Electrostatic Interactions and Reduction Potential Effects from Ionic Strength and pH Profiles in Electron Transfer Reactions of Spinach Plastocyanin

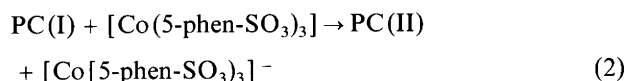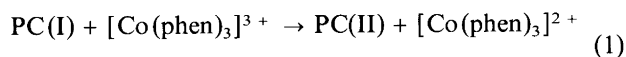Marianne V. Pedersen,[a] Inger Søtofte[b] and Jens Ulstrup[†,a]

[a] Chemistry Department A, Building 207, Technical University of Denmark, DK-2800 Lyngby, Denmark and [b] Chemistry Department B, Building 301, The Technical University of Denmark, DK-2800 Lyngby, Denmark

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We have investigated the ionic strength and pH dependence of the rate constants for electron transfer between spinach plastocyanin and the two structurally closely related tris-1,10-phenanthroline and tris-5-sulfonato-1,10-phenanthroline cobalt (III) complexes. The former complex is triply positively charged, the latter electrically neutral. By consideration of the combined electron-transfer pattern the observed ionic strength and pH variation can be resolved into reduction potential and inter-reactant interaction (work term) effects. Comparison of the latter with simple models for electrostatic interaction between cobalt(III) and charged protein surface groups shows that electron transfer between the negatively charged plastocyanin and the positively charged cobalt(III) is likely to occur at both adjacent and remote sites. This supports previous views of dual-path plastocyanin electron transfer based on other kinds of data.

Plastocyanins (PC) are a group of blue single-copper proteins (MW ≈ 10 kDa) central in the photosynthesis of plants, green algae and blue–green bacteria.[1-4] The charge distribution of most PCs is strongly asymmetric, with most of the overall negative charge accumulated in areas remote (ca. 12–18 Å) from the Cu atom.[5-7] The electron-transfer (ET) patterns of PCs towards small molecular reaction partners,[8] the natural partners cytochrome f[9-12] and photosystem I,[13-16] and in electrochemical processes[17-19] have been broadly investigated. A key issue has been dual-path ET of PC with one ET site adjacent to Cu and another, longer path along Cu/Cys84/Tyr83 through the protein, ending among the negative charges at the remote site.[6,8,20]

The asymmetric charge distribution offers an interesting probe for electrostatic interaction ('work terms') between PC and positively charged reaction partners, important to protein–surface interactions in general. Electrostatic surface probing (e.g. pH profiles[21,22]), however, reflects the combined effects of reduction potential ($E°'$) and inter-reactant changes. In this work we provide some new data which facilitate distinction, namely pH and ionic strength profiles, between the two reactions

$$PC(I) + [Co(phen)_3]^{3+} \rightarrow PC(II) + [Co(phen)_3]^{2+} \tag{1}$$

$$PC(I) + [Co(5\text{-phen-}SO_3)_3] \rightarrow PC(II) + [Co[5\text{-phen-}SO_3)_3]^- \tag{2}$$

(phen = 1,10-phenanthroline). Ionic strength and pH effects of the former incorporate both $E°'$ and work-term changes. The latter involves an electrically neutral reaction partner in which observed effects can be assigned largely to $E°'$ of PC and used to separate the combined effects in the former.

Experimental

Plastocyanin from fresh spinach leaves was isolated and purified by reported procedures.[23] The absorbance ratio was $A_{278}/A_{597} = 1.18$. The Co(III) complexes were prepared and characterized as previously.[24-27] The 5-position of the sulfonate substituent was verified crystallographically using reported methods.[28-31] Bond lengths and angles agree with those of other 1,10-phenanthrolines. Elemental analysis corresponding to [Co(5-phen-SO_3)_3]·8H_2O is: C: 44.0; N: 8.41; S: 9.64; H: 2.96. Found: C: 43.7; N: 8.49; S: 9.72; H: 4.68. Other reagents were AnalaR grade. Buffers were acetate, MES (2-N-

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† To whom correspondence should be addressed.

morpholinoethane sulfonic acid) and Tris (tris-hydroxy-methylaminomethane). All solutions were made up in Milli-Q millipore water.

