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Binding of carboxylate anions at the non-heme Fe(II) of PS II. II. Competition with bicarbonate and effects on the Q_A/Q_B electron transfer rate

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Abstract

In the companion paper (Petrouleas, V., Deligiannalcis, Y. and Diner, B.A. (1994) Biochim. Biophys. Acta 1188, 260–270) we examined the effects of a number of carboxylate anions on the EPR signals associated with the iron quinone complex, the ligand field parameters of the Fe^{3+}, and the redox properties of the iron. In this paper, we show that three representative anions, glycolate, glyoxylate, and oxalate, compete with NO, formate and bicarbonate for binding to the iron. Furthermore, the bound anions affect diversely the Q_A/Q_B electron transfer rate. Glycolate has an extreme effect, similar to what is observed with high levels of formate, and is characterised by a dissociation constant, K_d, ~0.5–0.7 mM. Oxalate gives a marked slowing of the rate of Q_A^- oxidation on all flashes but preserves a marked oscillation of the rate of period two. Glyoxylate appears to have an intermediate effect. These results offer new information on the stereochemistry of the binding of dissociable ligands to the non-heme iron of PS II and a tool for probing the redox chemistry of the iron and the electron transfer properties of the iron-quinone complex.

Keywords: Photosystem II; Iron-quinone complex; EPR; Carboxylate anion

1. Introduction

The Q_A to Q_B electron transfer rate in PS II (for a recent review on the iron-quinone complex of PS II see Ref. [1]) is severely slowed in the presence of molecules, such as formate [2,3] or NO [4], which displace bicarbonate, or following prolonged incubation in a bicarbonate free medium [4]. If dark-adapted chloroplasts, treated as above, are given a train of saturating flashes, then the rate of oxidation of Q_A^- following the second and subsequent flashes is found to be slowed to a greater extent than that following the first flash. Since electron transfer to the semiquinone, Q_B^-, on the second flash requires protonation of Q_B, various authors have proposed that bicarbonate is involved in the protonation pathway [1,5,6]. Our recent studies of cyanide binding at the PS II non-heme iron [7] suggest a rather indirect role of bicarbonate. Cyanide displaces the bicarbonate and yet the electron transfer rate is not affected except for an unusual inversion at high pH in the phase of the binary oscillations observed during successive electron transfers. This observation suggests that bicarbonate may not be a direct participant in a proton pathway, but may act indirectly, possibly by altering the pK_a of nearby protonatable group(s) [8].

In the companion paper, we examined the effects on the EPR signals and on the redox properties of the iron of a rather extensive list of carboxylate anions [9]. These anions have pronounced and diverse effects on the midpoint potential of the iron. While these effects can be correlated in part with the difference in the pK_a of the carboxylate groups of the anions, the variations in the pH dependence of the midpoint potential of the iron imply alteration in the pK_a values of protein residue(s) in the vicinity of the iron. It is likely that the same residues may be also involved in the protonation reactions accompanying the Q_A/Q_B electron transfer.

Abbreviations: PS II, Photosystem II; BBY, thylakoid membrane fragments isolated according to Refs. [14–16]; Chl, chlorophyll; Mes, 4-morpholinethanesulfonic acid.

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It would be of interest, therefore, to examine the effects of these carboxylate anions on the Q_A/Q_B electron transfer rate. Indications for an inhibitory effect of one of the present anions, glycolate, on the Hill reaction have been provided already in an early paper by Good [10].

We selected in this paper three of these anions for their rather extreme effects on the midpoint potential of the iron. Compared to the untreated iron with an E_m (Fe^{3+}/Fe^{2+}) at pH 7 of 400–420 mV and −60 mV/pH unit variation [11,12], oxalate raises the midpoint potential to $E_m > 500$ in the pH range 6 to 7.5, glycolate lowers it to 340 mV (very weakly pH dependent), and glyoxylate has an intermediate effect with an E_m of 390 mV at pH 6.1 and an E_m of 330 mV at pH 7.5 [9]. In the following, we show that these anions compete with known molecules (including bicarbonate) for binding to the iron and we subsequently study the effects on the electron transfer rates.

