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A HIGH-POTENTIAL REDOX COMPONENT LOCATED WITHIN CYANOBACTERIAL PHOTOSYSTEM II

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Summary

The calcium-dependent oxygen evolution activity of preparations of *Phormidium luridum* shows a marked selectivity in favor of ferricyanide over benzoquinone as Hill oxidant. In addition, the rate of oxygen evolution increases with increasing solution redox potential over the range +350 to +550 mV vs. the standard hydrogen electrode. These properties pertain to both 3-(3,4-dichlorophenyl)-1,1-dimethylurea-sensitive and -insensitive fractions of the total oxygen evolution activity. Neither changes in solution potential nor use of oxidants other than ferricyanide obviate the need for added Ca^{2+}.

To explain these observations, two models are proposed, each of which invokes the existence of a redox component located within Photosystem II and having a midpoint potential greater than +450 mV. In one model, the postulated species is a donor which competes with water for oxidizing equivalents generated by System II. In the other model, the 450 mV species is a high-potential primary acceptor of System II electrons.

Introduction

We have recently described the Ca^{2+}-dependent evolution of oxygen from cell-free preparations illuminated in the presence of ferricyanide [1,2]. The phenomenon is observable after French-press treatment of *Phormidium luridum* or other species of cyanobacteria. Cell breakage effects a release of endogenous calcium and, we believe, allows an inherent Ca^{2+} requirement of the Photosystem II reaction center or water-oxidation apparatus to become manifest.

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Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, 2-(*N*-morpholino) ethanesulfonic acid.

In this report, additional evidence will be presented that the French-press procedure selectively dismantles the cyanobacterial photosynthetic machinery. As in the case of Ca^{2+} dependence, the altered properties of the resulting preparations may provide insight into the functioning of the photosynthetic system in its native state. In particular, we will present evidence of a high redox-potential component located within Photosystem II. The component may or may not be an electron carrier along the main electron transport path involved in oxygen evolution.

Methods

P. luridum var. *olivacae* was grown and subjected to French-press breakage as described in detail elsewhere [2]. Whole cells were removed by centrifugation over a cushion of 0.4 M sucrose. Breakage and assay were carried out in the presence of 8 mg 'Pentex' defatted bovine serum albumin per ml of buffer containing 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) (Sigma) and 5 mM NaOH, pH 6.2. During the assay, 30 mM NaCl was present, as were 6—26 μg chlorophyll/ml.

Oxygen evolution was determined using an oxygen luminometer as described [2]. The practical detection limit for this system is 100 nmol/mg chlorophyll per h. The assay medium for French-press preparations contained 10 mM MES/NaOH, pH 6.2, 30 mM NaCl, and in a total volume of 2.1 ml, 16 mg albumin and 10—20 μg chlorophyll. Other additions were as indicated. Generally, samples were illuminated with continuous saturating light until a steady-state level of oxygen evolution was attained. Where indicated, 20-s pulses of continuous illumination were given, repetition of pulses providing an estimation of the rate of activity loss during illumination. If these losses exceeded 10% during the first pulse, the activity obtained at the onset of illumination was calculated and reported. For light sources, filters and determination of chlorophyll in French-press preparations, see ref. 2.

Solution redox potential determinations were made using a Radiometer combination platinum-calomel electrode and PHM-64 pH meter. Values are presented as mV vs. the standard hydrogen electrode, 240 mV higher than the measured potentials [3]. Stability of the redox potential, generally within 10 mV of the reported value, was ascertained by potential measurement before and after addition of chlorophyll-containing material and exposure to light.

$NaFe^{III}EDTA$ was obtained from Calbiochem, $Na_4SiW_{12}O_4$ (sodium silicotungstate) and $NaReO_4$ from Alpha-Ventron, and $NaVO_3 \cdot xH_2O$ ('sodium vanadate (pure)') from Eimer and Amend, N.Y.C. Benzoquinone was purified by sublimation, and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU; Dupont) by recrystallization from dimethylsulfoxide.

