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Effect of ionic strength on intra-protein electron transfer reactions: The case study of charge recombination within the bacterial reaction center

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ABSTRACT

It is a common believe that intra-protein electron transfer (ET) involving reactants and products that are overall electroneutral are not influenced by the ions of the surrounding solution. The results presented here show an electrostatic coupling between the ionic atmosphere surrounding a membrane protein (the reaction center (RC) from the photosynthetic bacterium *Rhodobacter sphaeroides*) and two very different intra-protein ET processes taking place within it. Specifically we have studied the effect of salt concentration on: i) the *kinetics* of the charge recombination between the reduced primary quinone acceptor Q_A^- and the primary photoxidized donor P^+; ii) the *thermodynamic equilibrium* ($Q_A^- \leftrightarrow Q_B^-$) for the ET between Q_A^- and the secondary quinone acceptor Q_B. A distinctive point of this investigation is that reactants and products are overall electroneutral. The protein electrostatics has been described adopting the lowest level of complexity sufficient to grasp the experimental phenomenology and the impact of salt on the relative free energy level of reactants and products has been evaluated according to suitable thermodynamic cycles. The ionic strength effect was found to be independent on the ion nature for $P^+Q_A^-$ charge recombination where the leading electrostatic term was the dipole moment. In the case of the $Q_A^- \leftrightarrow Q_B^-$ equilibrium, the relative stability of Q_A^- and Q_B^- was found to depend on the salt concentration in a fashion that is different for chaotropic and kosmotropic ions. In such a case both dipole moment and quadrupole moments of the RC must be considered.

1. Introduction

The presence in solution of an inert electrolyte is known to screen the electrostatic interactions between charged molecules. Such a screening is effective for distances above the screening length that is defined as the reciprocal of the Debye-Hückel (D-H) parameter κ that depends on the valence Z_i and concentration C_i of all the ions in solution according to: [1,2]

$$\kappa = \sqrt{\frac{2e^2\sum_i Z_i^2 C_i}{Dk_bT}} \quad (1)$$

where T is the absolute temperature, k_b the Boltzmann's constant, D the solvent dielectric constant, and *e* the elementary charge. According to Eq. (1), κ is proportional to the square root of the ionic strength.

Accordingly, in the case of bimolecular reactions, the presence of added salt slows-down reactions involving oppositely charged reactants and speeds-up reactions involving molecules of the same charge (the "kinetic salt effect"). Such a behaviour can be described within the formalism of the transition state assuming that the presence of electrolytes changes the activity coefficients of the reactants and of the activated complex [3].

For ions obeying the Debye-Hückel (D-H) theory, the activity coefficient of the i-th ion (γ_i) depends on κ according to:

$$\ln\gamma_i = -\frac{Z_i^2e^2}{2Dk_bT}\frac{\kappa}{1+\kappa R_i} \quad (2)$$

Where R_i is the radius of the i-th ion. Eq. (2) was the foundation of most the models rationalizing the dependence of the rate of electron transfer on ionic strength. The simplest model dates back to 1922 and

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is the Brønsted-Debye-Hückel equation often reported in the chemical kinetics textbooks to explain the "kinetic salt effect". Such a model has been subsequently refined to handle complex reactants such as proteins [4–6].

A slightly different approach was proposed by Wherland & Gray that incorporated in the Marcus theory of electron transfer (ET) the electrostatic energy of the charged reactants within the activated complex [7].

It must be emphasized that in case of zwitterions or proteins in a charge separated state, the overall charge is null ($Z_i = 0$) and the above reviewed models foretell that the reaction rate must be independent on the ionic strength.

Several improvements have been proposed to take into account, beside the interactions among net excess charges (monopoles) also interactions involving dipole moments of the proteins (see for example the references [4–6]).

The leading contribution in these models is the monopole term and the contribution of dipole moments influences mainly the reactant mutual orientation within the activated complex.

Such models have been extensively used to study electron transfer reactions between proteins and between proteins and small ligands and the study of bimolecular reaction rates as a function the ionic strength has been proposed as a tool to disentangle electrostatic and non-electrostatic contribution to the activation free energy [3]. A non-exhaustive list of examples are the electron transfer reactions between the following pairs: cytochrome c and inorganic complexes [7], self-exchange between oxidized and reduced cytochrome b_5, [8] Cytochrome c_2 and Cytochrome bc_1, [9] Cytochrome c and Cytochrome c Oxidase, [10] Photosystem 1 and Plastocyanin, [11] Plastocyanine and Cytochrome c, [12]. Also studied was the influence of ionic strength on the electron transfer from Cytochrome c2 to the photosynthetic Reaction Center [13,14].

The subject of the latter study, the Reaction Center (RC) is the membrane-bound protein responsible, in the photosynthetic bacteria, for the initial light-induced electron transfer reaction in photosynthesis [15–17].

