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PROTON ELECTROCHEMICAL GRADIENT AND PHOSPHATE POTENTIAL IN SUBMITOCHONDRIAL PARTICLES

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Summary

1. The aerobic uptake of inorganic ions, such as $^{86}Rb^+$ or $^{125}I^-$, by submitochondrial particles, is about one order of magnitude lower than the uptake of organic ions, such as acridines or 8-anilino-1-naphthalene sulphonate. The values of ΔpH, the transmembrane pH differential, and $\Delta\psi$, the transmembrane membrane potential are between 60 and 100 mV when calculated on the inorganic ions and between 150 and 240 mV when calculated on the organic ions. The discrepancy between the ΔpH and $\Delta\psi$ values from organic and inorganic ions is large at high but not at low ion/protein ratios.

2. In the absence of weak bases and strong acids the values of $\Delta\tilde{\mu}_H$, the proton electrochemical potential difference, are close to 100 mV and the magnitude of ΔpH and $\Delta\psi$ are similar. Weak bases decrease ΔpH and enhance $\Delta\psi$. Strong acids decrease $\Delta\psi$ and enhance ΔpH. Interchangeability of ΔpH with $\Delta\psi$ occurs at low concentrations of weak bases and strong acids. High concentrations of weak bases and strong acids cause depression of $\Delta\tilde{\mu}_H$.

3. Concentrations of weak bases capable of abolishing ΔpH, do not affect ATP synthesis. Concentrations of strong acids capable of abolishing $\Delta\psi$ affect only slightly ATP synthesis. Concentrations of weak bases and strong acids capable of causing a decline of $\Delta pH + \Delta\psi$ inhibit ATP synthesis.

4. Depression of $\Delta\tilde{\mu}_H$ is paralleled by inhibition of ATP synthesis and decline of ΔGp, the phosphate potential. Abolition of ATP synthesis occurs only when $\Delta\tilde{\mu}_H$ is below 20 mV. The $\Delta Gp/\Delta\tilde{\mu}_H$ ratio increases hyperbolically with the decrease of $\Delta\tilde{\mu}_H$.

Introduction

The assessment of the competence of $\Delta\tilde{\mu}_H$ to act as kinetic intermediate in energy transduction requires the determination of $\Delta\tilde{\mu}_H$ in a variety of systems

[1,2]. The role of ΔpH and $\Delta\psi$ in chloroplasts and chromatophores has been investigated either through indirect methods (uncoupling by NH_4Cl or by NH_4Cl + valinomycin) [3—7] or by direct methods (determination of ion distributions or of spectroscopic shifts) [8,17].

A discrepancy has been observed between measurements based on spectroscopic shifts and on ion distribution. For example in chloroplasts $\Delta\psi$ is zero on ion distribution and 100 mV on carotenoid shift; in chromatophores $\Delta\psi$ is 88 mV on ion distribution and 144 mV on carotenoid shift [2,8,17].

In submitochondrial particles [18—20] a comparison between the values of ΔpH and $\Delta\psi$ based on organic and inorganic ion distributions has been carried out only partially. Azzi et al. [21] calculated a $\Delta\psi$ of 165 mV using 8-anilino-1-naphthalene sulphonate. Rottenberg and Lee [22] calculated a ΔpH of 3.5 units on acridine fluorescence quenching and NH_4 uptake.

The purpose of the present study is two-fold. First, to compare organic and inorganic ions as tools for determining ΔpH and $\Delta\psi$ in particles. It appears that the values provided by the inorganic ions are lower than those provided by the organic ions at high but not at low ion/protein ratios. Second, to study the effect of permeant ions on $\Delta\tilde{\mu}_H$ and ΔGp. In accord with other reports in chromatophores [7], permeant ions cause a partial interconvertibility of $\Delta pH/\Delta\psi$ which explains the resistence of ATP synthesis in particles either to weak bases or strong acids. On the other hand ATP synthesis and ΔGp decline with the depression of $\Delta\tilde{\mu}_H$, although the depression of the latter is more extensive than that of the former. This leads to an increase of the $\Delta Gp/\Delta\tilde{\mu}_H$ ratio at the lower values of $\Delta\tilde{\mu}_H$. The observation is interpreted as evidence for a microscopic mechanism of energy coupling.