Results

As shown in Fig. 1, curve a, the ferricyanide-coupled oxygen evolution activity of the *P. luridum* French-press preparation is partially insensitive to DCMU; activity falls with increasing DCMU concentration, but approaches an asymptotic value of about 30% of the total. The size of the DCMU-insensitive

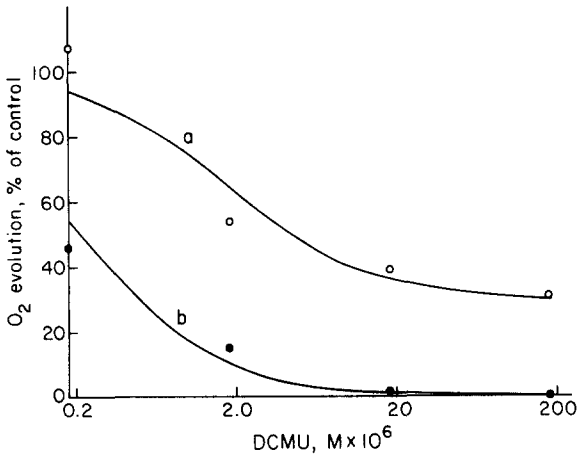

Fig. 1. Effect of DCMU on oxygen evolution from the French-press preparation and whole cells of *P. luridum*. Samples were illuminated with 20 s of saturating light after a 15-min dark incubation in the presence of DCMU as indicated, and at a chlorophyll concentration of 10 μg/ml. Activity losses during illumination were less than 10%. Open circles: French-press preparation isolated and assayed in buffer (see text) containing 33 mM $CaCl_2$, and assayed at 3.2 mM ferricyanide, E = 590 mV vs. standard hydrogen electrode. Closed circles: whole cells in 10 mM MES/30 mM NaCl/1 mM benzoquinone. Lines are calculated on the basis of first-order binding and inhibition by DCMU. For the preparation (curve a) 70% of the total activity was taken to be DCMU-sensitive and the DCMU binding midpoint 2.0 μM. For whole cells (curve b) the line is based on a maximal inhibition of 100%, with a midpoint of 0.2 μM. Control rates of oxygen evolution, obtained in the absence of inhibitor, were for the preparation 65 and for whole cells 112 μmol/mg chlorophyll per h.

fraction varies considerably between preparations [1], but is always much greater than that of whole cells, whether the latter are coupled to the reduction of benzoquinone (Fig. 1, curve b), ferricyanide or CO_2 [4].

As well as being partially insensitive to DCMU, the French-press preparation shows, in the DCMU-sensitive fraction of its oxygen evolution activity, a diminished affinity for the inhibitor (Fig. 1). Half-saturation of the sensitive fraction in the preparation was obtained at about 2.0 μM DCMU, vs. 0.2 μM in intact cells.

Both DCMU-sensitive and -insensitive fractions of oxygen evolution activity in the French-press preparations are Ca^{2+}-dependent; Ca^{2+}-independent oxygen evolution rarely exceeds 10% of the total [2] while 30—60% of the total is DCMU-insensitive (ref. 1 and Fig. 1). It follows that Ca^{2+} is needed at a step in the electron transport pathway before the site of action of DCMU.

