Notes

Carboxylate/Diphenylphosphate Exchanges in Asymmetric Diiron Complexes Modeling the Purple Acid Phosphatases Enzymes: Associated Redox Processes

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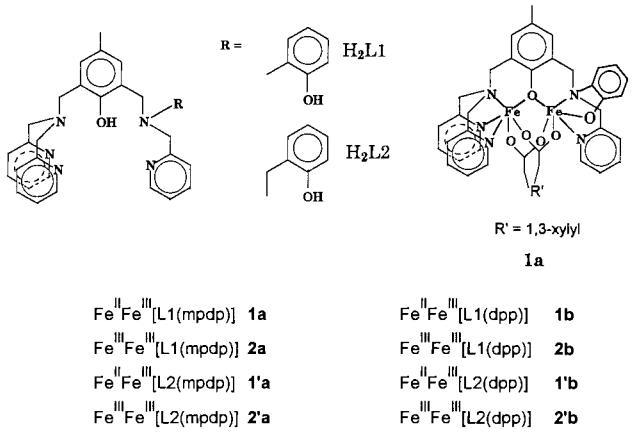
Introduction

Phosphate binding at the active sites of metalloproteins is taking place in many important biological processes and has been documented in a number of cases. Among these are proteins involved in phosphate ester hydrolysis and possessing a dinuclear metal site.[1] Of special interest in this respect are the iron-based purple acid phosphatases (PAP).[2,3] These enzymes are active for phosphate ester hydrolysis when the iron pair is in the mixed-valent state $Fe^{II}Fe^{III}$. However, addition of phosphate to the active form under aerobic conditions eventually gives the inactive $Fe^{III}Fe^{III}$ enzyme. In this process it is postulated that the $Fe^{II}(\mu$-OH)Fe^{III} core is transformed in Fe^{III}-(μ-OH)(μ-phosphato)Fe^{III}.[4]

To mimic the peculiar coordination of a terminal tyrosine residue to a single iron of the pair at the PAP active site, we designed asymmetric binucleating phenol ligands able to provide one atom of a diiron unit with a terminal phenolate ligand.[5,6] The bridging phenolate mimics the bridging hydroxide. The diiron complexes of this series of ligands therefore involve a triply bridging pattern: (μ-phenolato)bis(μ-carboxylato) or (μ-phenolato)bis(μ-phosphato) as illustrated in Scheme 1.

A number of diiron diarylphosphate complexes have been isolated in the past decade and their properties have been compared to those of their respective carboxylate counterparts.[7−11]

Scheme 1



1a

$Fe^{II}Fe^{III}$[L1(mpdp)]	**1a**	$Fe^{II}Fe^{III}$[L1(dpp)]	**1b**
$Fe^{III}Fe^{III}$[L1(mpdp)]	**2a**	$Fe^{III}Fe^{III}$[L1(dpp)]	**2b**
$Fe^{II}Fe^{III}$[L2(mpdp)]	**1'a**	$Fe^{II}Fe^{III}$[L2(dpp)]	**1'b**
$Fe^{III}Fe^{III}$[L2(mpdp)]	**2'a**	$Fe^{III}Fe^{III}$[L2(dpp)]	**2'b**

Nevertheless, only in very few instances has the carboxylate/diarylphosphate exchange be studied in some detail.[9] We report here such a study performed on the series of complexes depicted in Scheme 1. It shows that (i) the carboxylate/diphenylphosphate exchange is strongly dependent on the redox state of the diiron center and (ii) in the diferric complexes, reduction to the mixed-valent state is taking place along with the ligand exchanges.

Experimental Section

Synthesis. The ligands H_2L1 and H_2L2 and their complexes were synthesized as described previously.[6]

Spectroscopy and Electrochemistry. Electronic absorption spectra were recorded on a Lambda 9 Perkin-Elmer spectrometer in methanol and acetonitrile. NMR spectra were recorded in deuterated acetonitrile on Bruker AM 300 and AM 400 spectrometers. Mass spectra were obtained on a VG-ZAB-SEQ apparatus operating in the positive FAB mode. Aliquots of the reaction mixture were analyzed directly.