Experimental

Beef heart mitochondria and Mn-submitochondrial particles were prepared as described previously [23]. EDTA-submitochondrial particles were prepared as described by Lee and Ernster [24].

The uptake of 8-anilino-1-naphthalene sulfonate was measured either by centrifugation or by direct assay [21]. In the former case the suspension was centrifuged and the fluorescence of free dye in the supernatant measured after addition of 0.1% bovine serum albumin. The dye concentration was then calculated by reference with a titration curve obtained under identical conditions. In the latter case the fluorometer was set at 100% fluorescence with the sample containing the incubation medium supplemented with 5 μM dye and 0.1% bovine serum albumin. The incubation medium was added to the cuvette supplemented with 5 μM dye but without bovine serum albumin, and the fluorescence enhancement was measured after addition of submitochondrial particles and an oxidizable substrate, i.e. succinate. The amount of dye uptake was then calculated by assuming that the dye taken up by the particles undergoes a fluorescence enhancement similar to that occurring during binding to bovine serum albumin. In some cases the extent of fluorescence enhancement due to substrate addition was small in respect to the fluorescence of 5 μM dye supplemented with bovine serum albumin. In this case the fluorometer scale was amplified by a known factor which was then accounted for in the calculation of the uptake.

The uptake of the acridines was also measured either by centrifugation or by direct assay [23]. In the former case the suspension was centrifuged and the fluorescence of free dye in the supernatant was measured. The concentration was then calculated by reference with a titration curve obtained under identical conditions. In the latter case the fluorometer was set at 100% fluorescence with the sample containing the incubation medium and 5 μM dye. The medium was then supplemented with submitochondrial particles and an oxidizable substrate or ATP. The dye uptake was calculated by assuming that the dye taken up by the particles undergoes 100% fluorescence quenching. All measurements were carried out with an Eppendorf Fluorometer. Centrifugation was carried out for 30 min at 150 000 $\times g$ with an MSE Ultracentrifuge.

In a preceding paper the centrifugation procedure to measure the ion distribution has been discussed [28]. Table I shows a further comparison of the data on 9-aminoacridine uptake obtained either by direct assay or after centrifugation. It is seen that there is very good agreement between the two procedures. The experiment supports the validity of the centrifugation procedure to measure ion distributions.

The uptake of I^- and Rb^+ was determined with $^{125}I^-$ and $^{86}Rb^+$. The specific activity of each radioisotope was always higher than 10^5 cpm $\cdot$ nmol^{-1}. Centrifugation was started after 90—180 min of incubation, depending on the protein concentration. After centrifugation the supernatant was carefully drained off from the test tubes and the residual water from the walls removed with filter paper. The vials were counted in a Packard 2525 liquid scintillation spectrometer. The amount of active uptake was calculated by difference between a sample supplemented with an oxidizable substrate and a sample supplemented with $2 \cdot 10^{-6}$ M FCCP. The radioactivity in the uncoupler supplemented sample accounts for both the unspecific binding and the extraparticle water.

In order to calculate ΔpH and $\Delta\psi$ from the uptake of either organic or inorganic ions we have assumed that the volume of the inner space is 0.5 μl $\cdot$ mg^{-1} protein [27] and that binding is negligible. $\Delta\psi$ was then calculated by applying the Nernst equation to the distribution of strong acids. ΔpH was calculated on the $^{86}Rb^+$ distribution, the assumption being that, in the presence of an excess of nigericin, Rb^+ behaves as a weak basis.

ATP and ADP were measured as described in the preceeding paper [29].

TABLE I

COMPARISON BETWEEN DIRECT ASSAY AND CENTRIFUGATION PROCEDURE

The medium contained 0.1 M choline chloride, 10 mM $MgSO_4$ 2 mM ATP, 10 mM Tris-Cl pH 7.4, 30 μM 9 aminoacridine and variable amounts of protein as indicated.

