ionization of the ligands; the p$K$ values could be estimated as 10.7 and 12.1, respectively. However, the latter is so high that it is likely to represent an indirect effect correlated with protein denaturation.

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## Oxygen evolution by isolated chloroplasts with carbon dioxide as the hydrogen acceptor. A requirement for orthophosphate or pyrophosphate

Chloroplasts isolated in sugar media and incubated under aerobic conditions in the presence of bicarbonate will evolve oxygen in the light at rates comparable to those obtained in the presence of artificial hydrogen acceptors[1]. Simultaneous measurements of oxygen and carbon dioxide gave parallel progress curves, each with an initial lag, and values in agreement with the fixation of one molecule of carbon dioxide for each molecule of oxygen produced. In the absence of added bicarbonate, oxygen evolution started but fell off and ceased as the endogenous carbon dioxide became exhausted. At this point evolution could be restarted by the addition of bicarbonate[1]. Fig. 1 shows a comparable requirement for $P_i$ by chloroplasts prepared and incubated in phosphate-free media. Again, oxygen evolution started after an initial lag but then fell off, presumably as the endogenous phosphate was utilised. When net evolution

had ceased (*i.e.* when oxygen production and consumption were in balance) the addition of $P_i$ brought about an almost immediate resumption of evolution. The net oxygen evolution so induced bore a quantitative relationship to the inorganic phosphate added. It will be seen in Fig. 1 that approx. 3 molecules of oxygen were evolved for each molecule of phosphate added. A carbon dioxide-dependent incorporation of $^{32}P$ into sugar phosphates by isolated chloroplasts in similar reaction mixtures has already been demonstrated[2]. These results therefore imply that the reactions which predominated during the period of measurement were those leading initially to the synthesis of triose phosphate according to the overall equation

$$3CO_2 + 3H_2O + H_3PO_4 \longrightarrow \text{triose phosphate} + H_2O + 3O_2$$

It should be noted that the results have no direct bearing on the relationship between oxygen evolution and photophosphorylation[3,4]. However, if the reactions are (as we suppose) those of the carbon cycle, the synthesis of 1 molecule of triose phosphate would require the production and consumption of 9 molecules of ATP.

*In vitro*, photosynthetic carbon dioxide fixation (and its associated oxygen evolution) is catalysed at high rates only by chloroplasts with intact envelopes (see *e.g.* WALKER[5]). It would seem therefore (Fig. 1) that $P_i$ must penetrate the envelope with great rapidity (comparable with $CO_2$–bicarbonate) since the maximum rate of evolution was resumed within 30 sec of its addition.

Recently, exceptionally high rates of carbon dioxide fixation (155 $\mu$moles/mg chlorophyll per h) have been reported by JENSEN AND BASSHAM[6] for chloroplasts

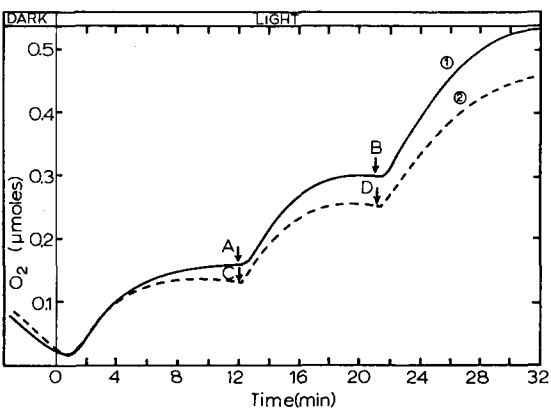


Fig. 1. Polarographic records of oxygen evolution by isolated spinach chloroplasts. Reaction mixtures (pH 7.5) contained in a total of 1.8 ml in $\mu$moles: *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid[6,7], 84.6; $MnCl_2$, 1.7; $MgCl_2$, 1.7; NaCl, 4.0; EDTA, 3.4; sodium isoascorbate, 3.4; sorbitol, 558; and chloroplasts (162 $\mu$g chlorophyll). Chloroplasts were prepared in sorbitol media[8] in which 0.05 M 2-(*N*-morpholino)ethane sulphonic acid[7] at pH 6.1 (*cf.* JENSEN AND BASSHAM[6]) was substituted for 0.1 M phosphate at pH 6.8. Oxygen was measured in a closed, stirred vessel at 20° with a stationary platinum electrode separated from the reaction mixtures by a teflon membrane[1]. Near-saturating red light was provided by a 150-W quartz–iodine slide-projector. A perspex filter (transmitting 90% of the incident light above 610 m$\mu$) and 4″ of water were interposed between the light source and the reaction vessel. Arrows indicate the addition of sodium orthophosphate (Curve 2, 0.05 $\mu$mole in 5 $\mu$l at C, 0.10 $\mu$mole in 10 $\mu$l at D) and sodium pyrophosphate (Curve 1, 0.025 $\mu$mole in 2.5 $\mu$l at A, 0.05 $\mu$mole in 5 $\mu$l at B). The vertical gap between the two curves is a consequence of slight loss of activity as the chloroplasts age. In this experiment the pyrophosphate Curve 1 was obtained immediately prior to the orthophosphate Curve 2. When the experiment was repeated, in the reverse order and with fresh chloroplasts, the orthophosphate curve came above the pyrophosphate curve by a similar margin.

isolated in sorbitol media and incubated in reaction mixtures containing pyrophosphate. The nature of the pyrophosphate stimulation was not clarified. Fig. 1 shows that when sodium pyrophosphate was substituted for orthophosphate it produced virtually the same effect (apart from a slightly longer interval between its addition and the attainment of the maximum rate) but at half the concentration. This would be in accord with a rapid and complete hydrolysis so that one molecule of pyrophosphate would act as two molecules of orthophosphate. However, in the absence of added substrate (other than carbon dioxide) orthophosphate can induce a marked lengthening of the initial lag in oxygen evolution at concentrations above $2.5 \cdot 10^{-4}$ M. Pyrophosphate is ineffective, in this respect, in concentrations as high as $2.5 \cdot 10^{-2}$ M. This is difficult to equate with a rapid and uncontrolled hydrolysis of pyrophosphate and the question of a more direct participation of pyrophosphate in photophosphorylation must remain open.

The possibilities that AMP might serve as a direct acceptor for pyrophosphate, or that ADP could react with pyrophosphate to give one molecule of ATP and one molecule of orthophosphate are worth examination. *In vivo* they could provide a mechanism for the utilisation of pyrophosphate produced in starch and sucrose synthesis. A full account of this work will be published at a later date.

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