Electrochemical measurements were done as described.[6] The reference electrode consisted of a silver wire immersed in a 10^{-2} M solution of silver nitrate in an acetonitrile solution 10^{-1}M in tetrabutylammonium perchlorate (TBAP). All potentials reported are relative to Ag/10 mM Ag^+ in CH_3CN + TBAP 0.1 M and can be converted to SCE and NHE by adding 0.30 and 0.52 V, respectively.

Results and Discussion

The ligand exchanges have been investigated by means of several techniques: electronic absorption spectroscopy, NMR, mass spectrometry, and electrochemistry. For the sake of clarity, we first describe the diphenylphosphate/carboxylate exchange process.

(i) Diphenylphosphate/Carboxylate Exchange. When 1 equiv of disodium 1,3-*m*-benzene dipropionate (Na_2mpdp) was added to an acetonitrile solution of **1b**, the quantitative

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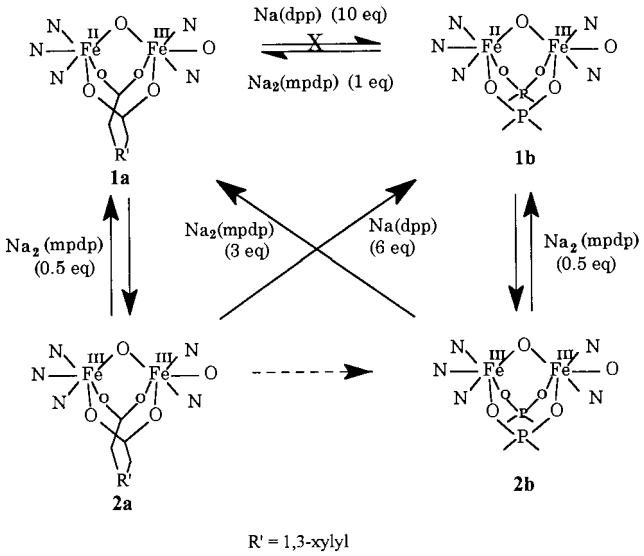
Scheme 2



transformation of **1b** into **1a** was achieved within a few minutes. NMR analysis of the reaction mixture in deuterated acetonitrile confirmed the replacement of the diphenylphosphate (dpp) ligand, whose characteristic resonances[6] disappeared. It showed that 1 equiv of Na_2mpdp is sufficient to realize the quantitative transformation of **1b** in **1a** (Scheme 2). It must be noted that the reaction could not be followed by electronic absorption spectroscopy since **1b** and **1a** have too similar spectra.

The reaction of **2b** with Na_2mpdp is more complex because, apart from the bridging ligand exchange, the reduction of the bis-ferric center to the mixed-valent state is observed. Indeed, addition of the carboxylate causes an immediate color change, which corresponds to the displacement of the phenolate → Fe(III) charge-transfer band from 640 to 550 nm. The final spectrum matches the one of **1a**.[6] To confirm the redox change associated with the ligand exchange, the reaction was studied by electrochemistry. Upon addition of Na_2mpdp, the initial $Fe^{II}Fe^{III}/Fe^{III}Fe^{III}$ couple in the cyclic voltammogram of **2b** ($E_{1/2}$ = 0.21 V; Figure 1A, curve a) is replaced by the one typical of **1a** ($E_{1/2}$ = 0.11 V; Figure 1A, curve b). Moreover, the formation of the $Fe^{II}Fe^{III}$ species is clearly demonstrated by voltamperometry experiments conducted at a Pt rotating disk electrode (Figure 1B). The two initial reduction waves of **2b** were replaced by a single reduction wave and an oxidation wave typical of the electroactivity of **1a**.[6]

