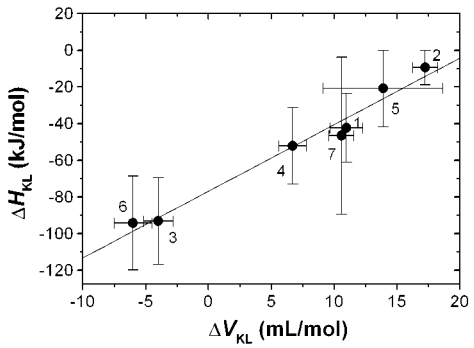

Figure 1. ΔH_{KL} vs ΔV_{KL}, for the $K_{510} \rightarrow$ L process for: **1**: pSRII-WT, 50 mM NaCl, **2**: pSRII-WT, 150 mM NaCl, **3**: pSRII-WT, purple membrane lipids (PML) 20:1, **4**: pSRII-His (His tagged for purification purposes), *n*-dodecyl-β-D-maltoside (DM), 10 mM NaCl, **5**: pSRII-His, DM, 150 mM NaCl, **6**: pSRII-His, PML 20:1, **7**: pSRII-trHtrII, PML 20:1. All samples in phosphate buffer 25 mM. Excitation was with an 8-ns laser pulse at 500 nm. The several-temperatures method was applied with samples **1**, **2**, **4**, and **5** (no transducer) in the range 11−56 °C and with **7** (pSRII-trHtrII) in the range 4−30 °C. The results from the two-temperatures method were used for **3** and **6**.[5]

A linear correlation was previously found between ΔH_i and ΔV_i for the formation and the decay of transient species upon photoinduced intra- and intermolecular electron-transfer reactions in aqueous solutions in the presence of various monovalent salts.[10] The linear correlation was the result of an enthalpy−entropy compensation reflecting the fact that in those reactions both enthalpy and entropy changes were strongly dominated by the interactions of the chromophore with the solvent.[11] In both cases, the spectra of ground and intermediate states were unaffected by the added salts. The free energy of the monitored reaction was in both cases constant along the salt series.

In the present case, the spectra of the ground state is affected neither by the variation of the media for pSRII (WT and his-tagged) nor by the presence of the ligated transducer in pSRII-trHtrII. The spectra of K_{510} (and those of L) are identical for samples **1**, **2**, **4**, **5** (unpublished), **3**,[12] **6**,[13] and **7**[9] (see Figure 1

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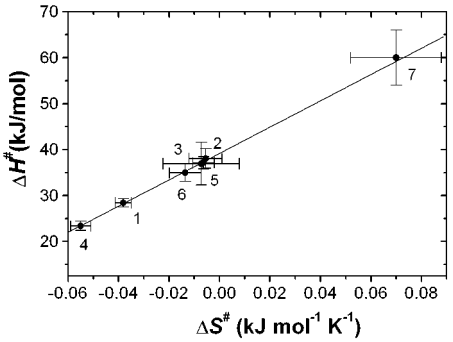

Figure 2. Eyring activation parameters for the $K_{510} \rightarrow L$ decay, derived from the *T*-dependence of the μs decay determined by LIOAS. *T* ranges: 11−56 °C for **1**−**6**,[5] 4−30 °C for **7**. Numbering as in Figure 1.

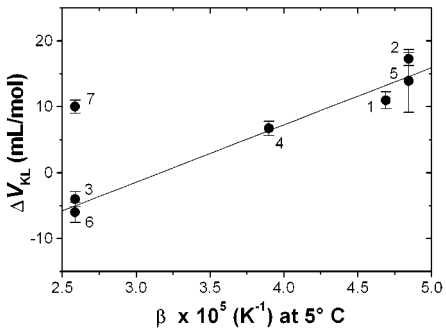

Figure 3. ΔV_{KL} vs β for the pSRII samples (numbering as in Figure 1). The β values were calculated with the measured ($c_p\rho/\beta$) and ρ (all at 5 °C), and with a constant $c_p = c_p$ for H_2O. The line through the points is a linear regression and has no physical meaning.

caption for the samples numbering). Furthermore, the lifetime of K_{510} at 25 °C, $\tau_K = (1.3 \pm 0.2)$ μs, is the same for all samples (including **7**) without any recognizable trend. These observations strongly indicate a constant free energy for the $K_{510} \rightarrow L$ reaction, ΔG_{KL}, along the mild media variation. Therefore, again in this case it is possible to use Gibbs eq ($\Delta H_{KL} = \Delta G_{KL} + T\Delta S_{KL}$) to calculate $\Delta G_{KL} = -(77 \pm 3)$ kJ/mol, directly from the intercept in Figure 1 (i.e., at $\Delta V_{KL} = 0$). The fact that the values for pSRII-trHtrII fall into the same correlation as those for the transducer-free samples (Figure 1, within the experimental error with **7**) indicates that the chromoprotein−transducer interaction is similar in nature to the chromoprotein−solvent interaction.

