MEMBRANE POTENTIAL AS A DRIVING FORCE FOR ATP SYNTHESIS IN CHLOROPLASTS

SHIMON SCHULDINER, HAGAI ROTTENBERG and MORDHAY AVRON
Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel

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1. Introduction

ATP synthesis driven by ion gradients has been observed in chloroplasts and mitochondria. Jagendorf and Uribe [1] showed that a pH gradient can drive ATP synthesis in chloroplasts. In a comparable experiment with valinomycin treated mitochondria, only a very small amount of ATP was synthesized [2]. K^+ efflux from preloaded mitochondria was shown to be coupled to the synthesis of up to 20 μmoles ATP/g protein [3, 4].

These experiments were interpreted [1, 4, 5] as a manifestation of the ability of the proton electrochemical gradient (proton motive force) to drive ATP synthesis as proposed by Mitchell [6]. The suggestion that in mitochondria the membrane potential is the dominant component of the proton motive force [7, 8], whereas in chloroplasts ΔpH is dominant [7, 9] is in agreement with the above mentioned experiments. However, it should be possible in both systems to utilize both forms of energy. That is, one should be able for example to drive ATP synthesis in chloroplasts by an appropriate membrane potential. If it is assumed that this membrane potential can drive ATP synthesis only by coupling it to a H^+ efflux, the best conditions for observing such an effect would be realized when enough protons are available in the osmotic space of the chloroplasts, while nevertheless, the proton concentration gradient is too small to drive the phosphorylation by itself. Chloroplasts can be loaded with protons either by buffering the interior of the chloroplast at an appropriate pH [1] or by light induced proton uptake with or without additional internal buffers [10, 11]. The extent of ΔpH created can be controlled by varying the light intensity [9]. To be effective, the membrane potential across the chloroplast membrane should be positive inside, and can be created by providing a high external concentration of an ion pair in which the cation is much more permeant than the anion. This situation is realized by the addition of KCl in the presence of valinomycin (see [12, 13]).

In this communication we demonstrate that ATP synthesis is highly stimulated by a KCl gradient in post illumination [15] or in acid–base [1] type experiments, under conditions in which ΔpH is suboptimal. The imposed potassium gradient increased the synthesis of ATP by as much as 40–60 nmoles ATP/mg chlorophyll. The stimulation was fully abolished when the K^+ gradient was decreased by increasing the internal concentration of K^+ through preincubation of the chloroplasts with K^+ and valinomycin.

2. Methods

Chloroplasts from lettuce leaves were prepared as previously described [16] except that they were washed and resuspended in a solution containing 0.1 M sorbitol and 0.1 mM $Mg(H_2PO_4)_2$, pH 5.6 (final pH with chloroplasts 7.0). For acid–base experiments the resuspension was in 0.01 M sorbitol and 0.1 mM $(H_2PO_4)_2$.

ATP synthesis was measured as previously described [17] and post-illumination ("Xe") experiments were performed as in [18], except for the time of illumination and light intensities which were varied as specified. Acid–base experiments were performed as described [1].

Table 1

Dependence of the yield of ATP in post-illumination ATP synthesis, on the composition of the medium.

Additions to the dark stage	ATP formed (nmoles × mg chl^{-1})	
	−valinomycin	+ 3 μM valinomycin
No additions	11.6	13.1
Sorbitol, 80 mM	9.1	10.1
Choline Cl, 40 mM	15.3	15.4
NaCl, 40 mM	18.0	–
KCl, 40 mM	26.0	40.0

The reaction mixture of the light stage contained in a final volume of 1.2 ml: sorbitol, 200 mM; pyocyanine, 20 μM; $Mg(H_2PO_4)_2$, 0.3 mM, pH 7.0, and chloroplasts containing 290 μg chlorophyll. In the dark stage the volume was 1.5 ml (final vol 2.7 ml) and the final conc. (in 2.7 ml) were: $Mg(H_2PO_4)_2$ 0.8 mM (containing 1.3×10^7 cpm ^{32}P); ADP, 1 mM; Tris HEPES, pH 8.0, 5 mM and others as indicated in the table. Chloroplasts were illuminated for 30 sec with 2×10^5 ergs × cm^{-2} × sec^{-1} (650–750 nm). Fifteen seconds after injection into the dark stage the reaction was stopped by the addition of TCA to a final conc. of 3%.

