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A Ca^{2+}-STIMULATED INCORPORATION OF PHOSPHATE INTO ATP IN CHLOROPLASTS; THE PROBLEM OF ALLOTOPY

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Summary

Under continuous illumination, a Ca^{2+}-induced incorporation of phosphate into ATP is found in chloroplasts, with properties similar to the Mg^{2+}-catalyzed ATP—P_i exchange. Thus, the chloroplast coupling factor has no allotopic properties towards divalent cations.

Until recently, the behaviour of chloroplast coupling factor 1 (CF_1) towards divalent cations was classified as 'allotopic' [1]: the activities of the membrane-bound form, like photophosphorylation and light-induced ATPase, were Mg^{2+}-dependent [2,3], whereas the ATPase of purified CF_1 was dependent on the presence of Ca^{2+} [4]. Two years ago Nelson et al. [5] showed that isolated CF_1, under appropriate conditions, could also exhibit Mg^{2+}-catalyzed ATPase.

Considerations of symmetry would require the existence of membrane-bound Ca^{2+}-dependent activities as well. It occurred to us that the reason why these had not been observed might be the unique energetic feedback features of the system: chloroplast ATPase characteristically requires a high-energy conformation, and during Mg^{2+}-ATPase, this conformation under suitable conditions can be maintained by the ATPase reaction itself [6—8]. In other words, Mg^{2+} fulfills at least a dual function here: as a cofactor in the turnover of the ATPase enzyme, and in coupling it to the formation of the high-energy state required for maintaining that turnover. It might very well be that Ca^{2+} could substitute for Mg^{2+} in the first function, like it does with

Abbreviations: CF_1, chloroplast coupling factor 1; DCCD, dicyclohexyl carbodiimide.

TABLE I

COMPARISON OF Mg^{2+}- AND Ca^{2+}-STIMULATED PHOSPHATE INCORPORATION IN CHLOROPLASTS

Spinach chloroplasts were prepared as in ref. 7, but in a medium containing 100 mM KCl, 0.5 mM Na-EDTA and 5 mM Na-tricine (pH 8.2). The reaction mixture contained, in addition, 10 μM pyocyanin, 3 mM P_i containing about 10^6 cpm of $^{32}P_i$, and 10 mM DTE and 5 mM $MgCl_2$ or $CaCl_2$ as indicated. Chlorophyll concentration was 50 μg/ml, temp. 25° C, pH 8.0. After a 5 min preillumination period (Stage A) the incorporation reaction was started by addition of ATP to a final concentration of 5 mM. After again 5 min in the light or the dark (Stage B) the reaction was terminated by addition of trichloroacetic acid (final concentration 4%), and $^{32}P_i$ incorporation into the organic fraction determined essentially as in ref. 12. In Expt 1, less than 5% of the ATP was broken down by dark Mg^{2+}—ATPase during the course of the exchange. Figures given in μmoles P_i incorporated per h per mg chlorophyll.

Stage		Divalent cation present		
A	B	Mg^{2+}	Ca^{2+}	None
Experiment 1				
+ DTE	dark	6.2	0	0
+ DTE	light	10.3	5.9	0.8
Experiment 2				
— DTE	light	10.4	1.0	—
+ DTE	light	18.8	7.3	—
+ DTE	light + DCCD 10 μM	15.1	5.7	—
+ DTE	light + DCCD 50 μM	5.7	0.8	—

the purified enzyme, but not in the latter. In that case, it should be possible to observe a Ca^{2+}-stimulated turnover of the bound ATPase enzyme under conditions of an externally applied high-energy state.

In Table I it is shown that, indeed, under continuous illumination a Ca^{2+}-stimulated phosphate incorporation into ATP can be induced in chloroplasts, with 40—60% of the activity of the Mg^{2+}-stimulated ATP—P_i exchange. Like the latter, it is greatly enhanced by preillumination in the presence of the di-sulphydryl compound dithioerythritol [1,9,10] and inhibited by dicyclohexyl carbodiimide (DCCD) (Expt 2); but whereas the Mg^{2+}-stimulated exchange after induction continues in the dark by virtue of simultaneous ATPase activity [10], the Ca^{2+}-stimulated reaction is strictly dependent upon continuous external energy supply in the form of light (Expt 1). As yet, it is not clear whether this Ca^{2+}-stimulated phosphate incorporation represents a true ATP—P_i exchange or an ATPase reaction followed by ATP-synthesis. In view of the absolute requirement for Mg^{2+} in photophosphorylation, the latter possibility seems less probable, unless some firmly-bound Mg^{2+} would suffice here. Besides, we were not able to show any significant Ca^{2+}-ATPase activity in chloroplasts by varying the energy state of the chloroplasts over a wide range by background illumination and/or addition of uncoupler. The light-dependent Ca^{2+}-ATPase found by Bennun and Avron [11] might however be relevant in this respect.

The important point is that under the proper energetic conditions also membrane-bound CF_1 exhibits Ca^{2+}-stimulated activity. The problem of the 'allotopy' of CF_1 towards different cations thereby is changed into a problem of the effect of divalent cations on energy conservation.

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