The mechanism of the reaction has been investigated by NMR. A spectrum of **2b** in solution with CD_3CN shows broad unresolved features in the range 0−100 ppm, which is usual for weakly antiferromagnetically coupled diferric complexes. Addition of 0.5 equiv of Na_2mpdp gives rise to the well-resolved spectrum that is characteristic of the mixed-valent complex **1b**[6] illustrated in Figure 2a (bottom). Further addition of 0.5 equiv of Na_2mpdp furnishes an equimolar mixture of **1b** and **1a** which is formed at the expense of **1b** (Figure 2b, middle). Upon further addition of 1 equiv of dicarboxylate, the quantitative formation of **1a**, as judged from the disappearance of the characteristic protons of **1b** (Figure 2c, top), takes place. This experiment provides unequivocal evidence that the reduction of **2b** occurs before the substitution of the diphenylphosphate ligands by carboxylate. Formation of **1b** is confirmed by visible spectroscopy since, upon addition of 0.5 equiv of dicarboxylate, the phenolate → Fe(III) CT band moved from 640 to 550 nm. Further addition of Na_2mpdp induced the ligand exchange, and 2 equiv was needed to ensure the quantitative formation of **1a**. Mass spectrometric analysis confirmed the ligand exchange: the initial peak of the $[Fe_2(L1)(dpp)_2]^+$ cation at 1139 was replaced by the one of $[Fe_2(L1)(mpdp)]^+$ at 861.

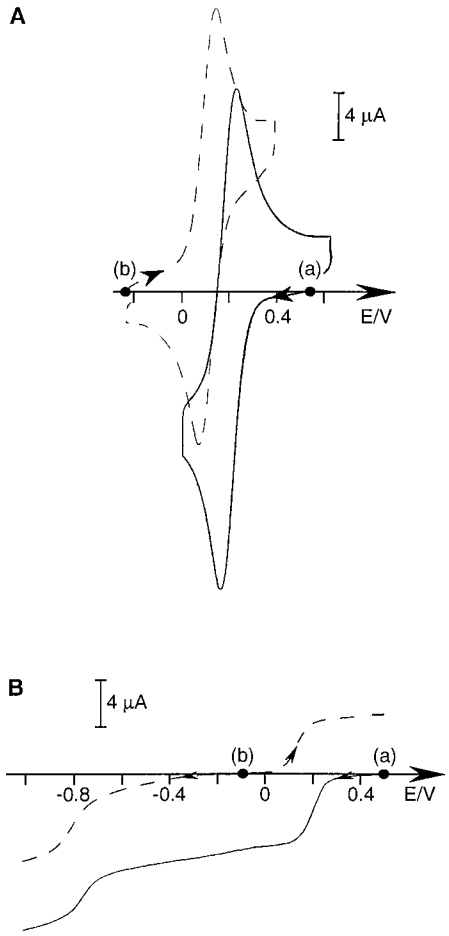


Figure 1. Cyclic voltammograms at 100 $mV \cdot s^{-1}$ (A) and rotating disk voltammograms at 600 $tr \cdot min^{-1}$ and 10 $mV \cdot s^{-1}$ (B) in CH_3CN containing 0.1 M tetra-*N*-butylammonium perchlorate at Pt electrode (0.2 cm^2 and 0.03 cm^2, respectively) for **2b**: (a) initial solution (0.73 mM) and (b) after the addition of 1.5 molar equiv of Na_2(mpdp) in MeOH (7 mM).