Absorption spectra were recorded on a Milton Roy Spectronic 3000 diode-array spectrophotometer. Kinetic data were collected using a High-Tech SF53 stopped-flow instrument combined with OLIS4300S (On-Line-Instruments-System, Jefferson, GA) data acquisition software. The Co complexes were present in 15–50-fold excess. The temperature was 25.0°C, the ionic strength for the pH profiles, μ, was 0.1 M (NaCl), while different pH values were used in the ionic-strength investigations.

Results and discussion

Figure 1 shows pH profiles for reactions (1) and (2). The former agree with previous reports.[21,22] The latter has not been reported before. The rate constants, k_{obs}, follow

$$k_{obs} \approx k_1/(1 + K_a[H^+]) \tag{3}$$

corresponding to the fast equilibrium

$$PC(I) + H^+ \rightleftharpoons PC(I) \cdot H^+ \tag{4}$$
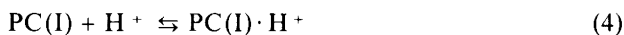
and insignificant protonated PC reaction. k_1 is the rate constant of normal, deprotonated PC; K_a is the equilibrium constant of eqn. (4). Single protonation is also the best fit when the number of protons in eqn. (4) is varied. Collective remote-site protonation is thus insignificantly reflected. pK_a is 5.18 for eqn. (1), compared to 5.27 reported,[21,22] and 4.89, i.e. smaller, for eqn. (2). This is understandable, as the former value reflects both a change in $E°'$ of PC, and Co(III) repulsion from the pro-
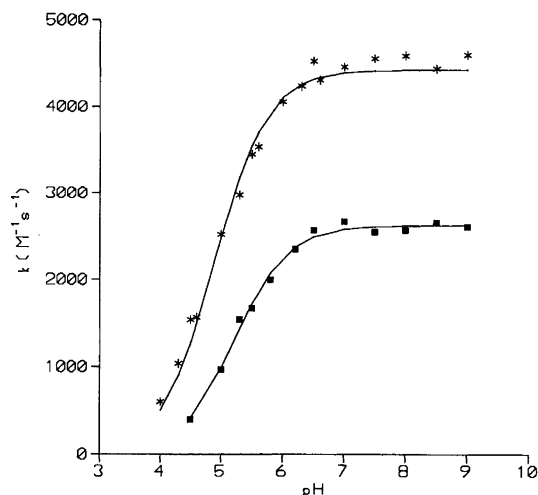
tons, while the latter solely reflects the $E°'$-effect. Figure 2 shows the ionic strength profiles at pH 7.0. At pH 4.0, close to the isoelectric point of PC (4.2), both k_1 values depend weakly on μ (not shown), while k_1 drops significantly with increasing μ at higher pH, where the protein is negatively charged.

We introduce the following form of the ratio between the rate constants of the sulfonated, k_1^{sub} [eqn. (2)] and unsulfonated, k_1^u [eqn. (1)] Co complex

$$k_1^{sub}/k_1^u \approx (\kappa_{el}^{sub}/\kappa_{el}^u) \exp(- {}^1\!/_2\Delta\Delta G°/2k_BT)$$

$$\exp[(w_r^u + w_p^u r)/2k_BT] \tag{5}$$

This form, rooted in ET theory[32] is discussed elsewhere.[11] $\Delta\Delta G°$ is the reaction Gibbs free-energy difference of the two reactions (99 mV), w_r^u is the reactants' and w_p^u the products' work terms for eqn. (1), κ_{el}^{sub} and κ_{el}^u are the electronic transmission coefficients, k_B is Boltzmann's constant and T is the temperature. The reorganization Gibbs free energy, effective vibrational frequency and reaction zone are assumed to be the same for eqns. (1) and (2), and work terms for eqn. (2) are disregarded. Eqn. (5) and the data offer the following view of the interactions on the PC surface.