To examine the possibility that the effects of Ca^{2+} on oxygen evolution rate were due to interactions with ferricyanide, an effort was made to find alternative oxidants which would support oxygen evolution. In fact, the preparation was highly selective in its ability to utilize exogenous electron acceptors. In this regard it resembles an earlier *P. luridum* preparation based on solubilization of photosynthetic membranes by a non-ionic detergent [4]. Table I shows that of several inorganic oxidants tried, only three, ferricyanide, ferric-EDTA, and orthovanadate were found to be effective, and two supported only low rates of oxygen evolution. A number of oxidants not listed, including K_2IrCl_6, inacti-

TABLE I

Ca^{2+} DEPENDENCE OF LIGHT-SATURATED RATES OF OXYGEN EVOLUTION COUPLED TO VARIOUS ELECTRON ACCEPTORS

French-press preparations of *P. luridim* assayed in the presence of oxidants indicated, concentrations given in mM, except for $NaVO_3$, which is given in μg/ml. If an oxidant failed to support oxygen evolution, ferricyanide was added to test for inactivation of the oxygen-evolving apparatus; in no case presented was the ferricyanide-coupled activity greatly changed by the presence of the other oxidant. An asterisk indicates instability of the measured redox potential, though the latter was always above 500 mV vs. standard hydrogen electrode. Preparation no. 1: steady-state rate under continuous illumination, with or without 3.8 mM $CaCl_2$. Preparation no. 2: illumination with 20 s of continous light, with or without 33 mM $CaCl_2$, and corrected for activity losses (10—20%, see Methods).

	Oxygen evolution (μmol/mg chlorophyll per h)		*E* (mV) (vs. standard hydrogen electron
	No Ca^{2+}	With Ca^{2+}	
Preparation no. 1			
$K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ (0.3, 0.3)	0.6	6.5	470
$NaFe^{III}EDTA$ (30)	0.4	1.1	*
$KMnO_4$ (0.3)	n.d.	<0.1	*
$Na_4SiW_{12}O_4$ + DCMU (0.1, 0.09)	n.d.	<0.1	*
Preparation no. 2			
$K_3Fe(CN)_6$ + $K_4Fe(CN)_6$ (3.2, 0.2)	1.6	9.4	540
$NaVO_3 \cdot XH_2O$ (430)	0.6	1.9	540
$NaReO_4$ (3.2)	n.d.	0.8	545
$KClO_4$ (3.0)	n.d.	0.7	560

n.d., not determined.

vated ferricyanide-coupled oxygen evolution. This was not the case with any of the acceptors appearing in Table I (see legend).

It is important to note that in no instance did substitution of another oxidant for ferricyanide increase oxygen evolution activity in the absence of added Ca^{2+} (Table I) i.e., no oxidant tested was able to bypass the Ca^{2+}-dependent step. This excludes Ca^{2+}-ferricyanide interaction as the origin of Ca^{2+} stimulation of oxygen evolution rate [2].

The lipophilic oxidant benzoquinone supported only low rates of oxygen evolution in French-press preparations (Table II) though it did not inhibit ferri-

TABLE II

LIGHT-SATURATED RATES OF OXYGEN EVOLUTION COUPLED TO BENZOQUINONE AND TO FERRICYANIDE

French-press preparations of *P. luridim* were assayed in either 0.10 mM each benzo- and hydroquinone, or in 0.30 mM each ferri- and ferrocyanide. Centrifugation over a 0.4 M sucrose cushion was applied in the "low whole-cell" preparation, but not in the others. DCMU concentration used was 10 μM.

	Oxygen evolution (μmol/mg chlorophyll per h)		
	Quinone	Quinone + DCMU	Ferricyanide
no $CaCl_2$	1.0	<0.1	0.48
11 mM $CaCl_2$	1.3	<0.1	10.6
11 mM $CaCl_2$, low whole-cell preparation	0.4	n.d.	9.4

n.d., not determined.

