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Supporting Material

Effect of Anions on the Binding and Oxidation of Divalent Manganese and Iron in Modified Bacterial Reaction Centers

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SUPPLEMENTARY MATERIAL

Construction of the metal binding sites

The triple mutant containing the replacement of Phe to His at M197, Leu to His at L131, and Leu to His at M160 positions possesses all four possible hydrogen bonds between P and the BRC protein including the naturally occurring one between the L168 His and the 2-acetyl group of the L half of P. In addition to these mutations, the Arg residue at M164 position has been replaced with Tyr. This substitution eliminates the positive charge that could electrostatically hinder the binding of the divalent cation. The metal binding sites were created by introducing carboxylic residues near the M173 Glu, which is at an analogous position with Asp 170 in the D1 protein of PSII that is believed to be essential to bind the first manganese in the cluster (20,21). The metal binding mutants contain all previous four mutations and the following further substitutions: in the M1 mutant Met M168 to Glu and Val M193 to Glu, in the M2 mutant Met M168 to Glu and Gly M288 to Asp, in the M3 mutant Val M193 to Glu and Gly M288 to Asp, and in the M4 mutant Met M168 to Glu, Val M193 to Glu, and Gly M288 to Asp.

Data analysis

The dissociation constant for metal binding was determined based on a model developed earlier (24) and modified for the manganese and iron binding site near the dimer (12). The fraction of the BRC with bound metal that donated an electron to P^+ can be expressed as a function of the total added metal ion concentration $[M^{2+}]$ and the concentration of the reaction center [RC] as

$$R_M = \frac{[M^{2+}] + [RC] + K_D - \sqrt{([M^{2+}] + [RC] + K_D)^2 - 4[RC][M^{2+}]}}{2[RC]} \qquad \text{(S1)}$$

where K_D is the dissociation constant. In the analysis applied to the kinetic data R_M is given as the fraction of the fastest kinetic component, which is attributed to the metal oxidation while for the steady-state measurements $1\text{-}R_M$ is given as the fraction of P^+. The BRC concentration used in these studies was around 1 μM, which sets the lower limit of determining the value of K_D to ~1 μM.

It should be noted that this analysis assumes that the bound manganese is also capable of serving as a secondary electron donor to P^+. For electron transfer to occur, several parameters, such as the oxidation potential of the bound metal ion and the distance between the metal and P^+, must be tuned for optimal values for efficient electron transfer. If the manganese binds to the BRC but is not able to donate electron to P^+, it would appear as if it failed to bind.

In some measurements, especially at lower pH values, R_M does not reach unity even at the highest applied metal concentrations (13). This requires the addition of an offset $\Delta A_\infty/\Delta A_0$, where ΔA_∞ and ΔA_0 are the absorbance changes at 865 nm at the highest applied metal concentration and without any metal, respectively. With this modification for the steady-state measurements the fraction of P^+ can be given as

$$f(P^+) = 1 - R_M + \frac{\Delta A_\infty}{\Delta A_0} \qquad \text{(S2)}$$

The pH dependence of K_D was fitted with the previously introduced model assuming an electrostatic compensation of the binding of the divalent metal ion by proton release involving protonatable residues (13):

$$K_D = K_D{}^0(1+10^{pK(c)-pH}+(10^{pK(c)-pH})^2) \quad \text{(S3)}$$

where *pK(c)* is the negative logarithm value of the proton dissociation constant of the amino acid side chain in the binding cluster of the BRC and $K_D{}^0$ is the dissociation constant at high pH values where the binding becomes independent of pH. Eq. S3 assumes the participation of two amino acid side chains, with very close *pK* values representing the entire metal binding cluster. If only one side chain is involved in the electrostatic compensation then the last, quadratic term of the equation is omitted and the equation reduces to the following form:

$$K_D = K_D{}^0(1+10^{pK(c)-pH}) \quad \text{(S4)}$$

The effect of the concentration of added anions on the dissociation constant was modeled using:

$$K_D = K_D{}^0\frac{(1+10^{pA-pAK}+(10^{pA-pAK})^2)}{(1+10^{pA-pAK'}+(10^{pA-pAK'})^2)} \quad \text{(S5)}$$

Here pA, on the analogy of pH, is the negative logarithm value of the molar anion concentration and *pAK* and *pAK′* are the negative logarithm values of the anion dissociation constants to manganese and/or to the binding site. The anion can facilitate the binding of the metal at pA values between *pAK′* and *pAK*. Eq. S5 assumes that two anions are binding to the metal as ligands, while the assumption of only one anion per binding site results in the following simplified equation:

$$K_D = K_D{}^0\frac{(1+10^{pA-pAK})}{(1+10^{pA-pAK'})} \quad \text{(S6)}$$

Iron binding and oxidation

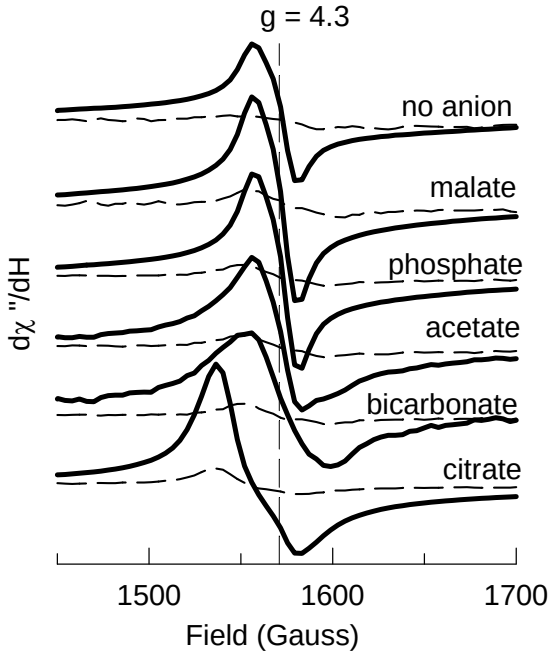

Figure S1. Light-minus-dark, X-band EPR difference spectra (thick solid lines) in the g = 4.3 region of the M1 mutant in the presence of added ferrous ion and in the presence of different anions. The spectra recorded in the dark (thin dashed lines) are also shown.
Conditions: Temperature: 120 K, magnetic field modulation frequency: 100 kHz, amplitude: 0.4 mT, microwave power: 10 mW, microwave frequency: 9.41 GHz, sweep rate:1.2 mT/s.

Anion	$^{*}K_D$ (μM)	$^{\dagger}k_{FeP}$ (s^{-1})	$^{\ddagger}\Delta_{P\text{-}T}$ (Gauss)
-	30	104	24
malate	28	109	27
phosphate	32	96	24
acetate	13	133	27
bicarbonate	1	275	44
citrate	17	121	51

Table S1. Properties of iron binding and oxidation in the presence of different anions in the M1 mutant at pH 7.0.

[*]Dissociation constant of the iron binding determined as described in Fig. 1,
[†]Rate constant of the electron transfer from the bound ferrous ion to the oxidized dimer at 100 μM added Fe^{2+} and 100 mM anion concentration except 15 mM bicarbonate,
[‡]Peak-to-trough line width of the g = 4.3 EPR signal of the high spin Fe^{3+}.