In the RC, photon absorption promotes electron transfer from a bacteriochlorophyll dimer pair (P) acting as the primary electron donor to the primary acceptor, Q_A, a ubiquinone molecule bound at a site close to the opposite side of the complex. This primary charge separation is stabilized by electron transfer from Q_A^- to a second ubiquinone molecule, bound at the Q_B site of the RC, which is located symmetrically to the Q_A site on the same cytoplasmic side of the membrane. In vivo, the photoxidized donor, P^+, is rapidly re-reduced by a soluble cytochrome c2, so that a second charge separation can take place across the RC, leading to the double reduction and protonation of Q_B, which leaves the RC in its quinol state, QH_2 [15,16].

The RC is yet the testing ground for the understanding of biological electron transfer because, beside the above mentioned electron transfer to cytochrome c2, it is the chief-actor of several intra-protein electron transfer reactions [16–18]. It should be noted that both the photo-induced forward charge separation ($h\nu + PQ \rightarrow P^+Q^-$) and the dark charge recombination ($P^+Q^- \rightarrow PQ$) that take place under some conditions cannot be described by the models developed for bimolecular electron transfer. Indeed, these are monomolecular ET reactions taking place among fixed redox centers within the same protein. The single reactant/product is overall neutral although it can contain separated charges.

Accordingly, the possible influence of ionic strength on these intra-protein charge recombination has been one of the few aspects of the RC functioning that was not investigated in detail. Only few non-systematic investigations, by Maroti et al., somehow addressed this point almost two decades ago [19–21].

In general, the impact of the surrounding on monomolecular intra-protein ET is an hot topic and a coupling mediated through the mechanical or dielectric response of the milieu [22–26], the chemical nature of the detergents [27] or through the solvent activity [28] was proven in the case of RC.

The purpose of the present investigation is to explore the *electrostatic* coupling between the ionic atmosphere surrounding the protein RC and the charge recombination processes taking place inside it. This has been tackled by studying the rate of charge recombination as a function of the ionic strength using as added salt the chlorides of the alkali metals (first group in the Periodic Table). The use of different salts is required in order to discriminate between the orthodox electrostatic effects implicit in the standard D-H treatment where the only difference among ions with the same valence is their size and the so called ion-specific effects [29,30]. A specific influence of the non-electrostatic nature of ions is presently assessed for several aspects of protein functionality and the effectiveness of different ions can be ordered according to the series first described by Franz Hofmeister, in the last decades of XIX century, that classified salts on the basis of their ability to solubilise or precipitate a water soluble protein [31]. The impact of ion nature on ion-specific effects depends on their degree of hydration. A traditional classification divides the ions into two families: ions with a high degree of hydration are called *kosmotropic* ions while those with a low degree of hydration are said *chaotropic* ions [29].

2. Experimental

RCs from *Rhodobacter sphaeroides* were isolated and purified according to Gray et al. [32]. In all preparations, the ratio of the absorption at 280 and 800 nm was between 1.2 and 1.3. This isolation procedure provides RCs with a Q_B content of about 60%.

RC was suspended in 10 mM Tris-HCl buffer at pH = 8.00, Lauryl-Dimethyl-Amino-Oxide (LDAO) 0.025% (w/v), hereafter TL buffer.

For each salt studied, two RC buffered mother solutions have been prepared; namely, one at the maximum salt concentration (see further) and one with buffer only, both at the same RC concentration ($1.0 \le [RC] \le 1.7$ μM, depending on RC batch of purification). These two solutions represent the extremes of the concentration interval explored, the intermediate solutions being prepared by properly mixing the two. This strategy not only allowed the minimization of RC concentration differences among samples but also ensured the reproducibility of the sample preparations. The RC concentration in each sample has been checked by Vis spectroscopy being known its molar extinction coefficient at 802 nm ($\varepsilon_{802\ nm} = 0.288\ \mu M^{-1}\ cm^{-1}$), a band not influenced by the RC redox state. For the fluorescence measurements, samples were diluted to have a [RC] ≅ 0.1 μM in order to minimize self screening effects.

Chloride salts of alkali metals have been used in order to cover a wide range in kosmotropic and chaotropic ions within the Hofmeister series. The reduced KCl solubility in the presence of LDAO (4.59 M in pure water; 3.20 M in TL) constrains its maximum concentration at 3.20 M for all the other salts the maximum concentration was 4.00 M.

After the measurements, the samples at [salt] = 1.00 M, were dialyzed (3 kDa cut-off dialysis membrane) against TL buffer for about 24 h with two changes of buffer (in any case the volume ratio was 1:100) to check the reversibility of any ions' effect on the protein. The efficiency of the dialysis procedure was checked by measuring the conductivity of the dialysis buffer. The buffer after the second dialysis showed in any case conductivity values typical of the TL buffer alone.

The activity of water (a_W) of the different salts' solutions were measured at 25 °C by means of a water activity meter (PA_wkit, Decagon Devices Inc.) previously calibrated with suitable standards (tolerance ± 0.02 a_W).

UV–Vis-Near IR spectra have been acquired with a Varian Cary-1 spectrophotometer.

Light induced redox changes of the primary donor of RC were monitored by a home-built kinetic single beam spectrophotometer