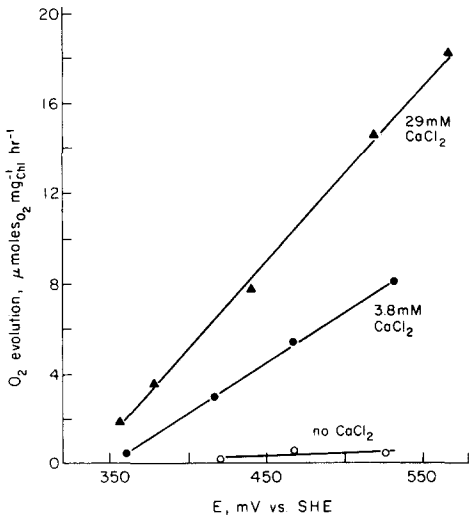

Fig. 2. Effect of solution redox potential on light-saturated rates of oxygen evolution. French-press preparation of *P. luridum* was assayed in the usual buffer containing 0.3 mM ferricyanide and $CaCl_2$ as indicated. Ferrocyanide concentration was varied from 0 to 27 mM. The indicated potential was determined after assay of samples.

cyanide-coupled activity by more than 10% (data not shown). Table II indicates that the benzoquinone-coupled activity was independent of external Ca^{2+} concentration, was DCMU-sensitive and could be selectively diminished by centrifugation of the preparation over 0.4 M sucrose. It is likely that oxygen evolution from the French-press preparation in the exclusive presence of benzoquinone arises from a relatively intact fraction of the preparation, i.e., one which has retained endogenous Ca^{2+} and a System II reaction center/primary acceptor which is inaccessible to added oxidants. Apparently, this fraction couples poorly to ferricyanide, since the total ferricyanide-coupled activity is scarcely diminished by removal of the bulk of benzoquinone-coupled activity (Table III).

Fig. 2 shows that over the range of 350—550 mV vs. standard hydrogen electrode, the oxygen evolution activity of the *P. luridum* French-press preparation follows the solution redox potential established by the ratio of the ferri- and ferrocyanide. At equal concentrations of ferri- and ferrocyanide, 10-fold variation in ferricyanide concentration has no effect on oxygen evolution rate (ref. 2 and Table VI) strongly suggesting that Fig. 2 is an equilibrium titration of a rate-controlling redox component within the preparation. Damage inflicted by oxidants beyond 550 mV prevented attainment of an activity plateau, but it is evident from the data presented that the midpoint of this redox component is at least 450 mV. These findings are similar to those reported previously for a detergent-based *P. luridum* preparation of lower maximal activity [1].

From Fig. 2 it is evident that Ca^{2+} concentration and solution redox potential are distinct in their influence upon oxygen evolution activity; Ca^{2+} dependence shows no sign of diminishing at high solution potential, and at saturating (29 mM) Ca^{2+}, sensitivity to potential is most pronounced.

Discussion

The French-press preparation described here possesses three remarkable properties related to electron transport. First, some 30% of the oxygen evolution activity is insensitive to DCMU (Fig. 1). Second, both DCMU-sensitive and -insensitive fractions of the total activity are highly selective in favor of ferricyanide over benzoquinone as Hill acceptor (Table II). Finally, the light-saturated rate of oxygen evolution increases markedly with the solution redox potential over the range 350—550 mV vs. standard hydrogen electrode (Fig. 2).

Two models, illustrated schematically in Fig. 3, might explain these unusual characteristics. In the first model (Fig. 3a), a redox carrier having a midpoint potential of about 450 mV is capable of donating electrons to the photooxidized System II reaction center in competition with water. Oxidation of this component, which by hypothesis could only carried out by certain one-electron oxidants, would permit water oxidation and the flow of electrons through a conventional System II reducing-side scheme. After the primary acceptor, the electron flow apparently proceeds through both DCMU-sensitive and -insensitive mechanisms. The latter mechanism would be analogous to that proposed previously [4] and by other workers [5—8]. According to this first model, then, redox sensitivity and Hill oxidant selectivity would be due to a component not on the main electron path between water and the terminal Hill acceptor.