The finding that the reduction occurs after consumption of the first 0.5 equiv of Na_2mpdp strongly supports that the dicarboxylate ligand is the actual reducing agent. This is in good agreement with the stoichiometry of the redox process involved, because a monocarboxylate (RCO_2^-) is known to be irreversibly oxidized according to a one-electron process (reactions a−c) at moderate anodic potential. For instance, we found that mpdp is irreversibly oxidized at 0.86 V vs Ag/Ag^+.

$$RCO_2^- \rightarrow RCO_2^{\bullet} + e^- \quad \text{(a)}$$

$$RCO_2^{\bullet} \rightarrow R^{\bullet} + CO_2 \quad \text{(b)}$$

$$R^{\bullet} \rightarrow 1/2\ R_2 \quad \text{(c)}$$

The involvement of the dicarboxylate as the reducing agent is confirmed by the following experiments:

(a) Addition of diphenylphosphate, which is liberated during the redox process, and methanol, used to solubilize the dicarboxylate, does not cause reduction of **2b**. Indeed, electronic absorption spectroscopy and electrochemical experiments in-

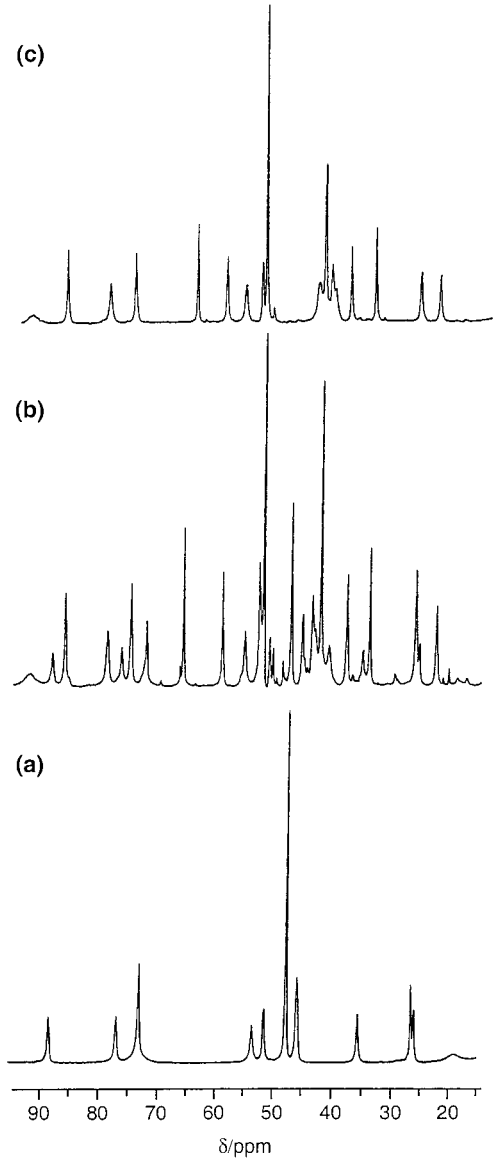


Figure 2. NMR spectrum of a CD_3CN solution of **2b** in the 15−95 ppm domain after the addition of Na_2(mpdp): (a) 0.5 equiv, (b) 1 equiv, and (c) 2 equiv.

dicate that an acetonitrile solution of **2b** was unaffected by addition of both reagents.

(b) Addition of 0.5 equiv of dicarboxylate to a solution of the corresponding dicarboxylate complex **2a** leads to its complete reduction into **1a**.

Involvement of the carboxylate was definitely proven by using phenylacetate as the bridging ligand. Indeed, its oxidation (1.43 V vs SCE[12]) is known to produce bibenzyl through coupling of the two benzyl radicals (reaction c). Therefore, the phenylacetate analogue of **2a** was prepared, and we observed that it reacted with sodium diphenylphosphate, producing bibenzyl, which was detected by chromatography and NMR after completion of the reaction.