2. Materials and methods

Chloroplasts were prepared, according to Avron [13], from market spinach. The chloroplasts were suspended in 50 mM Tris-HCl (pH 7.5) containing 10 mM NaCl, 0.4 M Sucrose and 5% DMSO and stored at −80°C. Spinach BBY membranes were isolated by standard methods [14–16].

The rates of relaxation of the chlorophyll fluorescence yield following saturating actinic flashes were measured in chloroplasts in the absence of an exogenous electron acceptor using a flash detection spectrophotometer as described by Diner and Petrouleas [4]. The set of blocking filters, to protect the sample photodiode described in the earlier publication, were replaced by a Schott LF470, a Schott KV550 and a Corning 2–64 together transmitting at > 670 nm. The fluorescence relaxation represents, to a first approximation, the rate of electron transfer between the primary and the secondary quinone electron acceptors, Q_A and Q_B. No correction was made for energy transfer. The fluorescence relaxation data were analysed by a triexponential curve fitting routine of the Macintosh Kaleida Graph 2.1 program (Abelbech software) using a Marquardt algorithm. The third phase was so much slower (> 100 s) than the other two rates as to be effectively a constant for the time scale of the experiment.

EPR measurements were obtained with a Bruker ER200D spectrometer interfaced to a PC and equipped with an ESR900 Oxford cryostat.

In the NO competition studies samples pre-treated with the carboxylate anions were bubbled gently inside the EPR tubes with a mixture of NO and N_2 in a 1:4 v/v ratio.

3. Results

3.1. Competition with NO for binding to the iron

Binding of NO to the non-heme iron elicits a characteristic light sensitive EPR signal at $g = 4$ [17]. Fig. 1 shows this Fe^{2+}-NO EPR signal induced by treatment with 0.3 mM NO in the absence and in the presence of 40 mM oxalate, glyoxylate, or glycolate. All of the anions reduce substantially the Fe^{2+}-NO signal, indicating displacement of NO from the iron.

3.2. Competition with formate

Binding of formate induces in the state $Q_A^-Fe^{2+}$ a strong EPR signal at $g = 1.84$ [18]. This signal is shown in Fig. 2 for a sample treated with 100 mM formate at pH 6.2. The signal is not very intense because the conditions were not optimal for binding of this anion (the treatment was aerobic and the pH somewhat high). In the presence of 40 mM of either oxalate, glyoxylate or glycolate the $g = 1.84$ signal is replaced by the $Q_A^-Fe^{2+}$ signals characteristic of these anions in the absence of formate [9,19]. This is particularly obvious for glyoxylate and oxalate. The relatively intense $g =$

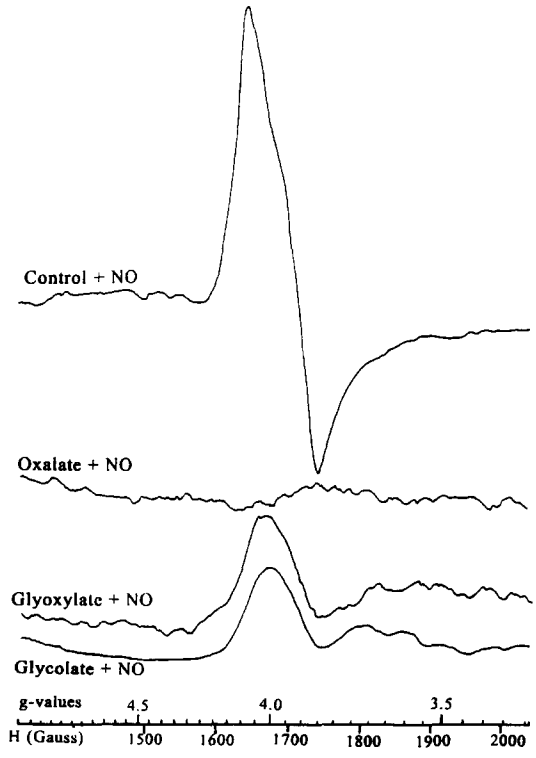

Fig. 1. Competition between NO and three anions for binding as reflected in the size of the Fe^{2+}-NO EPR signal at $g = 4$. BBY membranes at pH 6.3 (70 mM Mes) were bubbled gently with a mixture of NO/N_2 gas at a 1:4 ratio in the absence (control) or the presence of the indicated anions. EPR conditions: $T = 4.5$ K, microwave power 31 mW, modulation amplitude 16 G.

