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Kinetics of Demetalation of a Lead Porphyrin As Influenced by DNA

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The kinetics of the demetalation of (tetrakis(1-methylpyridinium-4-yl)porphine)lead(II) (PbTMpyP) by EDTA was investigated at 25 °C as a function of concentration, pH, and ionic strength. The rate law is first order in both the lead porphyrin and EDTA with the second-order rate constant showing a pH profile consistent with two acid forms for each of the reactants being important over the pH range considered. The dependence of the rate constant on ionic strength leads to the conclusion that the "effective" charge of the metalloporphyrin is nearly +2. DNA also induces the demetalation reaction. Under the conditions of the experiments considered here, the reaction is first order, yet the rate constant for the DNA-assisted demetalation is about 10^4 larger than that for spontaneous demetalation. Adding both DNA and EDTA to PbTMpyP leads to the formation of the free-base porphyrin (H_2TMpyP) with a rate law that is first order in both [PbTMpyP] and [EDTA] and has an inverse dependence on [DNA]. The effect of added NaCl is more complicated than that observed for EDTA alone, a maximum in the rate appearing at $\mu \sim 0.3$ M. These results can be interpreted as indicating that the reaction of the multivalent anion (EDTA) with the porphyrin–nucleic acid complex proceeds primarily via a pathway in which the cationic metalloporphyrin first dissociates from the DNA surface and is then attacked by the anion while free in solution.

Introduction

Reports abound dealing with the kinetics of metalloporphyrin formation under a variety of experimental conditions.[1] Less available are results for demetalation reactions, and most of these involve acid solvolysis or transmetalation although a few examples of demetalation via redox exist.[2,3] A systematic approach to the rates and mechanisms of these processess in aqueous solution has been attempted by using synthetic meso-substituted porphyrins with charged peripheries. Early studies seemed to indicate that the rate laws for metalation/demetalation processes involve an anion term, at least for certain cationic porphyrins. However, more recent reports led to the conclusion that the rate laws for positive and negative porphyrins have the same form; i.e., $\text{rate}_I = k_I[H_2P][M^{2+}]$ for incorporation of a divalent metal ion and $\text{rate}_A = k_A[MP][H^+]^2$ for acid solvolysis.[8-14] The previously reported anion catalysis appears to be attributable primarily to non-specific ionic strength effects.

The existence of a number of relatively unstable metalloporphyrins has suggested to us still another strategy for investigating demetalation reactions. Under appropriate conditions, a chelating ligand such as EDTA might well be able to compete effectively with the porphyrin moiety for a metal ion.[32] For example, the stability constants for Pb^{2+} and Cd^{2+} with tetrakis(1-methylpyridinium-4-yl)porphine (H_2TMpyP^{4+}; see Figure 1) have been determined as

$$M^{2+} + H_2TMpyP^{4+} \rightleftharpoons M(TMpyP)^{4+} + 2H^+$$

$$K_M = \frac{[M(TMpyP)^{4+}][H^+]^2}{[M^{2+}][H_2TMpyP^{4+}]} \quad (1)$$

$K_{Pb} = 3.2 \times 10^{-8}$ M and $K_{Cd} = 2.0 \times 10^{-8}$ M at 25 °C, $\mu = 0.2$.[15] Under similar conditions the stability constants[16] for $M^{2+} + EDTA^{4-} = MEDTA^{2-}$ are 9.5×10^{15} M^{-1} for Cd^{2+} and 5.8×10^{17} M^{-1} for Pb^{2+}. Thus, for the equilibrium

$$H_2O + PbTMpyP^{4+} + HEDTA^{3-} \rightleftharpoons PbEDTA^{2-} + H_2TMpyP^{4+} + OH^- \quad (2)$$

in which hydrolysis of the lead complexes is not considered

$$K_T = \frac{[PbEDTA^{2-}][H_2TMpyP^{4+}][OH^-]}{[PbTMpyP^{4+}][HEDTA^{3-}]} \sim 9$$

and thus, the above analysis suggests that, at pH 8–9 in the presence of a large excess of $HEDTA^{3-}$, the transfer of lead(II) will be virtually complete. The present report investigates the kinetics of this transfer process.

