DEMONSTRATION OF ACID–BASE PHOSPHORYLATION IN CHROMATOPHORES IN THE PRESENCE OF A K^+ DIFFUSION POTENTIAL

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1. Introduction

Hind and Jagendorf [1] reported that chloroplasts can synthesize a small amount of ATP in total darkness when transferred from an acid to an alkaline pH. The yield of ATP obtained in this acid–base phosphorylation [2] as well as in postillumination ATP synthesis in chloroplasts [3] was found to be dependent on the number of protons accumulated inside the particles [4–8] and on the size of the pH gradient (Δ pH) formed across their membrane [2,3,9]. When the Δ pH dropped below a value of 2.5 pH units almost no ATP synthesis was observed in either the acid–base [2,10] or the postillumination [9,10] type experiments. The very low level of postillumination ATP synthesis found in chromatophores [11–13] has also been traced to the low Δ pH created during illumination, since an increase in this Δ pH resulted in a pronounced increase in the yield of ATP [13]. The increase in light-induced Δ pH was realized by adding to the light stage either permeant anions, e.g. thiocyanate or perchlorate, or permeant cations, such as KCl in the presence of valinomycin.

Recently it has been reported that at suboptimal conditions for postillumination and acid–base phosphorylation in chloroplasts ATP synthesis could be stimulated by an artificially induced K^+ diffusion potential [9,10,14]. Similarly, the low level of postillumination ATP formed in chromatophores in the absence of permeant anions or cations in the light stage was found to be markedly stimulated by a K^+ diffusion potential created by the addition of valinomycin+ KCl to the dark stage [15].

In this communication we have examined the system of acid–base phosphorylation in *Rhodospirillum rubrum* chromatophores. Almost no phosphorylation was obtained, even under conditions which were found to be optimal in chloroplasts [2,6], namely when succinate was present in the acid stage and the pH difference between both stages was above 3.0 pH units. These conditions are therefore insufficient for an active acid–base phosphorylation in chromatophores. A large amount of ATP (50–60 μmoles/mg bacteriochlorophyll) was however synthesized when valinomycin and KCl were added to the base stage. But in chromatophores, unlike in chloroplasts [10], the stimulation by the K^+ diffusion potential increased with an increase in the Δ pH. This stimulation was dependent on the supply of protons, since it required the presence of succinate or other compounds that can penetrate and buffer the internal space of the chromatophores in the acid stage.

2. Experimental

The growth of *R. rubrum* cells, the isolation and storage of chromatophores was carried out according to Gromet–Elhanan [16].

Acid–base experiments were performed as described by Jagendorf and Uribe [2]. In the acid stage chromatophores containing 200 μg bacteriochlorophyll were added to a solution containing 50 mM sodium succinate at pH 5.0 in a final volume of 0.95 ml. After 2 min at room temperature the following components were added in 0.9 ml to give in the base stage the following final concentration in 1.85 ml: 53 mM Tricine–NaOH pH 8.5, 2.7 mM $MgCl_2$, 1.3 mM ADP,

Table 1
Stimulation of acid–base phosphorylation by a K^+ diffusion potential

Additions to the base stage	ATP formed	
	–valinomycin	+valinomycin
	nmoles/mg	bacteriochlorophyll
None	3.5	4.1
KCl, 100 mM	3.3	57.5
KCl, 90 mM, KSCN, 10 mM	–	10.7

Conditions were as described under Experimental. Where indicated 10 μM valinomycin was added to the base stage.

0.88 mM sodium phosphate (containing 2×10^7 cpm ^{32}P), 20 mM glycose, 10 U of hexokinase and enough NaOH to bring the final pH to 8.5. After 30 sec at room temperature the reaction was stopped by the addition of perchloric acid to a final concentration of 3%. After centriguation 0.3 ml of 5 N-H_2SO_4 were added to 1.2 ml of supernatant. The samples were treated for 10 min at 100°C [13] and assayed for ATP^{32} according to Avron [17].