1.84 signal in the latter case has a width which is significantly broader than the formate-induced signal and is characteristic of the binding of oxalate.

3.3. Competition with bicarbonate

In an earlier report [19], it was shown that addition of 5 mM bicarbonate to a sample pre-treated with 10 mM glyoxylate (without removal of glyoxylate) shifts partially the Fe^{3+} signals toward their positions in an untreated sample. This indicates that bicarbonate competes with glyoxylate for binding. Fig. 3 illustrates a somewhat different experiment, demonstrating the competition between bicarbonate and glycolate. In a control sample poised to an ambient potential of 400 mV at pH 6.3, the Fe^{3+} EPR signal is very small due to the relatively high midpoint potential of the iron at this pH [11,12]. Addition of bicarbonate does not modify the spectrum (spectrum not shown). Treatment of a similar sample with 5 mM glycolate elicits a full size Fe^{3+} signal, since in the presence of this anion the midpoint potential of the iron is 340 mV [9]. The positions of the peaks are also shifted as expected for this anion. In a sample treated with 5 mM bicarbonate in addition to 5 mM glycolate, the Fe^{3+} peaks are intermediate both in size and in position.

3.4. Effect of the anions on the Q_A/Q_B electron transfer rate

Chloroplasts were incubated in the dark for 2–3 h at 10 μg Chl/ml in buffer A (0.3 mM Sorbitol, 30 mM Na phosphate (pH 6.33), 10 mM NaCl and 5 mM $MgCl_2$), containing either 30 mM $NaHCO_3$, oxalate, glycolate or glyoxylate. Electron transfer rates in the iron-quinone complex were assayed by giving a train of saturating actinic flashes 600 ms apart and measuring, using probe flashes, the chlorophyll fluorescence yield at various times following the actinic flash. The kinetic traces are shown in Figs. 4 and 5 and the rate components are deconvoluted in Table 1. The fluorescence yield rise at small times in some of the traces (see, e.g., the glycolate traces in Fig. 4B) is due to rate limiting steps on the donor side of PS II associated with the reduction of $P680^+$. These are particularly marked in the S3 to S4 transition of the oxygen evolving complex and where Q_A^- oxidation is sufficiently slow to see the donor side limitation.

A 10 min incubation at pH 6.33 with either 10 or 30 mM $NaHCO_3$ shows the typical oscillations of period two (see points at 500 μs) for the oxidation of Q_A^-, fast (4–7 ms^{-1}) on odd numbered flashes, and slower (2–3 ms^{-1}) on even numbered flashes (Fig. 4A, Table 1). In the deconvolution shown in Table 1, the rapid fluorescence relaxation is less apparent on the third flash as it is superimposed on the strong fluorescence quenching associated with the $S_3 \rightarrow S_0$ transition of the PS II donor side [20] in the submillisecond time range. With increased incubation time to 2 h (Fig. 4B), the relaxation rate slows on odd numbered flashes (see points at 500 μs), remaining the same on even numbered flashes, to the point were the periodicity changes phase by 180°. Similar behaviour is observed if chloroplasts are preincubated for 1 h with formate to displace bicarbon-