Also considered in the present study of demetalation is the influence of DNA. The subject of interest here is the catalytic (and inhibitory) patterns demonstrated by DNA on reactions between ions and how these patterns reflect the bonding modes of ions to nucleic acids.[17] The reactions of DNA with H_2TMpyP^{4+} and some of its metal derivatives have been extensively studied.[18-21] The interactions of these porphyrin species with nucleic acids involve electrostatic binding, external (groove) binding, and/or intercalation. Spectroscopic signatures for each of these bonding modes have been determined[19,20] and estimates obtained for stability and rate constants for these interactions.[18-22] On the basis of these previous studies, we had reason to believe that the interaction of DNA with the free-base porphyrin (H_2TMpyP^{4+}) would be more profound than that with $PbTMpyP^{4+}$. Therefore, a driving force for demetalation is expected to be introduced with the addition of DNA to the lead derivative. Such is indeed the case, and the kinetics of this DNA-driven demetalation process is considered here. Finally, the effect of simultaneously adding the two demetalating reagents, EDTA and DNA, to solutions of the lead porphyrin is considered. In this manner the impact of the nucleic acid on the ligand-me-

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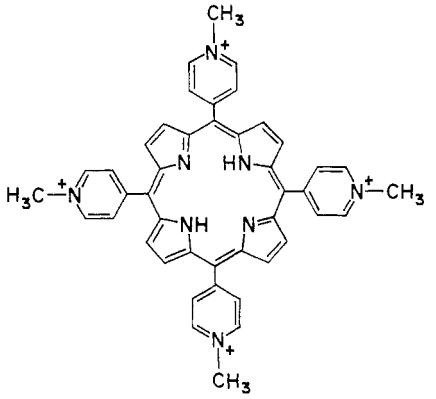

Figure 1. Structure of the free-base form of tetrakis(1-methylpyridinium-4-yl)porphine, H_2TMpyP^{4+}.

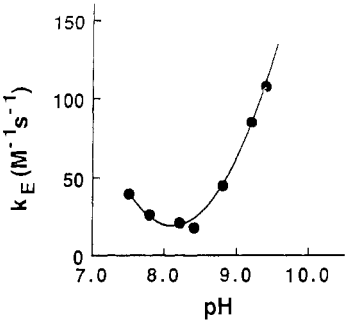

Figure 2. pH dependence of the demetalation of PbTMpyP by EDTA. The second-order rate constants ($k_E = k_{expt}/[EDTA]$) were obtained at 25 °C, μ = 0.1 and [EDTA] = 5.00 mM, λ = 475 nm. The solid curve shown is obtained from eq 5 with k_1 = 55 M^{-1} s^{-1}, k_2 = 900 M^{-1} s^{-1}, k_3 ~ 0 M^{-1} s^{-1}, k_4 = 1000 M^{-1} s^{-1}, and pK'_a = 7.7.

diated demetalation step can be considered with respect to the predictions of polyelectrolyte theory.[23]

Experimental Section

The lead(II) derivative of tetrakis(1-methylpyridinium-4-yl)porphine (PbTMpyP) was prepared as the perchlorate as previously reported.[24] Concentrations were determined spectrophotometrically by using ϵ = 1.5 × 10^5 M^{-1} cm^{-1} at the Soret maximum of 475 nm.[15] TAPS (3-[tris(hydroxymethyl)methylamino]-1-propanesulfonic acid) was obtained from Aldrich Chemical Co.; ethylenediaminetetraacetate in its disodium form and calf thymus DNA were purchased from Sigma Chemical Co. The DNA was purified as previously described,[19] and the integrity of the nucleic acid duplex was determined by comparing the UV and circular dichroism spectra with previously published results.[25] Concentrations of DNA are expressed in base pairs/L determined spectrophotometrically[25] by using ϵ^{260} = 1.31 × 10^4 M^{-1} cm^{-1}. All other chemicals were reagent grade as obtained from Fisher Scientific and were used without further purification.

Spectral and kinetic measurements were carried out on a Varian 2200 UV/vis spectrophotometer; circular dichroism spectra were taken on an Aviv 60DS spectrometer, and rapid kinetic measurements were performed with a Nortech stopped-flow apparatus interfaced to an IBM XT microcomputer. The stopped-flow operating system was designed by Jon Choi of this department.