Table 2

Dependence of the stimulation of ATP synthesis on the proton concentration gradient.

pH Light stage	pH Dark stage	ΔpH*	ATP formed (nmoles × mg chl^{-1}) Choline Cl	KCL + valinomycin	KCl + Val / Choline
6.5	8.5	3.3	19.6	29.9	1.5
6.5	7.7	2.5	5.1	39.9	7.8
7.5	8.5	3.1	6.6	20.2	3.1
7.5	7.8	2.4	3.4	22.4	6.6

The reaction mixture of the light stage contained in a final vol of 1.2 ml: sorbitol, 100 mM; choline-Cl, 66 mM; pyocyanine, 15 μM; phenylene-diamine-di HCl, 5 mM, brought to the indicated pH by titration with Tris base; MES-Tris buffer, 5 mM, at the pH indicated, and chloroplasts containing 160 μg chlorophyll. In the dark stage the final vol was 2.7 ml and the final conc. were: $Mg(H_2PO_4)_2$, 0.8 mM (containing 1.6×10^{-1} cpm ^{32}P); ADP, 1 mM; Tris HEPES, 5 mM; choline Cl 100 mM; or KCl, 100 mM plus 3 μM valinomycin. Final pH was as indicated adjusted by addition of Tris base or HCl. Chloroplasts were illuminated 2 min with 9×10^3 ergs × cm^{-2} × sec^{-1} (650–750 nm). Other details as under table 1.

*ΔpH here is the difference between the chloroplasts' internal pH in the light stage [14] and pH in the dark stage.

3. Results and discussion

Table 1 shows that under suboptimal conditions, that is when the external pH for the light stage was high (7.0) and for the dark stage low (8.0), yields of ATP obtained in post-illumination ATP synthesis were considerably increased when a potassium salt together with valinomycin were added to the dark stage. It can be seen that neither sodium nor choline or sorbitol could replace potassium. However, sodium, in the presence of nonactin gave yields similar to those obtained with potassium in the presence of valinomycin [19].

Table 2 shows that the relative stimulation of the synthesis of ATP by potassium and valinomycin was higher, the lower the proton concentration gradient created. The size of the gradient was varied by either changing the pH of the dark stage or that of the light stage. All experiments in this table were performed utilizing low intensity. Phenylene diamine was included to increase the absolute yield [11].

Table 3 shows that when the light intensity is reduced, thus reducing ΔpH, the relative stimulation by KCl+valinomycin is again higher the smaller the ΔpH. In both experiments the protons present in the chloroplasts did not induce synthesis of a considerable amount of ATP, when the proton gradient was too small. However, when such a limiting proton gradient was supplemented with a diffusion membrane potential considerable higher yields were obtained. It is also evident that under optimal conditions for post-illumination ATP synthesis (dark pH 8.5, high light intensities), no stimulation was obtained with KCl + valinomycin.

Stimulation of the synthesis of ATP by membrane potential induced by means of a K^+ gradient was also observed in the dark when the protons were supplied by incubating the chloroplasts with acids that can penetrate and buffer the internal space (acid–base conditions, table 4). Here again, when the protein con-

Table 3

Dependence of the stimulation of ATP synthesis by KCl + valinomycin on the light intensity.

pH of the dark stage	Light intensity (ergs × cm^{-2} × sec^{-1})	ΔpH*	ATP formed (nmoles × mg chl^{-1}) Choline Cl	KCl + Valinomycin	KCl + val / Choline
7.5	3.5×10^5	3.0	61.1	143.9	2.4
7.5	9×10^3	2.4	3.5	26.4	7.5
7.5	2.5×10^3	1.2	0.1	6.1	6.1
8.5	3.5×10^5	4.0	154	160	1.0
8.5	9×10^3	3.4	9.4	29.4	3.1
8.5	2.5×10^3	2.2	<0.1	9.6	>100

Conditions as in table 2, except for the pH of the light stage which was 6.5, dark stage and light intensities which were as indicated. ΔpH* calculated as described in table 2.