Table 1
Effect of the anions on the Q_A/Q_B electron transfer rate

Condition	flash 1	flash 2	flash 3	flash 4	flash 5
Bicarbonate	5.2(69)	2.6(76)	3.0(64)	2.9(77)	3.1(72)
30 mM, pH 6.33, 10 min	0.61(19)	0.26(11)	0.50(24)	0.43(14)	0.34(15)
Bicarbonate	7.0(69)	3.3(69)	4.2(57)	3.0(73)	4.2(64)
30 mM, pH 7.55, 10 min	0.71(18)	0.44(19)	0.67(28)	0.44(16)	0.58(22)
Bicarbonate	2.0(64)	2.2(73)	1.4(70)	2.0(78)	1.9(67)
30 mM, pH 6.33, 2 h	0.29(24)	0.28(14)	0.23(19)	0.27(14)	0.26(20)
Glycolate	1.8(26)	0.35(33)	0.50(27)	0.54(27)	0.37(30)
30 mM, pH 6.33, 2 h	0.091(38)	0.055(36)	0.072(41)	0.072(42)	0.069(38)
Glyoxylate	1.5(46)	0.99(52)	0.72(58)	1.1(57)	0.91(51)
30 mM, pH 6.33, 2 h	0.13(35)	0.063(27)	0.053(26)	0.069(28)	0.063(30)
Oxalate	0.90(60)	0.64(43)	0.75(56)	0.85(48)	0.94(48)
30 mM, pH 6.33, 2 h	0.096(22)	0.066(37)	0.094(25)	0.096(35)	0.099(31)
Bicarbonate	4.6(68)	2.7(70)	2.2(69)	2.4(75)	2.9(67)
30 mM, pH 7.55, 2 h	0.43(18)	0.33(17)	0.25(15)	0.28(13)	0.34(17)
Glycolate	3.5(63)	1.9(65)	2.4(55)	2.2(68)	3.5(57)
30 mM, pH 7.55, 2 h	0.28(16)	0.13(8.6)	0.32(18)	0.35(8.5)	0.36(16)
Glycolate	4.2(63)	2.0(70)	2.1(62)	2.2(72)	2.8(61)
30 mM, pH 7.55, 2 h	0.37(19)	0.12(10)	0.26(18)	0.41(11)	0.29(18)
Oxalate	3.7(62)	2.1(71)	2.6(58)	2.3(69)	2.6(65)
30 mM, pH 7.55, 2 h	0.47(21)	0.10(11)	0.41(22)	0.45(13)	0.24(15)

The first two phases of a trixponential fit to the fluorescence relaxation data of Fig. 4. The rates (in ms^{-1}) are indicated for each flash and condition, immediately followed by the percent contribution of that component to the total relaxation. The third phase, with a rate of < 0.001 ms^{-1}, is effectively a constant on the time scale of the flash experiment.

ate and then resuspended in 10 mM bicarbonate in the absence or at very low concentrations of formate (Fig. 5, see below). The origin of the change in phase is unclear.

A 2–3 h incubation with oxalate (pH 6.33, Fig. 4B, Table 1) instead of bicarbonate gives a marked slowing of the rate of Q_A^- oxidation on all flashes, though the rates are substantially faster on odd than on even numbered flashes as in fresh bicarbonate-treated chloroplasts, showing a marked oscillation of period two (see points at 1 ms).

Incubation with 30 mM glycolate (pH 6.33, Fig. 4B, Table 1) shows a substantial slowing of the electron transfer rates, most marked on the second and subsequent flashes as is well known for formate (see below). The principal kinetic components (Table 1) for the first flash are 1.8 and 0.091 ms^{-1} and for the second and subsequent flashes 0.35–0.50 and 0.055–0.75 ms^{-1}. The lower level of F on the first flash is most likely due to the spontaneous oxidation of the iron in the presence of this anion [9]. The $t_{1/2}$ for the Q_A^- to Fe^{3+} electron transfer rate at this pH is 7 μs or less [11], much faster than the time resolution of the present experiment.

Incubation with 30 mM glyoxylate (pH 6.33, Fig. 4B, Table 1) shows a marked slowing of electron transfer, showing nearly no sign of an oscillatory relaxation pattern or pronounced slowing of electron transfer on the second and subsequent flashes as with glycolate.

A similar set of incubations were carried out at pH 7.55 (Fig. 4C). The effects on electron transfer were much less marked at this pH. This reduced effect probably stems from the much higher affinity of bicarbonate for binding to the reaction centre and the consequent difficulty in its displacement by the carboxylate anions. Nonetheless, of the three carboxylate anions tested, glycolate still gave the most marked slowing of electron transfer, slower on the second and subsequent flashes as at lower pH. Also, a 2 h incubation with $NaHCO_3$ showed essentially the same behaviour as a 10 min incubation with the periodicity of two retained in its usual phase but with depressed amplitude.