Results

The kinetics of demetalation of PbTMpyP by EDTA to form the free-base porphyrin H_2TMpyP^{4+} show a complicated pH dependence (cf. Figure 2). In this presentation the uncharged symbols PbTMpyP and EDTA are used to represent the sum total of all the various acid/base forms of these species that may exist in solution under the specified conditions. At low pH (7.5–8.5) a monophasic kinetic profile is obtained for which the rate constants show a modest decrease with increasing pH [k_E (≡k_{expt}/

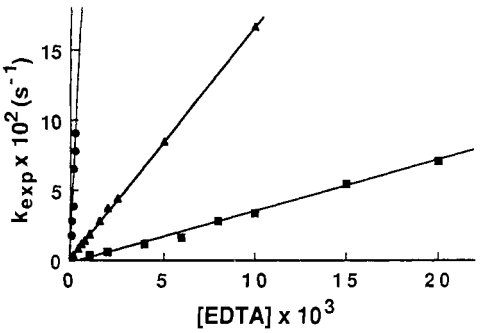

Figure 3. Experimental rate constants for the demetalation of PbTMpyP as a function of [EDTA]: (●) μ = 0.01 M; (▲) μ = 0.1 M; (■) μ = 0.5 M. Conditions: $[PbTMpyP]_0$ = 5–9 μM, pH 8.4, 25 °C, λ = 475 nm.

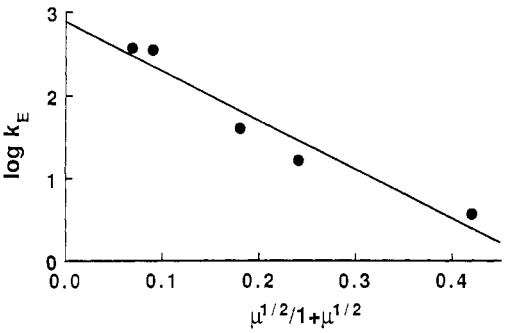

Figure 4. Plot of log k_E (=k_{expt}/[EDTA]) vs $\mu^{1/2}/(1 + \mu^{1/2})$ at 25 °C, pH 8.4. The slope of the line is −6.1 ± 0.8 and the intercept is 2.9 ± 0.2.

[EDTA], vide infra) = 39 M^{-1} s^{-1} at pH 7.5, 26 M^{-1} s^{-1} at pH 7.8, 20 M^{-1} s^{-1} at pH 8.2, and 19 M^{-1} s^{-1} at pH 8.4]. As the pH is increased beyond 8.5, the kinetic profiles become biphasic with the faster effect comprising about 80% of the total color changes observed at 475 nm. Whereas the rate constant for the small, slow effect shows no pH dependence, the larger, faster effect increases markedly, going from 45 M^{-1} s^{-1} at pH 8.8 to 107 M^{-1} s^{-1} at pH 9.5. We selected pH 8.4, with 10 mM TAPS used as buffer, for most of our studies. Under these conditions less than 1% of the metalloporphyrin is spontaneously demetalated at equilibrium.[15]

The EDTA-assisted removal of Pb(II) from the porphyrin was studied as a function of EDTA concentration and ionic strength at pH 8.4, 25 °C. The kinetics were monitored routinely at 475 nm, the Soret maximum of the metalloporphyrin. Occasionally, the reaction was observed also at 422 nm, the Soret maximum of the free-base porphyrin; excellent agreement was obtained between the rate constants at the two wavelengths. Whereas both AuTMpyP[21,26,27] and PdTMpyP[28] have been reported to undergo photochemical reductions in the presence of EDTA, no such process was observed for PbTMpyP, perhaps because of the rapid demetalation brought about by the diamine. The half-life for the photochemically induced reduction of AuTMpyP by 2 mM NADH at pH 7 is, for example, on the order of minutes.[27] The reactions studied here proved to be monophasic over at least 3 half-lives and to be first order in lead porphyrin and first order in EDTA, which was present in large excess (cf. Figure 3). As may be seen from Figure 3, the rate constant for the reaction shows a marked ionic strength dependence; a summary of the results is shown in Table Ia. A plot of log k vs $\mu^{1/2}/(1 + \mu^{1/2})$ for these data is shown in Figure 4, yielding a slope of −6.1 ± 0.8 and an intercept of 2.9 ± 0.2.