Amount of protein (mg)	Dye uptake (μM)	
	Direct assay	Centrifugation
0.12	2.8	3.3
0.240	7.7	6.6
0.480	11.3	11.6
0.720	14.2	15.2
0.960	16.0	17.0

Both in the experiments where the uptake of organic and inorganic ions was determined after centrifugation and the experiment of ATP synthesis the incubation medium was bubbled with oxygen for at least 10 min.

In the experiment of Fig. 8 the values of $\Delta\tilde{\mu}_H$ have been calculated theoretically in the following manner. As discussed by Rossi and Azzone [30] for the intact mitochondria, it may be assumed that: (a) water is at thermodynamic equilibrium and (b) NH_4^+ and NO_3^- become the major intraparticle ions. Assumption (a) may not hold in case the breaking tension of the membrane is so large as to allow a large increase of the internal osmotic pressure. The activities of matrix NH_4^+ and matrix NO_3^- are then each taken, as 50% the osmolarity of the medium. $\Delta\tilde{\mu}_H$ in the submitochondrial particles in presence of increasing concentrations of NH_4^+ and NO_3^-, assumed at electrochemical equilibrium, is then (cf. also Walker [31]):

$$[H^+]_i = [H^+]_0 [NH_4^+]_i/[NH_4^+]_0 \quad (1)$$

$$\Delta pH = \log \frac{1}{2} \frac{[Osm]_0}{[NH_4^+]_0} \quad (2)$$

$$\Delta\psi = \log \frac{1}{2} \frac{[Osm]_0}{[NO_3^-]_0} \quad (3)$$

$$\Delta\tilde{\mu}_H = \Delta\psi - 59 \text{ mV } \Delta pH \quad (4)$$

Eqns. 1 and 2 imply that amines equilibrate freely across the membrane according to ΔpH. Eqn. 3 implies that NO_3^- equilibrates freely according to $\Delta\psi$.

Results

Determination of ΔpH and $\Delta\psi$

Papa et al. [32] measured an uptake of SCN^- of 13 nmol · mg^{-1} protein in the presence of 10 mM $[SCN^-]_0$, in energized Mg^{2+}-particles. Fig. 1 shows a correlation between I^- concentration and I^- uptake. The uptake increased almost linearly with the increase of $[I^-]_0$ and reached 12 nmol · mg^{-1} protein at 5 mM $[I^-]_0$. $\Delta\psi$ decreased from 76 mV at the lower $[I^-]_0$ to 54 mV at 5 mM $[I^-]_0$. The scattering of values, among various preparations, was between 60 mV and 100 mV. From the data of Papa et al. [33] a $\Delta\psi$ of 24 mV may be calculated.

8-Anilino-1-naphthalene sulfonate is generally considered to be an indicator of the electrostatic potential either at the membrane interphase or across the membrane. Azzi et al. [21] calculated a $\Delta\psi$ of 165 mV (in energized particles) by calibrating the 8-anilino-1-naphthalene sulfonate response with known gradients of KCl (+ valinomycin). Fig. 2 shows the uptake of the dye at various dye/protein ratios. The extent of dye uptake was markedly dependent on the dye/protein ratio (cf. Nordenbrand and Ernster [33]), ranging from 0.04 nmol · mg^{-1} protein at the lower ratios to more than 2 nmol · mg^{-1} protein at the higher ratios. The values for I^- and dye uptake were similar when measured at low anion/protein ratios and very different at high anion/protein ratios. $\Delta\psi$ increased parallel to the uptake, reaching values of about 180 mV at the higher dye/protein ratios. At a low dye/protein ratio $\Delta\psi$ was about 85 mV, close to