Table 4

Stimulation of acid–base phosphorylation under suboptimal conditions by a membrane potential.

Succinate concentrations	Acid pH	Base pH	ATP formed (nmoles × mg chl^{-1}) Choline-Cl	KCl + Val	KCl+Val / Choline
12.5 mM	4.3	8.5	66	85	1.2
12.5 mM	4.3	7.5	17	52	3.0
12.5 mM	5.4	7.5	1.5	30	20
12.5 mM	6.0	7.5	<0.1	5	>50
50 mM	6.0	7.5	1.0	25	25

Chloroplasts containing 210 μg chlorophyll were added to a solution containing Tris-succinate at the stated concentration and pH, in a final vol of 1 ml. After 30 sec the following components were added in 1.7 ml to give the following final conc. (in 2.7 ml): $Mg(H_2PO_4)_2$, 0.6 mM (containing 1.2×10^7 cpm ^{32}P); ADP, 0.88 mM; MES–Tris, pH 7.5, 1.8 mM; choline-Cl, 90 mM or KCl, 90 mM plus valinomycin 10 μM, and enough Tris base to bring the final pH to the desired value. The reaction was stopped after 15 sec by addition of trichloroacetic acid to a final conc. of 3%. Both stages were carried out at 4°.

centration gradient itself was big enough (acid pH 4.3 and basic pH 8.5), the membrane potential created did not stimulate ATP synthesis. However, when the gradient was lower than 2.5–2.8 pH units, most of the ATP synthesis was due to the added membrane potential.

As can be seen, under such suboptimal conditions (acid stage pH 5.4–6.0) the amount of ATP formed was further increased by increasing the succinate concentration several fold.

Fig. 1 shows that preloading chloroplasts before the experiment with K^+ caused a decrease in the amount of ATP formed. Thus, as expected, decreasing the K^+ gradient by increasing the initial internal K^+ concentration decreases the yield obtained. A decrease of 50% w: already seen when the preincubation concentration was 10 mM KCl (external KCl concentration – 100 mM). When the ratio of external to internal KCl dropped to 2 or less, the inhibition was total. The figure indicates that the amount of extra ATP formed was directly related to the K^+_{out}/K^+_{in} ratio, which is consistent with the assumption that the phosphorylation is stimulated by the KCl diffusion potential.

The actual value of the membrane potential created can be estimated only very roughly since we do not know the permeability constants of potassium and chloride and also because the potential is transient. Of interest as energy source is not only the initial value of the membrane potential but the integral of the potential during the period of phosphorylation. This period must be quite short since all the ATP synthesized is present already 5 sec after mixing the chloroplasts with KCl [19].