The demetalation reaction was also considered in the absence of EDTA but with calf thymus DNA added. When PbTMpyP is mixed with DNA, a very rapid color change occurs as the Soret

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Table I. Rate Constants for Demetalation of PbTMPyP at 25 °C

(a) Varying EDTA, No DNA, pH 8.4

μ, M	k_E, M^{-1} s^{-1}	μ, M	k_E, M^{-1} s^{-1}
0.0050	356 ± 21	0.10	16.7 ± 0.1
0.010	343 ± 18	0.50	3.68 ± 0.1
0.050	40.1 ± 2.7		

(b) No EDTA, Varying DNA, μ = 0.1 M

10^4[DNA]$_0$, M	pH	10^4k_D, s^{-1}	10^4[DNA]$_0$, M	pH	10^4k_D, s^{-1}
1.85	9.0	0.68	3.70	8.4	1.5
2.15	8.5	4.2	1.85	8.0	3.7
0.924	8.4	2.0	2.15	8.0	5.6
1.85	8.4	1.4			

(c) Varying EDTA, 0.200 mM DNA, pH 8.4

μ, M	k_{ED}, M^{-1} s^{-1}	μ, M	k_{ED}, M^{-1} s^{-1}
0.050	0.0760 ± 0.0046	0.40	4.22 ± 0.14
0.10	0.650 ± 0.013	0.50	3.52 ± 0.01
0.25	2.90 ± 0.070	1.0	2.15 ± 0.03
0.30	4.65 ± 0.16		

(d) 2.50 mM EDTA, Varying DNA, pH 8.4

μ, M	10^4[DNA]$_0$, M	k_{ED}, M^{-1} s^{-1}	10^4[DNA]$_0$, M	k_{ED}, M^{-1} s^{-1}
0.10	1.00	2.83	5.15	0.250
	2.00	0.650	7.48	0.168
	4.06	0.278		
0.25	1.00	13.7[a]	1.00	13.7
	2.00	6.56[a]	3.55	2.86
	3.71	2.45[a]	4.50	2.10
0.50	1.00	5.00	4.50	3.30
	3.55	3.42	7.48	2.55

[a] [EDTA] = 1.00×10^{-3} M.

band of the metalloporphyrin shifts from 475 to 480 nm with little hypochromicity. Spectral changes of this magnitude are characteristic of externally (groove) bonded metalloporphyrins.[19] However, this PbTMpyP·DNA complex is not stable, and in a much slower step, the Soret band at 480 nm decreases as one at 430 nm develops with the appearance of three isosbestic points between 380 and 520 nm (Figure 5). Previous work has shown that at μ = 0.1 the Soret band of the H_2TMpyP·DNA complex is at 430 nm,[19,22] consistent with the suggestion that the slow process being observed here is for the demetalation of PbTMpyP. Furthermore, the CD spectrum of the product matches that obtained for H_2TMpyP·DNA under similar conditions.[19] Any redistribution of the resultant free-base porphyrin on DNA subsequent to demetalation has been previously shown to be very rapid.[20] The results of these demetalation experiments in the presence of DNA are given in Table Ib.

Finally, several series of experiments were conducted in which the demetalation reaction was studied in the presence of both DNA and EDTA (cf. parts c and d of Table I). In qualitative terms, the reaction to remove Pb(II) from the porphyrin moiety is faster than in the presence of DNA alone but slower than when EDTA but not DNA is present. The rate law is first-order in [PbTMpyP] and [EDTA], shows an inverse dependence on [DNA] and depends upon ionic strength in a complicated fashion (Table Ic,d and Figure 6). Linear plots such as the one shown in Figure 6 were obtained for each of the ionic strengths considered with the intercepts near or at zero (μ = 0.10, −0.28 ± 0.26; μ = 0.25, −0.05 ± 0.05; μ = 0.50, 0.18 ± 0.01) and slopes of (8.6 ± 0.6) × 10^3 s at μ = 0.10, (1.2 ● 0.2) × 10^3 s at μ = 0.25, and (2.9 ± 0.2) × 10^2 s at μ = 0.50.