3. Results and discussion

Table 1 shows that phosphorylation following acid–base transition was very low in chromatophores but it could be considerably increased by the addition of valinomycin together with KCl to the base stage. Neither KCl nor valinomycin alone had any effect, and when Cl^- was partially replaced by a permeant anion like SCN^- [18,19] ATP formation was markedly inhibited (table 1). It is therefore concluded that the imposition of a K^+ diffusion potential can stimulate acid–base phosphorylation also in chromatophores. In this system, as in postillumination ATP synthesis in chloroplasts [9] and chromatophores [15], the degree of stimulation depends on the level of the diffusion potential, since it decreased in the presence of permeant anions which reduce the magnitude of the diffusion potential.

The relative stimulation by valinomycin+KCl in chloroplasts was higher the smaller the Δ pH between the acid and base stages [10]. Indeed, when the Δ pH was lower than 2.5 pH units most of the ATP synthesis was due to the added membrane potential [10, 14,20], while when it was higher than 3.5 pH units there was already a high yield of ATP in the absence of valinomycin and KCl and very little further stimulation was obtained by the added membrane potential [2,10,20]. In chromatophores, on the other hand, an increase from 0.5 to 3.5 in the pH difference between both stages did not lead to any significant increase in ATP synthesis in the absence of valinomycin+KCl (table 2). Moreover, the relative stimulation by the diffusion potential was higher the higher the pH difference between both stages and even at a Δ pH of 3.5 most of the ATP synthesis was due to the added diffusion potential (table 2). The drastic reduction in ATP synthesis at pH 4.5 in the acid stage was found to be caused by an irreversible aggregation of the chromatophores at this pH and therefore all further experiments were carried out at pH 5.0 in the acid stage.

The above results indicate that, while in chloroplasts a transition from pH 5.0 to 8.5 is already sufficient to drive ATP synthesis, in chromatophores these conditions are insufficient. A possible reason for this difference can be in a difference in the number of protons accumulated inside the particles. Acid–base phosphorylation was high in chloroplasts when the difference in pH between the acid and base stages was large and enough protons were supplied by incubating them with acids like succinate, that can penetrate and buffer their internal space in the acid stage [2,4]. Uribe and Jagendorf have also reported [4,6] that a portion of the succinate that penetrated into the chloroplasts in the acid stage was retained on the transition from pH 4.0 to 8.5 and the amount of ATP formed after this acid–base transition was related to the amount of stored succinate. Since the ability to store the succi-

Table 2
Dependence of the stimulation of phosphorylation by the diffusion potential on the pH of the acid stage

pH of the acid stage	ATP formed		+val + KCl
	−valinomycin −KCl	+valinomycin +KCl	−val −KCl
	nmoles/mg	bacteriochlorophyll	
4.5	3.2	13.6	4.2
5.0	3.9	57.0	14.6
5.5	3.6	35.3	9.8
6.0	2.1	–	–
7.0	2.5	7.0	2.8
8.0	2.6	5.8	2.2

Conditions were as described under Experimental, except that in the acid stage 50 mM sodium succinate were present at the indicated pH. The final pH of the base stage was 8.5, and where indicated 10 μM valinomycin and 100 mM KCl were added to this stage.

nate and to form ATP decreased when chloroplasts particles were shrunken by exposure to sucrose they suggested that the internal osmotic space is an important factor in determining the amount of the stored organic acid, and consequently the capacity to accumulate protons and to form ATP.