It was remarked above that glycolate and formate appear to have similar effects on the acceptor side electron transfer. To compare the relative affinities of these two carboxylate anions, chloroplasts were incubated for 1 h at 25°C in the dark at 250 μg Chl/ml in Buffer A (pH 6.32) containing 10 mM Na-formate.

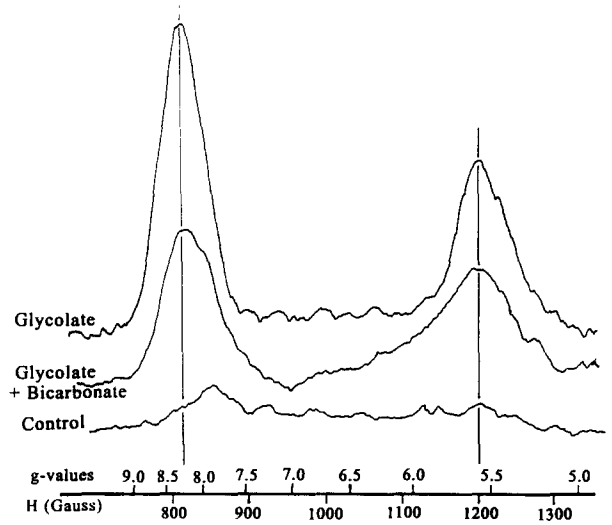

Fig. 3. Competition between bicarbonate and glycolate for binding as reflected in the Fe^{3+} EPR signals. Three BBY samples (pH 6.3, 50 mM Mes) poised with a ferri/ferrocyanide mixture at a potential of approx. 400 mV are treated with nothing additional or 5 mM bicarbonate (lowest trace), or 5 mM glycolate (upper trace) or with 5 mM bicarbonate in addition to 5 mM glycolate (middle trace). EPR conditions: T = 4.3 K, microwave power 8 mW, modulation amplitude 16G.

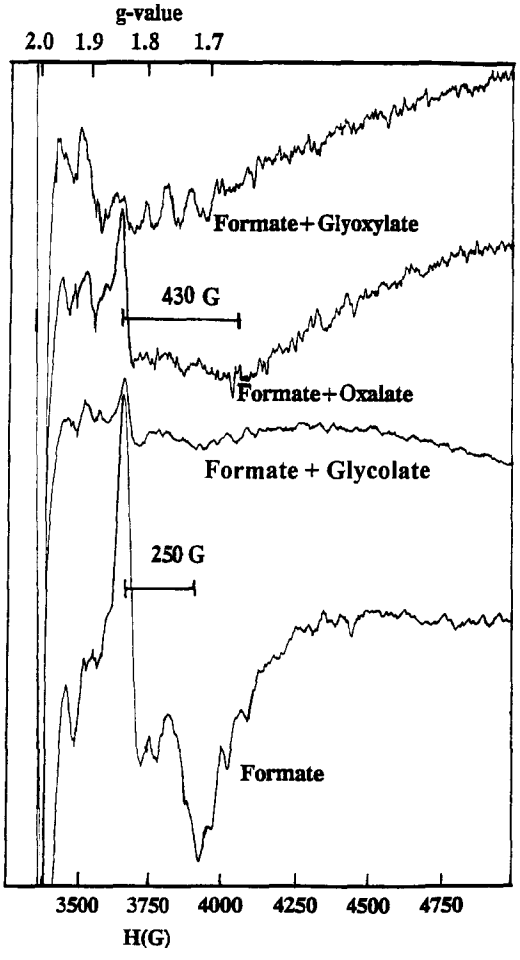

Fig. 2. Competition between formate and three anions for binding as reflected in the $Q_A^- Fe^{2+}$ EPR signal. BBY membranes at pH 6.2 (50 mM Mes) were treated with 100 mM formate in the absence or presence of three anions as indicated. The spectra were recorded after illumination at 200 K using the same conditions as in Fig. 1.

They were then diluted 25-fold to 10 μg Chl/ml into Buffer A containing 1 mM $NaHCO_3$, plus the indicated concentrations of formate or glycolate. The slowing of Q_A^- oxidation was evaluated as above. It is apparent from Fig. 5 that glycolate produces an equivalent slowing to formate (more marked on the second and subsequent flashes) but at one-third the concentration. As in the absence of bicarbonate, formate has been shown to have a dissociation constant, K_d, of approx. 2 mM [21]; the K_d for glycolate is approximately 0.7 mM, a value comparable to the ~0.5 mM estimate from the EPR studies [9].