Discussion

The demetalation of the lead(II) porphyrin by EDTA is first order in both reagents as indicated by the data plotted in Figure 3:

$$\text{rate} = k_{\text{exptl}}[\text{PbTMpyP}][\text{EDTA}] \qquad (3)$$

The pH range over which the reaction could be studied is limited

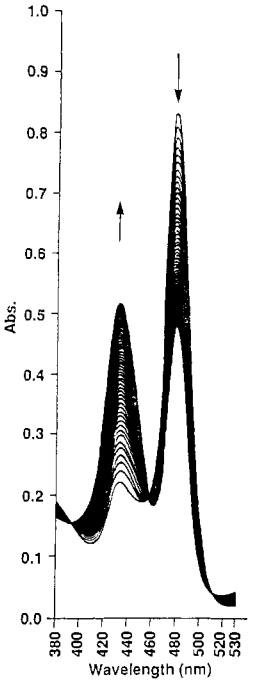

Figure 5. Demetalation of PbTMpyP in the presence of DNA. Conditions: [PbTMpyP]$_0$ = 6.6 μM, [DNA] = 185 μM, 10 mM TAPS, pH 8.4, μ = 0.1 M, 25 °C. The first spectrum was run 2 min after mixing, and spectra were run thereafter at 5-min intervals for several hours.

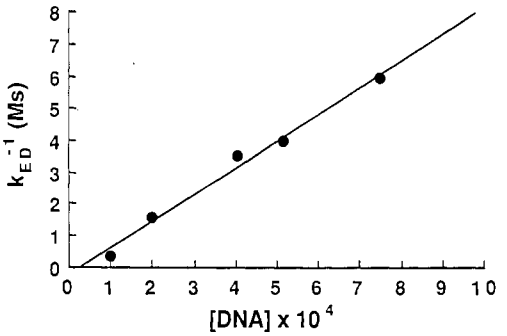

Figure 6. Plot of $1/k_{ED}$ vs [DNA] at [EDTA] = 2.5 mM, 10 mM TAPS, pH 8.4, μ = 0.1 M, and 25 °C.

by competition with the acid solvolysis pathway for this metalloporphyrin.[15] The instability of this porphyrin species has also precluded spectrophotometric titrations to determine its acidity, i.e.

$$\text{PbTMpyP}^{4+} \overset{K'_a}{\rightleftharpoons} \text{H}^+ + \text{(OH)PbTMpyP}^{3+}$$

However, the nature of the pH profile for the demetalation reaction (cf. Figure 2) suggests that at least two acid/base equilibria affect the kinetic process. We suggest the following series of steps to account for the hydrogen ion dependence of k_{exptl}:

$$\begin{aligned} \text{PbTMpyP}^{4+} + \text{HEDTA}^{3-} &\xrightarrow{k_1} \\ \text{(OH)PbTMpyP}^{3+} + \text{EDTA}^{4-} &\xrightarrow{k_2} \\ \text{(OH)PbTMpyP}^{3+} + \text{HEDTA}^{3-} &\xrightarrow{k_3} \\ \text{PbTMpyP}^{4+} + \text{EDTA}^{4-} &\xrightarrow{k_4} \end{aligned} \qquad (4)$$

This series of steps leads to

$$\frac{k_{\text{exptl}}}{[\text{EDTA}]} = k_E = \frac{\frac{[H^+]}{K'_a}\left(k_1\frac{[H^+]}{K_a} + k_4\right) + \left(k_3\frac{[H^+]}{K_a} + k_2\right)}{\left(1 + \frac{[H^+]}{K_a}\right)\left(1 + \frac{[H^+]}{K'_a}\right)} \qquad (5)$$

with[29] $K_a = [H^+][EDTA^{4-}]/[HEDTA^{3-}] = 4.57 \times 10^{-11}$. The best fit for the kinetic data was obtained with $k_1 = 55\ M^{-1}\ s^{-1}$, $k_2 = 900\ M^{-1}\ s^{-1}$, $k_3 \sim 0\ M^{-1}\ s^{-1}$, $k_4 = 1000\ M^{-1}\ s^{-1}$, and $pK'_a = 7.7$. Because of the large number of independent parameters, these values should be considered as estimates of the constants. It is interesting to note that the pK'_a obtained here is nearly identical[30] with the pK_a for Pb^{2+}. The theoretical curve shown in Figure 2 is obtained from eq 5 with the above parameters. On the basis of these values, the reaction at pH 8.4 proceeds about 48% by the 4+,3- interaction; 43% by the 3+,4- interaction and less than 10% by the 4+,4- pathway. However, the ionic strength dependence of the EDTA-assisted demetalation reaction yields a slope $\approx Z_A Z_B \sim -6$ (cf. Figure 4) implying that, as observed earlier,[14,31] porphyrins behave kinetically as if they carry less charge than predicted by their overall formal charges.