In chloroplasts, there appears to be a component similar to the one just postulated [9—11]. In each case, the species implicated has a midpoint of 450—480 mV, and is capable of reducing the photooxidized System II center under conditions where water oxidation is inhibited. The appearance of the same species in the oxygen-evolving preparation described here would open the possibility that this donor controls System II activity in vivo. French-press

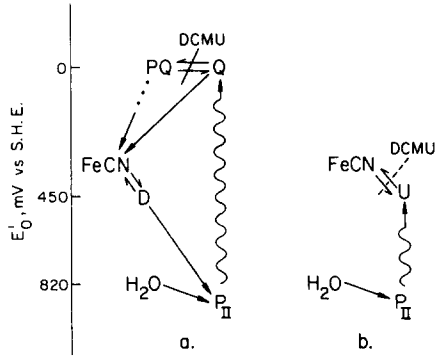

Fig. 3. Hypothetical schemes of electron transport in the *P. luridum* French-press preparation. The following species are positioned in the diagram according to midpoint potential: the 'primary' System II acceptor (Q), the plastoquinone pool including secondary acceptors (PQ), ferri/ferrocyanide (FeCN), the System II reaction center (P_{II}), and molecular oxygen/water at pH 7 (H_2O). As described in the text, the hypothetical donor (D) or acceptor (U) could have midpoint potentials somewhat more oxidizing (lower in the diagram) than shown. Wavy arrows indicate photo-assisted electron transfer; double arrows indicate equilibria established rapidly with respect to the turnover time of oxygen evolution. Partial DCMU sensitivity of electron transfer betweeen U and FeCN is indicated by a dashed line.

treatment might then unmask the redox control of oxygen evolution by increasing access of ferri- and ferrocyanide to the postulated donor.

In the second model, the titrated component lies on the reducing side of System II, conducting electrons from the reaction center to ferricyanide. Since over 90% of the oxygen evolution activity in these preparations is redox-sensitive (Fig. 1) the state of reduction of the proposed component must control both DCMU-sensitive and -insensitive oxygen evolution. In the context of a reducing-side model this would require the component to be located between the System II reaction center and the point at which DCMU blocks electron flow. However, because of its high midpoint potential, the proposed species cannot be the conventional System II acceptor; the latter is known in chloroplasts to have a potential below 0 mV [12].

Considerable evidence suggests the presence, in chloroplasts, of two one-electron acceptors whose reduction is DCMU insensitive [13—15]. Furthermore, the work of Ikegami and Katoh [13] indicates that one of these acceptors can be titrated with ferricyanide at a midpoint of 360 mV. It is possible that in the French-press *P. luridum* preparation, electron flow is constrained to proceed to ferricyanide via a similar high-potential acceptor (Fig. 3b). While, in chloroplasts, DCMU blocks access of ferricyanide to the 360 mV component, in the present case DCMU blockage would appear to be only partial, and involve a site distinct from that which normally blocks flow from the low potential acceptor. This difference would account for the two inhibition midpoints seen in Fig. 1.

In each of the models presented above, it is necessary to make the assumption that the redox carrier be capable only of one-electron reactions, otherwise it is difficult to account for the observed selectivity regarding Hill acceptors. Under the experimental conditions employed, ferricyanide and benzoquinone have similar midpoints for one- and two-electron reactions, respectively, but the one-electron potential of benzoquinone is very low [16]. While it might be expected that electron transport components prior to the site of DCMU inhibition be one-electron carriers [17,18] and hence couple poorly to quinone [8,19], in our experiments, neither DCMU-sensitive nor -insensitive oxygen evolution was supported by this oxidant. Each of our models invokes a one-electron component whose oxidation by ferricyanide is required for oxygen evolution. Whether this species is located on the oxidizing side (first model) or the reducing side (second model) of System II, cannot be determined at present. Flashing-light experiments, which may clarify this issue, are in progress.

In both models offered, the postulated component has some similarity to cyanobacterial cytochrome *b*-559 [20,21]. While in chloroplasts, this species has been excluded by other workers as either the titrated competitive donor [22] or the alternative acceptor [13], its role in the cyanobacterial preparation described here remains to be clarified.

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