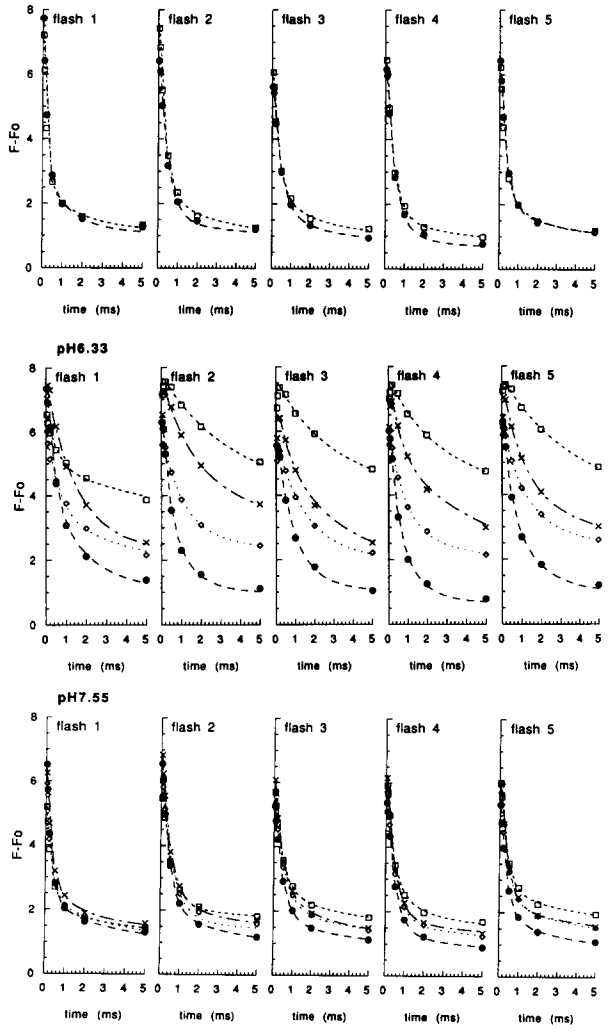

Fig. 4. Effect of incubation in the presence of various carboxylate anions and bicarbonate on the kinetics of relaxation of the chlorophyll fluorescence yield following actinic flash excitation. A train of five saturating actinic flashes were given 600 ms apart. Spinach chloroplasts were suspended at 10 μg Chl/ml in Buffer A plus either $NaHCO_3$ or the sodium salt of the other anions. The first time point is at 50 μs. (A) Incubation for 10 min in the dark at 25 K with 30 mM $NaHCO_3$ at pH 6.33 (●) and 7.55 (○). (B) Incubation for 2 h in the dark at 25°C in a sealed vial at pH 6.33 with (●) 30 mM $NaHCO_3$; (□) 30 mM Na-glycolate; (◇) 30 mM Na-glyoxylate and (×) 30 mM Na-oxalate. (C) Same as B except pH 7.55.

4. Discussion

4.1. Site of binding of the anions

The experiments in Figs. 1 and 2 demonstrated that all three anions displace NO or formate upon binding. Since bicarbonate competes with NO and formate for binding, one would expect that it also competes with the three anions. Indeed, the effects of glyoxylate and glycolate binding on the Fe^{3+} spectra [9] are reversed by the addition of bicarbonate (Fig. 3 and Ref. [19]). No similar reversibility could be demonstrated for oxalate, possibly due to a tighter binding of this anion. The chemical similarity of the present anions with bicarbonate and formate suggests that they all compete for the same binding site. As the iron has at least one exchangeable ligand [4,9,17] and the anions displace NO from its binding site on the iron, the anions bind most likely as ligands to the iron.