An examination of the data in Table Ib for the demetalation in the presence of excess DNA and no EDTA shows that under the conditions of the experiments the reaction appears first order. The rate constant at pH 8.4 and 25 °C ($k_D \sim 2 \times 10^{-4}\ s^{-1}$) can be compared with the acid solvolysis rate constant (k_r) under the same conditions. From the data of Haye and Hambright[15] and the general rate laws for metal incorporation and acid solvolysis (see Introduction), we obtain $k_r = k_A[H^+]^2 \sim 1 \times 10^{-8}\ s^{-1}$ at pH 8.4. Thus, the presence of DNA greatly enhances the rate of demetalation of PbTMpyP although the data appear first order. It is by now well-known that H_2TMpyP^{4+} and many of its metal derivatives interact extensively with DNA.[18-22] The rapid shift in the Soret maximum of PbTMpyP from 475 to 480 nm in the presence of DNA suggests external bonding for this metalloporphyrin consistent with earlier work in which the bonding of other axially liganded metalloporphyrins to nucleic acids was considered.[19-21] For the steps

$$\begin{gathered} PbTMpyP + DNA \overset{K_D}{\rightleftharpoons} PbTMpyP{\cdot}DNA \\ 2H_2O + PbTMpyP{\cdot}DNA \xrightarrow{k_D} Pb^{2+} + H_2TMpyP{\cdot}DNA + 2OH^- \end{gathered} \tag{6}$$

we obtain at fixed pH

$$\text{rate} = \frac{k_D K_D[PbTMpyP][DNA]}{1 + K_D[DNA]} \tag{7}$$

Under the conditions of these experiments,[18,19,22] it is likely that $K_D[DNA] > 1$ and the rate law of eq 7 simplifies to

$$\text{rate} = k_D[PbTMpyP] \tag{8}$$

with $k_D \sim 2 \times 10^{-4}\ s^{-1}$. Whether the rate enhancement of the demetalation by DNA involves a direct link to the lead center by a nucleic acid moiety such as, for example, a phosphate group of the backbone is unknown at present.

When DNA and EDTA are added simultaneously to a solution of PbTMpyP, the porphyrin/nucleic acid complex forms in a rapid step.[20] However, the demetalation by EDTA is much more rapid than the DNA-assisted process discussed above. A reaction sequence of the following type may be considered:

$$\begin{array}{ccc} PbTMpyP & \xrightarrow[k]{EDTA,\ 2H^+} & H_2TMpyP^{4+} + PbEDTA^{2-} \\ {\scriptstyle +DNA}\uparrow\downarrow{\scriptstyle -DNA} & & {\scriptstyle +DNA}\uparrow\downarrow{\scriptstyle -DNA} \\ PbTMpyP{\cdot}DNA & \xrightarrow[k']{EDTA,\ 2H^+} & H_2TMpyP{\cdot}DNA + PbEDTA^{2-} \end{array} \tag{9}$$

The above reaction scheme can be simplified when the effect of DNA on electrolytes is considered. The negative backbone of the filamentous nucleic acid has the effect of concentrating cations in its immediate environment, forming an ion condensation layer.[23] The concentration of anions, and especially highly charged ones, is expected to be depleted in this region. Therefore, the direct attack of PbTMpyP·DNA by EDTA probably does not contribute appreciably to the production of the demetalated species. The H_2TMpyP^{4+} produced in solution by EDTA attack on the dissociated metalloporphyrin would then combine very rapidly with DNA.[20] For the definition of K_D given in eq 6, the rate law for the simplified reaction pathway (eq 9) at a fixed pH is

$$\text{rate} = \frac{k[PbTMpyP][EDTA]}{1 + K_D[DNA]} \tag{10}$$

so that the observed pseudo-first-order rate constant, k_{obs} can be related to the above expression by

