P-193

WHAT ABOUT THE BICARBONATE EFFECT IN PHOTOSYSTEM II ?

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ABSTRACT: A significant difference exists between the reaction centers of Photosystem II (PSII) and those of oxygenic photosynthetic bacteria on the electron acceptor side: reactions in the Q_A-Fe-Q_B pocket are unaffected by formate in these bacteria, whereas in PSII, a bicarbonate reversible inhibition of electron flow from Q_A^- to Q_B^- occurs. This inhibition is ascribed to a possible role of bicarbonate in the protonation of Q_B^- (Q_B^{2-}). We shall summarize here the newer experiments on **(a)** the bicarbonate effect in selected D1 mutants which suggest the importance of the Q_B pocket (J. Cao, N. Ohad, J. Hirschberg and Govindjee, 1992, unpublished); and **(b)** the differential effects of various halogenated acetates which suggest a correlation with their partition coefficient and, thus, their hydrophobicity (C. Xu, Y. Zhu and Govindjee, 1992, unpublished). It appears from the existing data in the literature (see review [1,2]) that bicarbonate not only binds to Fe, but it may also H-bond to specific amino acids on both the D1 and D2 proteins of PSII.

1. INTRODUCTION

Inhibition of PSII by several inhibitors (e.g., formate, acetate, nitrite, azide and nitric oxide) is reversed uniquely by the addition of bicarbonate. This is known as *the bicarbonate effect* [1-4]. A significantly large bicarbonate-reversible inhibition is observed in the reduction of plastoquinone to plastoquinol [5-7]. In contrast, photosynthetic bacteria, that also reduce quinones to quinols, donot show this effect [8,9]. Michel and Deisenhofer [10] suggested that the reason for the difference between PSII and bacteria may lie in the glutamic acid (at position 234 on the M-subunit: M-E234) taking the role of bicarbonate. However, Wang et al. [11] showed that the bicarbonate effect could not be observed in any of the following mutants of *Rb. sphaeroides*: M-E234V, M-E234Q and M-E234G. Thus, the answer to the difference must lie elsewhere (see e.g. [12]). On the other hand, PSII mutants altered in single amino acids in the region of the Q_A-Fe-Q_B binding niche in the D2 [1,13] or in the D2 protein [14,15] show differential sensitivity to formate/bicarbonate confirming the view that the inhibitors and the bicarbonate bind in the Q_A-Fe-Q_B region of both the D1 and D2 proteins. In this paper, this concept is reinforced: Experiments of J. Cao, N. Ohad, J. Hirschberg and Govindjee (1992, unpublished) show that, in selected D1 mutants of *Synechococous* sp. PCC 7942, inhibition of PSII electron flow by formate increases in the order: Wild type (WT) < D1-F255Y < D1-S264A ~ D1-F255Y/S264A < D1-F255L/S264A. The difference between the latter two double mutants suggests the importance of the environment around D1-S264 in the bicarbonate effect (see section 2).

Although a site of bicarbonate effect exists between the electron donor "Z" and the electron acceptor Q_A, a major effect is on the electron acceptor side, between Q_A and the

plastoquinone pool. It has been observed that the bicarbonate-reversible inhibition by formate is maximal after the 2nd (or the 3rd) and subsequent flashes [6,7,16]. This has been interpreted to suggest [6,7] that one of the functions of bicarbonate, when bound, is to bring protons for the stabilization of Q_B^- (and Q_B^{2-}) (Also see [4,17]). The bound inhibitors are unable to provide the protons and, thus, inhibit the reactions at the Q_AQ_B complex. In this context, C. Xu, Y. Zhu and Govindjee (1992, unpublished; also see [18]) have investigated the effects of various halogenated acetates, with and without bicarbonate, on the Q_AQ_B reactions to understand the nature of this inhibitory process. These authors have observed the following hierarchy in the inhibition of Q_A^- to Q_B^- reaction: acetate ~ monofluoroacetate < monochloroacetate < monobromoacetate < dichloroacetate << trichloroacetate. The bicarbonate-reversibility was maximal with acetate and minimal with trichloroacetate. A correlation of this inhibitory effect with the partition coefficient (log P and, thus, hydrophobicity) of the acetates was observed. Furthermore, monochloroacetate (MCA) was observed to rephase the flash number dependence of $[Q_A^-]$ from even to odd flashes. This was interpreted to mean that the ratio of Q_B^- to Q_B increased in dark--a novel phenomenon--upon MCA addition (see section 3).

2. DIFFERENTIAL SENSITIVITY OF FORMATE IN HERBICIDE RESISTANT D1 MUTANTS OF SYNECHOCOCCUS sp. PCC 7942 (after J. Cao, N. Ohad, J. Hirschberg and Govindjee, 1992, unpublished)

Involvement of the D1 protein in the bicarbonate effect was already known from the interactions of herbicides and bicarbonate in PSII [19,20]. Several herbicide-resistant D1 mutants of *Synechocystis* sp. PCC 6714 showed differential sensitivity to formate in their Q_AQ_B reactions in the order: D1-S264A > WT > D1-F211S >> D1-F211S/A251V [14]. In *Chlamydomonas reinhardtii*, the order of sensitivity was: D1-S264A >> D1-F219I ≈ D1-F255Y > WT >> D1-A251V > L275F [15,21]. Thus, Q_AQ_B reactions were affected most in D1-S264A and the least in D1-L275F by formate treatment. These results suggest the importance of the top of the helix IV and V and the interhelical loop between them for the bicarbonate effect.