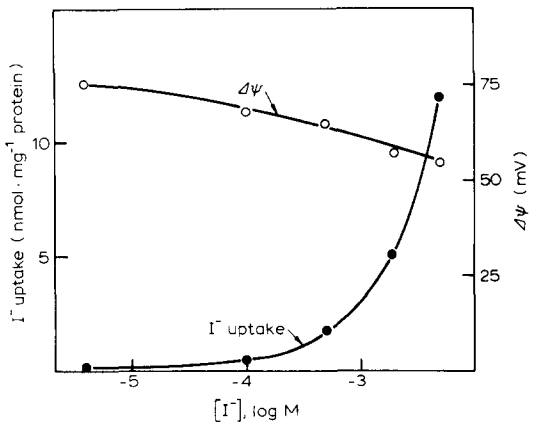

Fig. 1. Uptake of I^- and $\Delta\psi$. The medium contained 0.2 M sucrose, 25 mM Tris/acetate pH 7.6, 10 mM ammonium acetate, 3 mM succinate/Tris and 1.7 mg protein of Mn^{2+} submitochondrial particles treated with 1 μg oligomycin · mg^{-1} protein. The concentration of $K^{125}I$ was as indicated in the figure. The reaction was started by addition of the particles and the tubes centrifuged after 5 min incubation.

the values based on the I^- distribution. In the case of I^- $\Delta\psi$ was almost independent of the amount of protein.

The uptake of K^+ in nigericin treated submitochondrial particles has been measured by Cockrell and Racker [34] and by Montal et al. [18]. Montal et al. found in the range 0.1—1 mM KCl an uptake between 16 and 40 nmol · mg^{-1} protein. Rottenberg and Lee [24] reported an uptake of NH_4^+ of 20 nmol · mg^{-1} protein at 8 mM NH_4^+. By measuring the distribution of $^{85}Rb^+$ in nigericin treated mitochondria we have observed in Mn-particles an uptake which is about 3—5 times lower than that reported by Montal et al. [18]. On the other

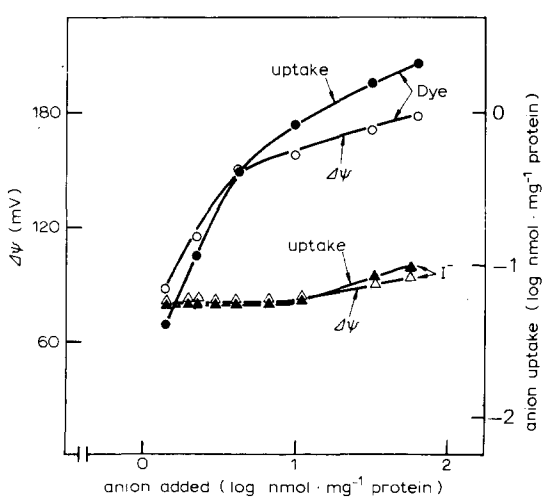

Fig. 2. Dependence of uptake of 8-anilino-1-naphthalene sulphonate and I^- on amount of protein. The medium was identical to that of Fig. 1. Ammonium acetate was omitted during the uptake of I^- and also in the uptake of 8-anilino-1-naphthalene sulphonate. Concentration of dye and I^- was 5 μM in all samples. The amounts of Mn-particles, treated with 1 μg oligomycin · mg^{-1} protein were as indicated in the figure.

hand the dependence of the uptake on $[K^+]_0$ was identical to that of Montal et al. [18].

9-Aminoacridine has been used to measure ΔpH in liposomes [35], in chloroplasts [9,10] in chromatophores [17] and submitochondrial particles [22]. A correlation has been found between extent of fluorescence quenching and methylamine distribution in chloroplasts and between fluorescence quenching and NH_4^+ uptake in submitochondrial particles [22]. Fig. 3 shows the uptake in energized particles of various acridine dyes at various dye/protein ratios. The dye uptake was strongly dependent on the dye/protein ratio for all the dyes and decreased in the order: acridine orange > atebrine > 9 amino-acridine. ΔpH ranged from values of 240 mV in the case of acridine orange at high dye/protein ratios to values of about 120 mV in the case of 9-amino-acridine and atebrine at low dye/protein ratios. Rottenberg and Lee [22] reported a ΔpH of 3—3.5 units with 9-aminoacridine at a dye/protein ratio of 10. This is very close to that shown in Fig. 3. The extent of dye uptake in Fig. 3 reflects an order of increasing lipophilicity which can be assessed from the number of hydrophobic groups on the acridine ring and by measuring the partition of the dye in H_2O/organic solvent mixtures (unpublished data).