The mode of binding is not yet established but some interesting comparisons can be made. The relatively small effects of glycolate, lactate, glyoxylate, and pyruvate on the EPR spectra of the Fe^{3+} (see companion paper [9]) suggest that these anions have a basic mode of binding that is similar to bicarbonate in the untreated system. The observed small shifts of the g-values probably reflect the difference in the group proximal to the carboxylate. The high pH nearly rhombic $g = 4.3$ Fe^{3+} EPR signal [9] would indicate a different conformation. The EPR signals in this latter case bear strong similarities to the spectra of transferrin [Ref. [22] and references therein]. The Fe^{3+} centre in transferrin shows a number of interesting analogies to the non-heme iron binding site of PS II: (a) both use bicarbonate (carbonate) as the natural ligand, (b) the anions examined in the present and in the companion paper appear to bind as ligands to both types of non-heme iron probably involving at least the carboxyl group, (c) there are probably two coordination sites available for the binding of dissociable ligands [7,22], and (d) both sites contain nearby basic residues that contribute positive charges that are thought to be implicated in anion binding. A lysine has been identified in the crystal structure of transferrin [23] and we have found in PS II that lysine 264 and arginine 265 of the D2 polypeptide are critical to bicarbonate binding [24].

In light of these similarities, it is tempting to draw an analogous model for the binding of the carboxylate anions in PS II to that proposed for transferrin. The tight binding of oxalate with respect to formate and bicarbonate, and its complete exclusion of NO would be consistent with bidentate ligation. Also the high pH, $g = 4.3$ resonance observed with some of the anions [9] would indicate bidentate ligation in analogy to the transferrin case [22,23]. The situation with bicarbonate, formate and the other anions at low pH is less clear.

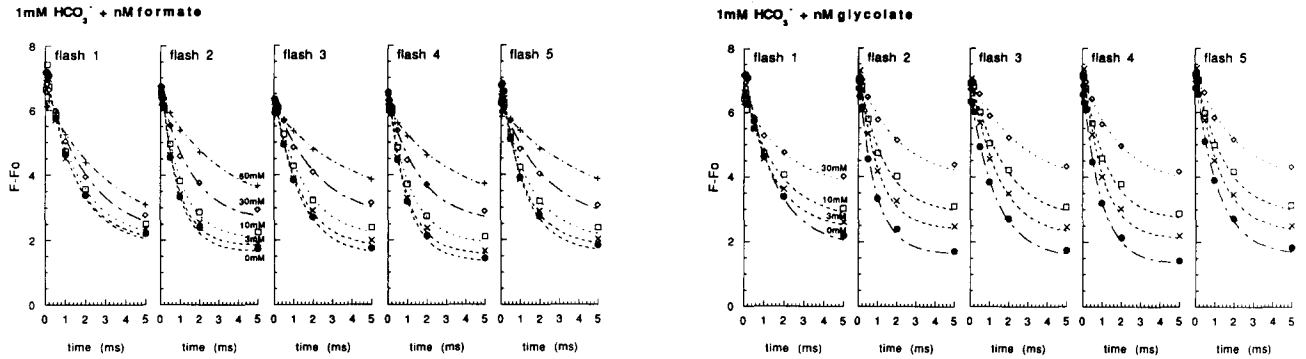

Fig. 5. Effect of various concentrations of formate and glycolate on the kinetics of relaxation of the chlorophyll fluorescence yield following actinic flash excitation in the presence of 1 mM $NaHCO_3$. A train of five saturating actinic flashes were given 600 ms apart. The first time point is at 50 μs. Spinach chloroplasts were suspended, at 250 μg Chl/ml, in Buffer A (pH 6.33, previously bubbled with N_2) plus 10 mM Na-formate. Incubation proceeded in the dark in a sealed vial for 1 h at 25°C at which time the chloroplasts were diluted 25-fold into buffer A (pH 6.33) containing 1 mM $NaHCO_3$ and the indicated concentrations of either (A) Na-formate or (B) Na-glycolate. Incubation proceeded in the dark for another hour at 25°C in a sealed vial after which the samples were analysed.