Cao, Ohad, Hirschberg and Govindjee, (1992, unpublished) have now shown that the bicarbonate-reversible inhibition of PSII electron flow (H_2O--> dimethylquinone/ferricyanide, with dibromomethylisopropylbenzoquinone present), at pH 6.8, in selected herbicide-resistant D1 mutants of *Synechococus* sp. PCC7942 follows the order (percent inhibition by 20mM formate): WT(23 ± 2%) < D1-F255Y (28±2%) < D1-S264A (35±2%) ≈ D1-F255Y/S264A (38±2%) < D1-F255L/S264A (49±3%). Although these differences are not very large, the differential sensitivity between the double mutants F255Y/S264A and F255L/S264A confirms the importance of not only D1-S264A, but also its environment for the bicarbonate effect.

3. DIFFERENTIAL EFFECT OF HALOGENATED ACETATES ON ELECTRON FLOW FROM Q_A^- TO Q_B^- (after C. Xu, Y. Zhu and Govindjee [18]; 1992, unpublished)

In order to understand the nature of the bicarbonate-reversible inhibition of PSII, Xu et al. [18]; unpublished, 1992) explored the bicarbonate binding niche by using different halogenated acetates with different hydrophobicities. Table 1 shows a correlation between the inhibitory activity of the halogenated acetates on the plastoquinone reduction (and equilibration between $Q_A^-Q_B \rightleftarrows Q_AQ_B^-$), the bicarbonate reversibility and the partition coefficient (and, thus, the hydrophobicity) of the inhibitors.

Table 1. A summary of results on halogenated acetates (after C. Xu, Y. Zhu and Govindjee, 1992, unpublished)

Chemicals	τ_{fast} (μs)	[slow component] / [fast component]	Bicarbonate Reversibility	pK_d	Dipole moment (Debye)	Log of partition coefficient
		$[Q_A^-]$ decay				
Acetate	610	0.9	+++++	4.7	1.61	-0.333
Mono-chloro-acetate	845	1.8	++++	2.8	3.25	+0.32
Dichloro-acetate	920	3.2	+++	1.3	2.53	+1.33
Trichloro-acetate	1,700	24.0	+	0.7	2.12	+1.54

Furthermore, monochloroacetate is a novel chemical in the sense that it rephases the flash number dependence of $[Q_A^-]$ with the maxima occurring at even to that at odd flashes suggesting an increase in the Q_B^-/Q_B ratio in the dark [18]. This novel phenomenon contrasts with the well known decrease in this ratio upon the addition of oxidants such as benzoquinone.

4. CONCLUDING REMARKS

The answer to the question raised in the title is: the bicarbonate effect is important for the understanding of the molecular mechanism of plastoquinone reduction in the reaction center (D1 and D2) of PSII. Differences with photosynthetic bacteria have evolutionary as well as mechanistic implications. Research in the laboratories of Alan Stemler, Jack Van Rensen, Bruce Diner, Johnathan Nugent and several others, including our own, promises to provide answers to several unsolved questions including the following: **(1).** What are indeed the active species of the inhibitors as well as the activator (bicarbonate) in view of the fact that charges on the binding proteins may change with pH? **(2).** What is indeed the precise binding niche of bicarbonate? (3). How does bicarbonate function in the protonation of $Q_B^-(Q_B^{2-})$? and **(4).** What is the importance of the bicarbonate effect between "Z" and "Q_A" (see ref. [22]) ? The binding site on the electron acceptor side of PSII is currently been attacked through the technique of site-directed mutagenesis [1,13] and this approach promises to provide answers within the next few years. Modeling of D1 and D2 in the region of $Q_AF_eQ_B$ binding niche will certainly aid in these studies. Our current target is to construct a mutant at the D1-R269 [4]. We consider it important to re-examine the question whether bicarbonate is a *strict* requirement for PSII. We already know that quinol reduction in photosynthetic bacteria does not require it [8,9,11]. Since bicarbonate is suggested to aid in protonation of Q_B^- (Q_B^{2-}), it may not be needed when there is plenty of protons around. Jursinic and Stemler [23] have concluded that when most anions are absent, bicarbonate is not required for efficient electron flow. No $C0_2$ was detected, upon

formate treatment, in these samples that were suspended in $C0_2$ free media, but they had high rates of electron flow. The question by the skeptics remains: Whether the $C0_2$ bound at the reaction center of PSII could be released in a reasonable time and in reasonable quantities to be detected by the methods employed? Since "$C0_2$ free" samples were used, it is possible that even if $C0_2$ from PSII sites was released, it could have rebound to multiple empty sites on the "$C0_2$ free" thylakoid membranes. On the other hand, it is also possible that *water* itself may bind to the site and provide the necessary protons when bicarbonate is absent (see Takahashi [12]). In *vivo*, however it is bicarbonate that remains bound under normal conditions [4]. The answer lies in the formulation of the detailed pathway of protonation for quinol formation.

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