(ii) Carboxylate/Diphenylphosphate Exchange. Studies of the reverse reactions showed that **1a** does not react with diphenylphosphate (Na(dpp)). Even with a 10-fold excess of Na(dpp), no reaction took place after 12 h. On the contrary, when **2a** was treated with 6 equiv of Na(dpp), quantitative

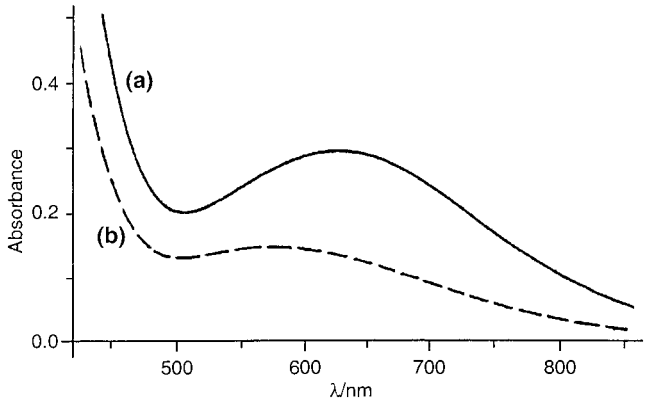


Figure 3. Electronic absorption spectrum of an acetonitrile solution of **2a** (solid line) and **1b** (broken line) formed upon the addition of 6 equiv of Na(dpp) to the initial solution.

replacement of the dicarboxylate was obtained as judged from the displacement of the phenolate → Fe^{III} charge-transfer transition from 616 to 550 nm (Figure 3), which corresponds to the formation of the mixed-valent diphenylphosphate complex **1b**. Therefore, reduction of the diferric center also occurred together with the bridging ligand exchange. We believe that the liberated dicarboxylate is the actual reducing agent (see above). It is likely that **2b** is a transient species that is reduced as soon as it is formed by the dicarboxylate which has been released. Reduction of **2a** by the dicarboxylate is inconsistent with the eventual formation of **1b** because (i) the diphenylphosphate derivatives are more easily reduced than are their carboxylate analogues[6] and (ii) as noted above, **1a** is insensitive to carboxylate diphenylphosphate exchange. Mass spectrometric analysis after isolation of the complex confirmed the ligand exchange.

Conclusion

The various transformations in the carboxylate/diphenylphosphate and diphenylphosphate/carboxylate exchanges are summarized in Scheme 2. It is worth noting that the complexes of ligand H_2L2 (**1′a**, **2′a**, **1′b**, and **2′b**, Scheme 1) behave in the same manner. Overall, they illustrate the strong preference of the mixed-valent state for carboxylates and the sensitivity of the diferric state, either the carboxylate or the diphenylphosphate derivatives, to reduction by carboxylates. The diphenylphosphate derivative is more difficult to oxidize than the carboxylate analogue by ca. 100 mV. This feature is at variance with the fact that the syntheses of the complexes under similar aerobic conditions furnish the diferric diphenylphosphato complexes and the mixed-valent carboxylates. This discrepancy may be explained by the use of an excess of carboxylate in the synthesis of the latter compounds which may reduce the complex. It is at variance also with the enzyme behavior because phosphate binding lowers the potential of uteroferrin by ca. 200 mV.[13] It must be kept in mind that the interaction of the PAP enzymes with phosphate consists of its addition and not the displacement of a carboxylate residue. Therefore, the decrease in the oxidation potential may be due to the replacement of terminal anionic ligands of the iron, possibly hydroxide.

The diiron (and ironmanganese) PAP enzymes appear peculiar in the family of hydrolytic enzymes because they rely on a redox active center to perform a nonredox function, phosphate

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hydrolysis. It is worth noting, however, that other biological functions have been proposed, and this question is not yet solved.[14−16] As we showed recently on model complexes, the presence of a terminal phenolate (tyrosinate) on an iron pair induces a thermodynamic destabilization of the diferrous state, explaining the loss of ferrous ions by the protein.[17] Moreover, as shown earlier and confirmed in the present work, the product of the hydrolysis reaction is able to deactivate the enzyme by promoting the oxidation of the diiron center. In this context, the redox regulation of the enzyme activity appears to be an attractive hypothesis.

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