The ability of bicarbonate and the carboxylate anions to displace NO would be consistent with bidentate ligation and the non-competition between NO and formate [4] consistent with monodentate coordination of the latter. However, the Mossbauer spectrum [19], the $Q_A^-Fe^{2+}$ EPR spectrum [18] and the high midpoint potential of the iron [11] in the presence of formate all resemble the non-heme iron of the bacterial reaction centres, which is coordinated in a bidendate fashion by glutamate for the two coordination positions not occupied by histidine imidazole [25]. It is probably premature to assign detailed coordination models for these anions, particularly as the coordination mode may depend on the redox state of the iron and the pH. In light of the discussion in the accompanying paper on the effects of the anions on the g-value of $Q_A^-Fe^{2+}$ and on the E_m of the Fe^{3+}/Fe^{2+} couple, it is likely that the anions bind to the non-heme iron and exert their effects in two ways: (1) through a displacement of bicarbonate and (2) by providing a dissociable proton or by strengthening or weakening an interaction between the non-heme iron and a critical amino acid residue(s) that stabilises Fe^{3+}. The nature and stereochemistry of the interaction remains to be worked out. It is worth pointing out, however, that in the case of the bacterial reaction centres, the coordinating glutamate is unlikely to be able to further stabilise Fe^{3+} by losing a proton. There are also two basic residues, D2-Lys264 and D2-Arg265 that are close by the non-heme iron in PS II and enhance the binding of bicarbonate [24]. Either of these could interact with the bound ligand and lower further the pK_a of the site that becomes deprotonated with the oxidation of the iron.

4.2. Effects on the electron transfer rate

A rather unexpected finding is that prolonged incubation of chloroplasts with 10 or 30 mM $NaHCO_3$ at pH 6.33 but not at pH 7.55 results in a 2- to 3-fold slowing of the rate of oxidation of Q_A^- on odd-numbered flashes. This change does not seem to arise from a slow reduction of Q_B to Q_B^- during the dark incubation period as incubation for 10 or 30 min with 10 μM $K_3Fe(CN)_6$ (data not shown) does not restore the initial phase of the oscillations. Also, replacement of the 50 mM $Na(H)PO_4$ buffer with 50 mM Mes-NaOH at the same pH yielded exactly the same result. No easy explanation can be offered for this effect. Perhaps it reflects some structural change in the reaction centre and a change in the binding affinity of plastoquinone.

The carboxylate anions produce a substantial slowing of the rate of oxidation of Q_A^-. Their effects are also very different. Glycolate has an extreme effect, similar to what is observed at high concentrations of formate or with NO in which the slowing of Q_A/Q_B electron transfer is most marked on the second and subsequent flashes [2–4]. Oxalate induces a significant but not as prominent slowing of the electron transfer rate but preserves the periodicity of two in the plot of rate versus flash number (Fig. 4B). Glyoxylate appears to have an intermediate effect. These different observations on the rate of electron transfer and the different effects of the anions on the EPR signals cannot therefore be explained solely by a displacement of bicarbonate. It has been proposed that bicarbonate participates in the protonation of Q_B^- [1,5,6]. Although it cannot be absolutely excluded that the carboxylate anions participate directly in the protonation of Q_B^{2-}, it is more likely that these molecules affect the pK_a of other protonatable group(s) involved in this reaction (see accompanying paper [9]). It is also likely that the same group(s) may be responsible at least in part for the pH dependence of the Fe(III)/Fe(II) midpoint potential.

Of all the anions that inhibit bicarbonate binding competitively (see for example Ref. [5]), formate has been considered the most effective. Snel and Van

Rensen [21] have even considered a physiological role of formate linking cell metabolism and photosynthetic electron transfer. The present results indicate that glycolate acts on electron transfer at a concentration at least 3-fold lower than that of formate with a K_m of about 0.5–0.7 mM. While it is tempting to propose that there is some regulation of electron transfer through the competition of glycolate and bicarbonate for binding to the non-heme iron, this mechanism does not appear to be supported by measurements at high pH. At pH 7.5, close to that existing physiologically on the stromal side of the membrane, glycolate binding is poor, even at 30 mM concentration. The stromal concentration of glycolate has been estimated to be much lower, on the order of 1 mM [26]. Therefore, unless binding of glycolate is enhanced by illumination, control of electron transfer through PS II by this carboxylate anion does not seem likely.

Despite the lack of any obvious physiological role for the carboxylate anions, they do provide us with information on the stereochemistry of binding of dissociable ligands to the non-heme iron and a tool for probing its redox chemistry.

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