$$\frac{k_{obs}}{[EDTA]} = k_{ED} = \frac{k}{1 + K_D[DNA]} \tag{11}$$

Thus a plot of $1/k_{ED}$ vs [DNA] is expected to be linear as is indeed found for the kinetic data reported here (cf. Figure 6) with a slope of K_D/k and an intercept of $1/k$. The values for K_D/k are found to be 8.6×10^3 s at $\mu = 0.10$, 1.2×10^3 s at $\mu = 0.25$, and 2.9×10^2 s at $\mu = 0.50$. We make a simplifying assumption to continue the analysis, which is likely to be a crude approximation only. Since the demetalation step involves *dissociated* $PbTMpyP^{4+}$ we assume that the presence of DNA in solution has no effect on the rate constant for the EDTA attack. In essence we are proposing that the reaction takes place sufficiently far from the macromolecule that its Coulombic influence is negligible. Then, $k = k_E$, and using the results shown in Figure 4, we calculate that $K_D = 2.3 \times 10^5\ M^{-1}$ at $\mu = 0.10$, $8.8 \times 10^3\ M^{-1}$ at $\mu = 0.25$, and $6.8 \times 10^2\ M^{-1}$ at $\mu = 0.50$. (From the data of Figure 4 we calculate that $k_E = 2.4\ M^{-1}\ s^{-1}$ at $\mu = 0.5$, which is in fair agreement with the value of $5.6\ M^{-1}\ s^{-1}$ calculated from the intercept of the plot of $1/k_{ED}$ vs [DNA] at this ionic strength.) The approximation made in eq 8 above that $K_D[DNA] > 1$ at $\mu = 0.10$ is seen to be justified under the conditions of our experiments. From polyelectrolyte theory,[23] log K_D is expected to decrease linearly with log [Na^+] (NaCl is the electrolyte used here), and such a plot is shown in Figure 7 for the limited data available here, leading to $\log K_D = 1.8 - 3.6 \log[Na^+]$. The unusual ionic strength (or [Na^+] dependence) of k_{ED} as shown in Table Ic can be interpreted in terms of the salt dependences of k_E and K_D in eq 11. As μ (or [Na^+]) increases, k_E and K_D both decrease but with different functional dependences. Thus, the appearance of a maximum in the rate constant, k_{ED}, as the NaCl concentration is varied is not unexpected. In principle, values of k_{ED} can be calculated at each μ and [Na^+] by using eq 11 and the results of Figures 4 and 7. When such calculations are attempted, the agreement between k_{ED} and k_{ED}^{calc} is good at low μ (e.g., $k_{ED} = 0.076\ M^{-1}\ s^{-1}$ and $k_{ED}^{calc} = 0.10\ M^{-1}\ s^{-1}$ at $\mu = 0.05$, $k_{ED} = 2.9\ M^{-1}\ s^{-1}$ and $k_{ED}^{calc} = 2.6\ M^{-1}\ s^{-1}$ at $\mu = 0.25$) but only fair at higher ionic strength ($k_{ED} = 3.5\ M^{-1}\ s^{-1}$ and $k_{ED}^{calc} = 2.0\ M^{-1}\ s^{-1}$ at $\mu = 0.5$ M) where the approximation that the DNA-bound lead porphyrin is not attacked by EDTA is likely

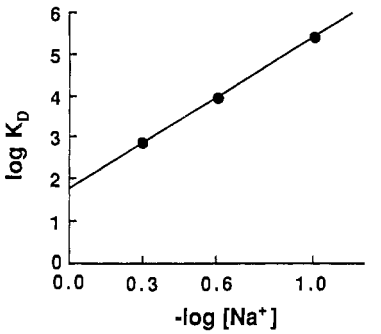

Figure 7. Plot of log K_D vs −log [Na^+] for data obtained from experiments on the EDTA-assisted demetalation of PbTMpyP in the presence of DNA (cf. eq 11).

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to be less valid. More rewarding is the appearance of a maximum in the k_{ED}^{calc} vs μ profile at $\mu \sim 0.3$, the same ionic strength at which the maximum occurs in the experimental data (cf. Table Ic). Thus, the admittedly oversimplified scheme (eq 9 with $k' \sim 0$) is adequate to account for most of the features of the experimental data. We conclude, on the basis of kinetic results obtained here, that the reaction of a multivalent anion with a drug–nucleic acid complex proceeds primarily via a pathway in which the drug molecule first dissociates from the DNA surface and is then attacked by the anion while free in solution.

