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INCREASE EFFECTED BY CALCIUM ION IN THE RATE OF OXYGEN EVOLUTION FROM PREPARATIONS OF *PHORMIDIUM LURIDUM*

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Summary

The presence of Ca^{2+} causes a twentyfold or greater increase in the rate of oxygen evolution by cell-free preparations of *Phormidium luridum*. The requirement for Ca^{2+} is specific; other divalent cations are much less effective or are inhibitory. The rate of the Hill reaction is maximal at 30 mM $CaCl_2$ in both detergent-free and Brij 35 preparations. The 3-(3,4-dichlorophenyl)-1,1-dimethylurea-insensitive component of oxygen-evolving activity in each preparation also shows the requirement for added Ca^{2+}. This indicates that Ca^{2+} is acting close to the oxygen-evolving reaction center of Photosystem II. Defatted bovine serum albumin increases the rate of oxygen evolution in the detergent-free preparation, but does not compete with Ca^{2+}, discounting fatty acid mediation of the effects of Ca^{2+}. Neither excess Hill acceptor nor uncouplers of photophosphorylation diminish the stimulatory effects of Ca^{2+}.

Divalent cations alter light energy distribution between the photosystems [1], and can increase light-saturated Photosystem II activity [2—8]. Ca^{2+} and Mg^{2+} have almost invariably been reported to be identical in their effects. In contrast, we report here a strong and specific requirement for Ca^{2+} in the evolution of oxygen by preparations from *Phormidium luridum*. Fredricks and Jagendorf [2] observed a similar phenomenon in *Anacystis nidulans*; however, the rate increase described below is far greater and the requirement far more than any previously reported.

Phormidium luridum var. *olivacae*, kindly provided by Dr. P. Thornber, were grown at 25°C under 10^4 ergs·cm^{-2}·s^{-1} fluorescent light, in Kratz and Myers medium D [9]. The EDTA concentration was twice that prescribed. Cells from actively growing cultures were harvested and washed in buffer

Abbreviations: EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

containing 10 mM MES/NaOH, 30 mM NaCl, and 1 mM EGTA at pH 6.1. Resuspension and all following steps employed the same buffer without EGTA. Experiments were performed using a broken-cell preparation. Washed *Phormidium* were passed once through a French pressure cell at 24 000 lb/inch^2, 5°C, and a chlorophyll concentration of 10—20 $\mu g \cdot ml^{-1}$. Low-speed centrifugation removed whole cells and dense debris. 1-ml aliquots were taken from the supernatant and diluted with 1 ml buffer containing any additions. To insure stable activity, samples were kept on ice in darkness for 2—4 h before measurement of oxygen evolution. Addition of a 1:1 mixture of potassium ferri and ferrocyanide 15 min prior to illumination provided the Hill acceptor, poised at 200 ± 20 mV v.s. S.C.E.

Illumination by a 650 W tungsten lamp permitted measurement of oxygen evolution rates which corresponded to at least 90% of light-saturated rates. A Zeiss heat filter, potassium chromate solution, and calibrated screen interceded between lamp and sample. Oxygen was detected using the oxygen luminometer [10]. Experiments were performed at 23—25°C and at a dark background of less than 5 ppm O_2. The chlorophyll content of broken-cell preparations was determined by measuring absorbance at 680 nm using a Cary 14 spectrophotometer with a scattered transmission accessory. This measurement was standardized by preparing broken cells from a suspension of whole cells whose chlorophyll content had been determined by methanol extraction [11]. The highest rates of oxygen production reported below are 40% of those reported for whole *Phormidium* cells coupled to ferricyanide or benzoquinone [16].

The effect of Ca^{2+} on light-saturated oxygen evolution from the broken-cell preparation is shown in Fig. 1. The dependence of rate on Ca^{2+} concentration up to 30 mM is approximately described by a hyperbolic saturation curve. Additional Ca^{2+} in excess of this concentration is inhibitory. Effects of other divalent cations are seen in Table I. Most remarkable is the Ca^{2+}/Mg^{2+} specificity; $CaCl_2$ is eight times as effective as $MgCl_2$ in stimulating the Hill

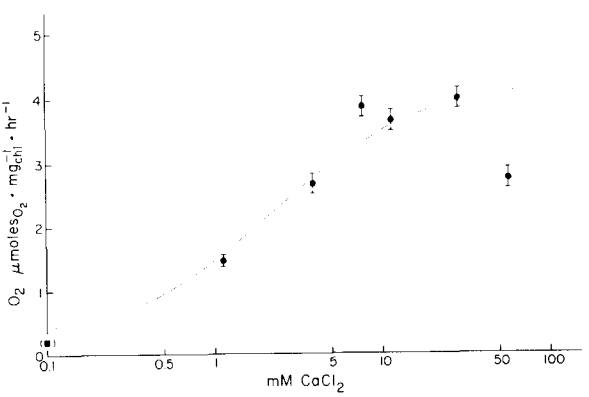

Fig. 1. Effect of Ca^{2+} on the rate of oxygen evolution from the broken-cell preparation. Point in parenthesis at extreme left indicates rate in the absence of added Ca^{2+}. Error bars reflect uncertainly in individual determinations. Line is theoretical, describing hyperbolic saturation of a single-ion binding process scaled vertically by extrema of observed rates with half-maximal binding at 2.4 mM. Assay mixture contained 10 mM MES, 30 mM NaCl, 0.30 mM each $K_3Fe(CN)_6$, and $K_4Fe(CN)_6$, 18 μg of chlorophyll in 2.1 ml total volume. The pH was 6.1, light intensity $6 \cdot 10^4$ ergs $\cdot cm^{-2} \cdot sec^{-1}$.