Ionic strength profiles. $E°'$ of PC is 14 mV more positive at $\mu = 0.10$ M than at 0.03 M.[33] Extrapolation by a $\mu^{1/2}$-dependence closely corresponds to the observed ionic strength profile of eqn. (2) and the reduction potential variation according to eqn. (5). The ionic strength vari-
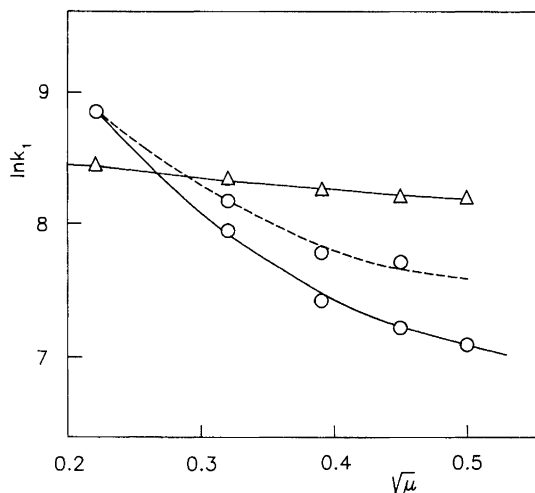

Fig. 1. pH profiles of the rate constants k_1 $(M^{-1} s^{-1})$ of reactions (1) (lower) and (2) (upper). PC concentration ≈ 5 μM. Co(III) concentrations 1.33×10^{-4} M [reaction (1)] and $(0.5–1.40) \times 10^{-4}$ M [reaction (2)]. Buffers (20 mM) were acetate (pH 4.0–5.0), MES (pH 5.3–7.0) and Tris (pH 7.5–9.0). Ionic strength 0.1 M (NaCl). 25°C.

Fig. 2. Ionic strength variation of k_1 $(M^{-1} s^{-1})$ (NaCl) for reactions (1) and (2). Circular symbols: reaction 1, pH 7.0. Triangular symbols: reaction (2), pH 7.0. The fully drawn curves show the experimental data. The dashed curve is the variation of k_{obs} for reaction (1) after correction for reduction potential variation of PC and [Co(phen)$_3$]$^{3+}$ by the data for reaction (2) in the upper curve and in Ref. 37. PC concentration ≈ 5 μM. Co(III) concentrations in the range (0.9–1.8) $\times 10^{-4}$ M [reaction (1)] and 0.66×10^{-4} M [reaction (2)]. 20 mM MES buffer (pH 7.0). 25°C.

ation of k_1 for the neutral complex is therefore entirely dominated by $E^{\circ\prime}$ of PC. The reduction potential corrected variation of k_1 with $\mu^{1/2}$ for eqn. (1) (pH 7.0) follows closely the Debye–Hückel form, i.e. ln k^{corr}_1 varies linearly with $\exp[-\kappa(r-a)]/(1+\kappa a)$, where κ is the inverse Debye length, and a and r are the average distance from $[Co(phen)_3]^{3+}$ to the nearest inert ion and to charged surface groups on PC, respectively. k^{corr}_1 here also includes ionic strength corrections for the reduction potential of $[Co(phen)_3]^{3+}$.[34] The best fit is obtained for an apparent PC charge of $-2.2\ |e|$, e being the electronic charge. $[Co(phen)_3]^{3+}$ is thus exposed to a high negative charge. As the electrostatic potential at the adjacent site is only weakly negative (cf. below) this points to the remote site as a competitive ET channel for $[Co(phen)_3]^{3+}$. The observed insignificant μ-dependence of k_1 at low pH is in line with these observations.