From Figure 1 and Gibbs eq, $X\ \Delta V_{KL} = T\Delta S_{KL}$. The slope in Figure 1, $X = (3.8 \pm 0.2)$ kJ/ml, is smaller than those previously measured in our laboratory: (12 ± 1) and (14.4 ± 0.8) kJ/ml for intra- and intermolecular electron-transfer reactions, respectively.[10,11] Obviously, the equilibrium shifted by the various media (detergent, diverse ionic strengths, membranes) in the present case is of a different nature than that in homogeneous aqueous solutions. Notwithstanding the errors, a compensation effect is found also between the activation parameters for K_{510} decay in the seven samples (Figure 2).

We attribute the enthalpy−entropy compensation effect in pSRII to subtle changes induced by the various environments (including the transducer in pSRII-trHtrII) in the equilibrium between fluctuating structures (substates) linked to the various possible chromophore conformations,[14] most likely resulting in different number and strength of hydrogen bonds and salt bridges. The type of states participating in the $K_{510} \rightarrow L$ transition depends on the medium, changing the magnitude of ΔH_{KL} and ΔS_{KL}, whereas the value of ΔG_{KL} is constant because it is intrinsic to the reaction.

The proportionality between ΔV_{KL} and the respective β values for transducer-free pSRII in all media at the same temperature (Figure 3) supports the above interpretation. It has been postulated that β correlates with the so-called compensating term (ΔV_{KL} in our case) for a system subjected to fluctuations. The example used was of a protein undergoing a particular reaction (e.g., the $K_{510} \rightarrow L$ transition) and subjected to fluctuating boundaries (in our case due to various protein substates) of the cavity where the reaction takes place.[14,15] A possible reason the value for pSRII-trHtrII falls out of the line in Figure 3 is that the substates participating in the $K_{510} \rightarrow L$ transition for this complex are different than for free pSRII.

The enthalpy content of K_{510} and of L for pSRII-trHtrII is (78 $\pm$ 18) and (32 $\pm$ 22) kJ/mol, respectively, the latter corresponding to 15% of the exciting energy (239.17 kJ/mol). This low value underlines the importance of entropic changes as driving force for the subsequent steps in the cycle. The large entropic contribution, for example, $T\Delta S_{KL}$ (31 kJ/mol) ~40% of ΔG_{KL} for pSRII-trHtrII (**7**) shows the weight of the reorganization of the environment during the $K_{510} \rightarrow L$ transition. Using eq 1 with $X = 3.8$ kJ/ml, for the $K_{590} \rightarrow L$ transition in BR with the measured $\Delta V_{KL} = -5.2$ mL/mol[16] (with $\Phi_K = 0.65$),[3] affords a $T\Delta S_{KL} = -20$ kJ/mol, in excellent agreement with the value previously calculated.[17] The nature of the interactions responsible for the compensation effect should then be similar for both proteins during the $K \rightarrow L$ step.

The constant $\Delta V_K = (10 \pm 1)$ mL/mol for samples **1**−**6** (no transducer) and the variable E_K point to energy changes intrinsic to K_{510} structure, not affecting its volume,[5] and documents the fact that the volume changes accompanying K_{510} formation are restricted to the chromophore protein cavity.[18] $\Delta H_K = (78 \pm 18)$ kJ/mol and $\Delta V_K = (12.5 \pm 1)$ mL/mol for pSRII-trHtrII are different from the values for pSRII−WT evidencing that already during K_{510} formation both properties are affected by the transducer.

The present is the first report of a direct determination of a time-resolved free energy change in a photoinduced relatively rapid reaction in photosensors. The data demonstrate how mild changes in the environment modify the thermodynamic profile of transient species decay, as long as the medium has a strong influence on it.

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