TABLE I

EFFECTS OF VARIOUS CATIONS AND DCMU ON RATE OF OXYGEN EVOLUTION

Conditions as in Fig. 1. DCMU, where indicated, was added about 2 h before assay.

Additions (mM)	Activity (μmol $O_2 \cdot mg^{-1}$ Chl$\cdot h^{-1}$)
None	0.27
$CaCl_2$, 7.6	4.6
$SrCl_2$, 7.7	0.90
$MgCl_2$, 8.0	0.56
$BaCl_2$, 7.0	0.10
$MnCl_2$, 8.0	0.14
$MnCl_2$, 8.0 + + $CaCl_2$, 7.6	1.9
DCMU,0.095 + $CaCl_2$, 7.6	3.0
DCMU,0.095	$\leq$0.07

reaction. The intermediate effectiveness of $SrCl_2$ and the inhibition by $BaCl_2$ suggest that ionic size may be important. Inhibition by $MnCl_2$ (50% at 8 mM) is independent of $CaCl_2$ and may due to substitution of Mn^{2+} for H_2O as donor to Photosystem II [12].

The broken-cell preparation carried out oxygen evolution in the presence of DCMU. Acitivity is lower but more stable in the presence of this inhibitor. Table I shows that the DCMU-insensitive component of oxygen evolution has a strong Ca^{2+}-dependence. This result indicates that Ca^{2+} is acting close to the oxygen-evolving center. Our conclusion is based on the assumption that DCMU-insensitive oxygen evolution involves only that portion of the electron transport chain preceeding the DCMU-sensitive site.

Oxygen evolution rate is increased by Ca^{2+} in the presence of 10 μM carbonylcyanide 3-chlorophenylhydrazone or atebrin, showing that the effect is not dependent upon tight coupling of electron flow to photophosphorylation [13].

Fatty acids are powerful inhibitors of the Hill reaction [14,15], and their aggregation by Ca^{2+} might result in an increased rate of oxygen production. If this were the mechanism of Ca^{2+} action, defatted bovine serum albumin would partially substitute for $CaCl_2$. We find (Table II) that added albumin increases oxygen evolution rates and improves stability under

TABLE II

EFFECT OF Ca^{2+} ON THE RATE OF OXYGEN EVOLUTION IN THE PRESENCE OF DEFATTED BOVINE SERUM ALBUMIN

Conditions as in Fig. 1, except that each sample contained 4 μg of chlorophyll. "Pentex" defatted bovine serum albumin (fraction V, Miles Laboratories) was added 2—4 h before assay.

Albumin/Chl (g/mg)	$CaCl_2$ (mM)	Activity (μmol $O_2 \cdot mg^{-1}$ Chl$\cdot h^{-1}$)
0	0	< 0.1
0	7.6	4.9
4.2	0	< 0.5
4.2	7.6	17.0
8.3	0	< 0.1
8.3	7.6	13.0

illumination; however, the increase in oxygen evolution rate caused by Ca^{2+} is even more pronounced in the presence than in the absence of albumin. Clearly Ca^{2+} and this fatty acid-binding protein do not compete.

The Hill reaction rate in the broken-cell preparation is saturated with respect to ferricyanide concentration at about 3 mM. Even under these conditions, stimulation by Ca^{2+} is undiminished. Apparently Ca^{2+} does not act by increasing permeability of the preparation to the exogenous electron acceptor.

The requirement for Ca^{2+} holds at all intensities along the light-saturation curve. Lower concentrations of Ca^{2+} are required for maximal activity at lower light intensities, however, indicating that Ca^{2+} may be affecting both the number of active units and the turnover kinetics. Flash experiments are needed to separate these two components of Ca^{2+} action.

Oxygen evolution rates of whole cells are not increased by the addition of Ca^{2+} to suspending buffer containing Hill acceptor. This is true even after cells have been washed with EGTA.

A previously published procedure [16] was employed to obtain cell-free preparations of *Phormidium* in the presence of Brij 35, a nonionic detergent. These preparations carry out oxygen evolution in the presence of DCMU and couple preferentially to high-potential one-electron oxidants. We find that the Ca^{2+} originally included in the assay buffer is required for full activity. The low activity seen in the absence of Ca^{2+} can be further depressed by treatment of the preparation with washed Dowex-50W. Activity is fully restored upon addition of Ca^{2+} before assay. The dependence of oxygen evolution rate on Ca^{2+} concentration is very similar to that observed in the detergent-free preparation.

The observations reported above indicate that Ca^{2+} is increasing the rate of oxygen evolution in these preparations by acting close to the point of water oxidation rather than on the reducing side of the site of DCMU inhibition. Added Ca^{2+} may be binding together soluble components [2,17] of the oxygen-evolving apparatus, or facilitating dark reactions which limit oxygen evolution at high light intensities, or both. Access of the exogenous Hill acceptor is not rate limiting, even under saturating light, and neither photophosphorylation nor fatty acid inhibition of electron transport appear to be involved. The large and specific effect of Ca^{2+} reported here may be of utility in the isolation and stabilization of purified oxygen-evolving reaction centers.

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