Effect of weak bases and strong acids on $\Delta\tilde{\mu}_H$

In Fig. 4 the energy linked dye response was studied in Mn- and EDTA-submitochondrial particles in the presence of various concentrations of ammonium salts of Cl^- and NO_3^-. In the presence of EDTA, addition of either NH_4Cl or NH_4NO_3 resulted in quenching of fluorescence, more marked in the presence of NO_3^-. With the Mn-particles, addition of NH_4Cl caused an increased 8-anilino-1-naphthalene sulphonate response, which was abolished at the higher NH_4Cl concentration. NH_4NO_3 caused first a small increase and then an inhibi-

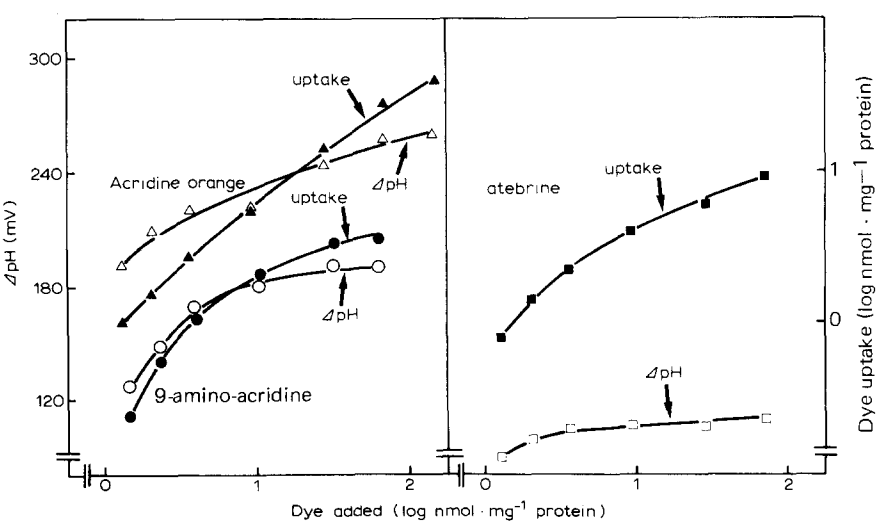

Fig. 3. Uptake of acridine orange, 9-aminoacridine and atebrine and ΔpH. The medium contained 0.1 M KCl, 5 mM $MgCl_2$, 5 mM Tris-Cl pH 7.0, 1 mM ATP, 5 μM dye and amounts of Mn-paritcles as indicated in the figure. The ordinate scale on the right and on the left refer to both sides of the figure.

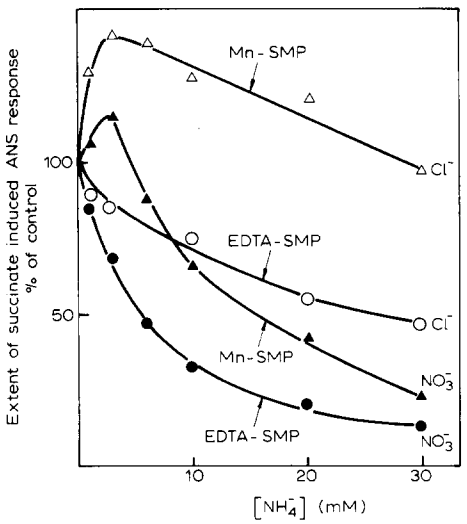

Fig. 4. Effect of NH_4Cl and NH_4NO_3 on 8-anilino-1-naphthalene sulphonate responses. The medium was identical to that of Figs. 1 and 2. The amount of oligomycin-treated submitochondrial particles was 1.4 mg.

tion. The results shown in Fig. 4 are presumably due to the effect of amines on ΔpH and of anions on $\Delta\psi$. The enhancement of fluorescence reflects an increase of $\Delta\psi$ due to the amine induced collapse of ΔpH. The more marked inhibition of fluorescence enhancement by NO_3^- compared to Cl^- reflect the higher degree of lipophilicity of NO_3^-. The more marked inhibition of fluorescence enhancement, in EDTA particles compared to Mn particles, suggests a higher degree of ion leakiness of the EDTA particles.