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Interpretation of Effects of pH on Electron-Transfer Reactions of *Chromatium vinosum* HIPIP with $[Co(phen)_3]^{3+/2+}$ and $[Fe(CN)_6]^{3-/4-}$ as Redox Partners

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Effects of pH 5.5–9.0 on rate constants for the $[Co(phen)_3]^{3+}$ and $[Fe(CN)_6]^{3-}$ oxidations of $HIPIP_{red}$ and the $[Co(phen)_3]^{2+}$ and $[Fe(CN)_6]^{4-}$ reductions of $HIPIP_{ox}$ have been studied, with the inorganic reactant in large excess. The trends observed yield acid dissociation pK_a values that can be assigned to the only histidine residue (His42). This assignment has been confirmed by studies using diethyl pyrocarbonate (DEPC) His42-modified $HIPIP_{red}$ and $HIPIP_{ox}$. Protonation of His42, which via Cys43 is directly attached to the Fe_4S_4, affects the reduction potential of the HIPIP active site. All the rate constant effects observed can be explained by variations in the reduction potential, supplemented (or otherwise) by the effect that protonation of His42 has on the overall charge and the interaction with the redox partner charge. For such a 1+ charge to be as influential, a reaction site close to His42 may be implicated, but this is not firmly established at this time. From structural information the reduced cluster has elongated Fe–S bond lengths of 2.38 Å (reduced) as compared to 2.22 Å (oxidized). Protonation of His42 results in an increase in $E°$ (30 mV) corresponding to a relative stabilization of $HIPIP_{red}$, consistent with a less tightly wrapped peptide so that expansion of the Fe_4S_4 core can occur more readily. On DEPC modification $E°$ decreases by 15 mV at pH 8.5, and $E°$ is no longer dependent on pH.

Introduction

High-potential iron–sulfur protein (HIPIP) from *Chromatium vinosum* (M_r 9300) has been the subject of extensive characterization.[1,2] It contains a single cuboidal Fe_4S_4 cluster that is coordinated to the S atoms of four cysteines (residues 43, 45, 63, and 77) of a single polypeptide chain (85 amino acids).[3] It has a pI of 3.88, and estimated charge (pH $\sim$ 7) of −3 (two Arg and one His included as positive charges) in the oxidized state. A distinctive feature is the high reduction potential (+350 mV for HIPIP from *C. vinosum*),[4] which is in contrast to that of the structurally closely related ferredoxins (−400 mV).[5] This is directly attributable to different one-electron-redox changes, involving the $Fe_4S_4^{3+/2+}$ (HIPIP) and $Fe_4S_4^{2+/+}$ (ferredoxin) states.[6,7] It is only with very strong reductants, such as e^-_{aq} in pulse radiolysis experiments, that the $Fe_4S_4^+$ superreduced HIPIP form is generated.[8] X-ray crystal structure information has been obtained for the oxidized and reduced states of HIPIP.[9,10] Structural differences that have been proposed to explain the different behavior of Fe_4S_4 clusters in HIPIP and ferredoxin proteins include the buried nature of the cluster in HIPIP and the extent of H-bonding of core S^{2-} ligands.[6]

Kinetic studies on redox reactions of HIPIP with a number of inorganic reagents as well as with other metalloproteins have been reported.[11–20] In all these reactions, it is known that HIPIP serves as a single-electron donor or acceptor. As yet little information has been forthcoming concerning the identity of binding sites for redox partners on HIPIP.

Here we report studies of *C. vinosum* HIPIP with two inorganic redox couples $[Co(phen)_3]^{3+/2+}$ ($E°$ = 370 mV) and $[Fe(CN)_6]^{3-/4-}$ ($E°$ = 410 mV)

$$HIPIP_{red} + [Co(phen)_3]^{3+} \rightleftharpoons HIPIP_{ox} + [Co(phen)_3]^{2+} \quad (1)$$

$$HIPIP_{red} + [Fe(CN)_6]^{3-} \rightleftharpoons HIPIP_{ox} + [Fe(CN)_6]^{4-} \quad (2)$$

in order to gain further insight into the mechanism of reaction and factors affecting reactivity. With the two inorganic oxidants

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