Previously, an increase in post-illumination ATP

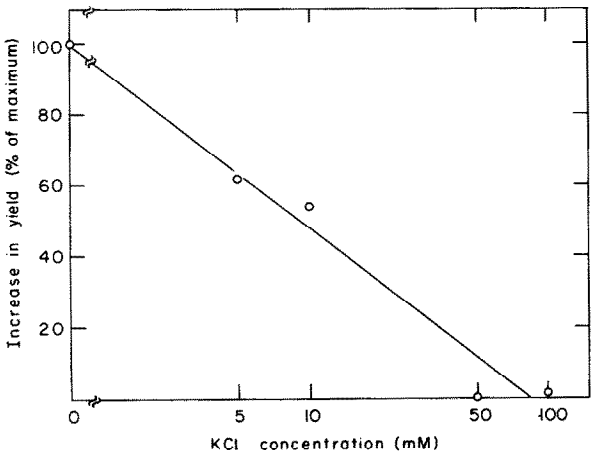

Fig. 1. Effect of varying KCl concentrations during preincubation on the yield in post-illumination ATP formation. Conditions as in table 2. Chloroplasts were preincubated in the dark in the reaction mixture described in table 2 and in the presence of the indicated concentrations of KCl and 3 μM valinomycin, for 1 hr. Phenylene diamine was added 2 min before turning the light on. The pH of the light was 6.5; dark stage, 7.5; light intensity was 9×10^3 ergs $\times$ cm^{-2} $\times$ sec^{-1} (650–750 nm). Without preincubation, ATP synthesis was 29.9 nmoles $\times$ mg chl^{-1} in the presence of KCl + valinomycin in the dark stage , and 1.3 nmoles $\times$ mg chl^{-1} in its absence.

formation in chromatophores [20] and subchloroplasts particles [21] by valinomycin and K^+ has been reported. However, in these studies, KCl + valinomycin were added to the light stage, and therefore the stimulation is most probably due to the increased extent of proton uptake and possibly ΔpH, and do not necessarily indicate utilization of the energy of a transient membrane potential.

Recent observations indicate that also in chromatophores the yield of ATP in post-illumination experiments can be increased when $K^+$and valinomycin are added to the dark stage [19]. This point against the suggestion that the low values of post-illumination ATP formation observed in chromatophores are due to the small number of protons taken in [20] but rather may indicate that the proton concentration gradient is too small.

The results described demonstrate that a membrane potential created by the addition of high external concentration of KCl can stimulate net synthesis of ATP. This synthesis can be most easily detected in cases in which a sufficient number of protons are present in the chloroplast but their concentration gradient is too smal to drive the synthesis by itself. After completion of this report an abstract [22] has appeared describing similar effects in the acid–base reaction.

References

[1] A.T. Jagendorf and E. Uribe, Proc. Natl. Acad. Sci. U.S. 55 (1966) 170.
[2] R.A. Reid, J. Moyle and P. Mitchell, Nature 212 (1966) 257.
[3] R.S. Cockrell, E.J. Harris and B.C. Pressmann, Nature 215 (1967) 1318.
[4] E. Rossi and G.F. Azzone, European J. Biochem. 12 (1970) 319.
[5] I.M. Glynn, Nature 216 (1967) 1318.
[6] P. Mitchell, Chemiosmotic Coupling and Energy Transduction, Glynn Research, Bodmin, Cornwall, England, 1968.
[7] G.D. Greville, Current Topics Bioenerg. 3 (1960) 1.
[8] B. Chance and M. Montal, Current Topics in Membranes and Transport 2 (1971) 100.
[9] H. Rottenberg, T. Grunwald and M. Avron, European J. Biochem. 25 (1972) 54.
[10] N Nelson, H. Nelson, Y. Naim and J. Neumann, Arch. Biochem. Biophys. 145 (1971) 263.
[11] M. Avron, Proc. 2nd. International Congress on Photosynthesis Res., eds. G. Forti, M. Avron and A. Melandri, Stresa, Italy (1971) in press.
[12] J. Barber, Biochim. Biophys. Acta 275 (1972) 105.
[13] S.J.D Karlish, N. Shavit and M. Avron, European J. Biochem. 9 (1969) 291.
[14] S. Schuldiner, H. Rottenberg and M. Avron, European J. Biochem 25 (1972) 64.
[15] G. Hind and A.T. Jagendorf, Proc Natl. Acad. Sci. U.S. 49 (1963) 715.
[16] M. Avron, Biochim. Biophys. Acta 40 (1960) 257.
[17] M. Avron, Anal. Biochem. 2 (1961) 535.
[18] Z. Gromet-Elhanan and M. Avron, Plant Physiol. 40 (196 1053.
[19] S. Schuldiner, H. Rottenberg and M. Avron, in preparatio
[20] J.B. Jackson, A.R. Crofts and L.V. Von-Stedingk, Europ J. Biochem. 6 (1968) 41.
[21] R. McCarty, FEBS Letters 9 (1970) 313.
[22] E.G. Uribe, Plant Physiol. 49 Suppl. (1972) 9.