pH profiles. The pH profiles offer the following more precise view:

(A) The ratio k^{sub}_1/k^u_1 at pH $\geq$ 7.0 is 1.70 and increases to 2.8 at pH 4.5. The Co(III) reduction potential difference would give 7.2. The further increase of k^u_1 must be caused by electrostatic attraction between PC and $[Co(phen)_3]^{3+}$, or different transmission coefficients (ET sites) for the two Co complexes. The corresponding ratio for positively charged cytochrome c is about 100,[35] in line with strong electrostatic repulsion between this protein and $[Co(phen)_3]^{3+}$.

(B) The attractive work term combination $w^u_r + w^u_p$ calculated by eqn. (5), disregarding the transmission coefficients, varies from -4.4 to -6.9 kJ in the pH range 4.5–7.0. This can be compared with -1.8 kJ for Coulomb attraction between a triply and a single charged ion separated by 10 Å {representative of $[Co(phen)_3]^{3+}$ and a local surface group}, screened by the dielectric constant 78. The electrostatic interaction and its change on remote-site deprotonation are therefore notable.

(C) Different ET sites of the Co complexes are reflected in the transmission coefficients. The adjacent site is electronically 50 times more favourable,[20] but the remote site is still competitive for positively charged reaction partners owing to the work terms. ET of $[Co(phen)_3]^{3+}$ solely at the remote site would alter the work terms by $2k_BT\ln(\kappa^{sub}_{el}/\kappa^u_{el}) \approx 19.3$ kJ, i.e., $w^u_r + w^u_p$ would then vary from -13.8 to 16.3 kJ in the pH range 4.5–7.0

(D) The pH profiles give a ratio of 3.1 between the rate constants at pH 7.0 and 4.5 for eqn. (2), and 6.2 for eqn. (1). The $E^{\circ\prime}$ increase of PC by 37 mV in this pH range[35] would give a ratio of 2.1, leaving ratios of 1.48 and 2.95 for eqns. (2) and (1), respectively, due to other effects of protonation than $E^{\circ\prime}$ changes. The factor 1.48 can only be traced to small structural changes associated with PC protonation, included in the reorganization Gibbs free energy. This leaves 2.95/1.48 $\approx$ 2.0 or 1.8 kJ for electrostatic repulsion between the protons and $[Co(phen)_3]^{3+}$. This quantity is 21.1 kJ if protonation is accompanied by a

shift of ET location from the remote to the adjacent site, cf. paragraph (C).

(E) The work terms can be compared with the following suitable models. The potential can be calculated by the Poisson–Boltzmann equation and chosen atomic charges;[37] or the protein and aqueous solution can be represented by two dielectric regions in which the boundaries induce a manifold of electrostatic image potentials in addition to the real potentials.[38] Using either view, the electrostatic potential at the adjacent site of reduced PC is weakly negative, the adjacent site work terms not exceeding ca. 3 kJ at pH 7 while they are 10–15 kJ at the remote site.

Comparison with the work terms from the pH profiles of eqns. (1) and (2) shows that ET of $[Co(phen)_3]^{3+/2+}$ solely at either the adjacent or the remove site gives notably larger values. The comparison is 6.9 vs. ca. 3 kJ at pH 7 for ET exclusively at the adjacent site, and 21–26 vs. 10–15 kJ for remote-site ET. Parallel, dual-path ET of $[Co(phen)_3]^{3+}$ and single, adjacent site ET of $[Co(5-phen-SO_3)_3]$ is therefore likely. From the figures the adjacent/remote site distribution ratio would be ca. 3/7, but the particular value is of minor importance and would be modified by details of the models. The important conclusion is that both ionic strength and pH profiles strongly point to ET of $[Co(phen)_3]^{3+}$ with comparable facility at the two sites. This is in line with previous suggestions based on other kinds of data and reasoning. Non-local dielectric effects would shift the site distribution in favour of the remote site.

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