As illustrated in table 3 an active acid–base phosphorylation in chromatophores was dependent even at a Δ pH of 3.5 on the addition of succinate to the acid stage and valinomycin+KCl to the base stage. Omission of either one of these supplements drastically reduced the yield of ATP. These results indicate that succinate can act as a buffer also in chromatophores, but its buffering capacity is not enough to drive ATP synthesis at a Δ pH of 3.5 pH units. In acid–base transition experiments the internal pH is expected to equal that of the outside pH, thus the most effective buffer should be a compound whose pK lies in the region of the outside pH. In chloroplasts, the pH of the acid stage was around 4.0 and the most effective buffer found was succinate with a pK = 4.2. In chromatophores the pH of the acid stage was kept around 5.0 and therefore compounds with a similar pK might be more suitable buffers. Indeed pyridine (pK = 5.2) was found to be more effective than succinate when they were used at similar concentrations, but even pyridine was not effective enough to drive the synthesis of a large amount of ATP in the absence of valinomycin+KCl (table 3).

Table 3
Dependence of the stimulation of phosphorylation by the diffusion potential on the presence of buffers

Additions to the acid stage	ATP formed	
	−valinomycin −KCl	+valinomycin +KCl
	nmoles/mg	bacteriochlorophyll
None	–	5.9
Succinate, 46 mM	3.1	28.1
Pyridine, 8.5 mM	3.0	18.9
Pyridine, 25 mM	4.3	–
Pyridine, 41 mM	4.6	40.2

Conditions were as described in table 2, except that the pH of the acid stage was 5.0 and when no buffer was added to this stage the pH was adjusted with HCl.

The results described provide further evidence for the suggestion that the membrane potential created by valinomycin+KCl can affect ATP synthesis by increasing the electrochemical gradient of protons. The low values of acid–base phosphorylation obtained in chromatophores in the absence of valinomycin+KCl even at a Δ pH of 3.5 seem to be due to an insufficient supply of protons in the presence of succinate or pyridine.

References

[1] Hind, J., and Jagendorf, A. T. (1965) J. Biol. Chem. 240, 3195–3201.

[2] Jagendorf, A. T. and Uribe, E. (1966) Proc. Natl. Acad. Sci. U.S. 55, 170–177.

[3] Hind, G. and Jagendorf, A. T. (1963) Proc. Natl. Acad. Sci. US. 49, 715–722.

[4] Uribe, E. and Jagendorf, A. T. (1967) Plant Physiol. 42, 697–705.

[5] Uribe, E. and Jagendorf, A. T. (1967) Plant Physiol. 42, 706–711.

[6] Uribe, E. and Jagendorf, A. T. (1968) Arch. Biochem. Biophys. 128, 351–359.

[7] Nelson, N., Nelson, H., Naim, J. and Neumann, J. (1971) Arch. Biochem. Biophys. 145, 263–267.

[8] Avron, M. (1971) In: Proceedings of IInd International Congress on Photosynthesis Research (Forti, G., Avron, M. and Melandri, B. A., eds.) Vol. 1, pp. 867–871, Dr. W. Junk, The Hague.

[9] Schuldiner, S., Rottenberg, H. and Avron, M. (1973) Eur. J. Biochem. 39, 455–462.

[10] Schuldiner, S., Rottenberg, H. and Avron, M. (1972) FEBS Letters 28, 173–176.

[11] Zaugg, W. S. and Vernon, L. P. (1966) Biochemistry 5, 34–40.

[12] Horio, T., Von Stedingk, L.-V. and Baltscheffsky, H. (1966) Acta Chem. Scand. 20, 1–10.

[13] Leiser, M. and Gromet-Elhanan, Z. (1974) J. Biol. Chem. in press.

[14] Uribe, E. G. (1973) FEBS Letters 36, 143–147.

[15] Gromet-Elhanan, Z. and Leiser, M. (1974) J. Biol. Chem. in press.

[16] Gromet-Elhanan, Z. (1974) J. Biol. Chem. 249, 2522–2527.

[17] Avron, M. (1960) Biochim. Biophys. Acta 40, 257–272.

[18] Gromet-Elhanan, Z. (1972) Biochim. Biophys. Acta 275, 125–129.

[19] Gromet-Elhanan, Z. and Leiser, M. (1973) Arch. Biochem. Biophys. 159, 583–589.

[20] Uribe, E. G. and Li, B. C. Y. (1973) Bioenergetics 4, 435–444.