Figs. 5 and 6 show the values of ΔpH, $\Delta\psi$ and $\Delta\tilde{\mu}_H$ measured on the distribution of inorganic ions: (i) $\Delta\psi$ was stimulated by NH_4^+. In the absence of Cl^- or

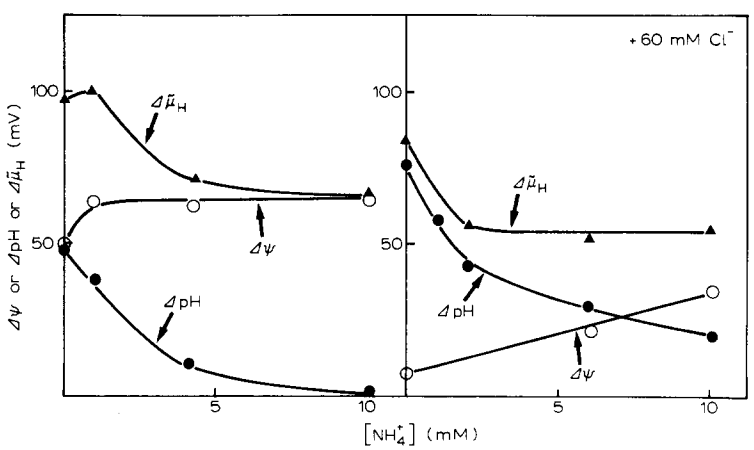

Fig. 5. Effect of NH_4^+ on $\Delta\tilde{\mu}_H$ in presence and absence of Cl^-. The medium contained 0.2 M sucrose, 2.5 mM Tris/acetate pH 7.6, 3 mM succinate/Tris. 1.5 mg of oligomycin treated Mn^{2+} particles. 2 μg nigericin in all samples. Anion uptake was measured in the presence of 100 μM $K^{125}I$ and cation uptake in presence of 100 μM $^{86}RbCl$. Each point is the average of at least three experiments. NH_4^+ was added as acetate salt.

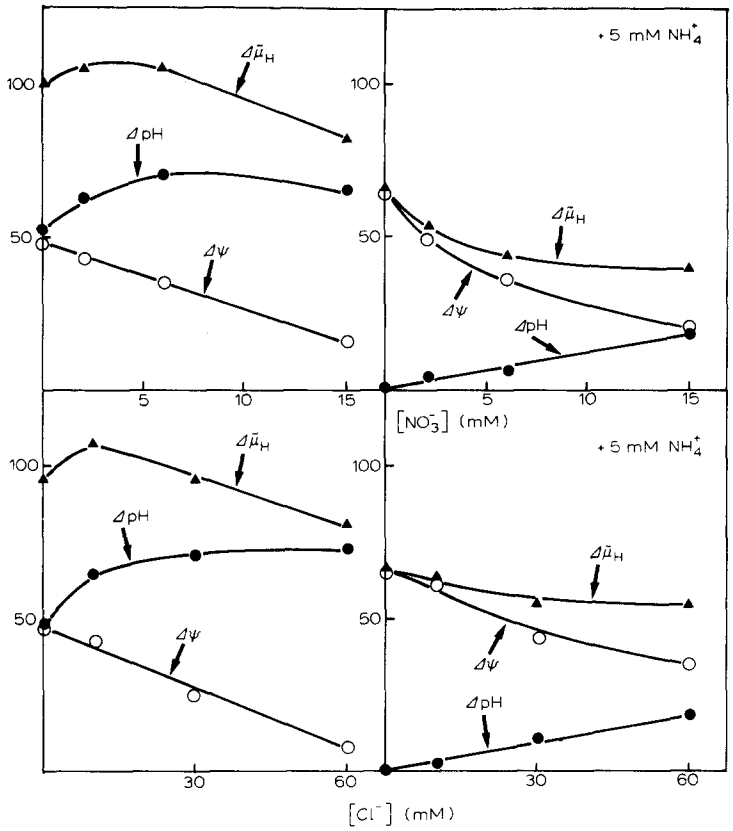

Fig. 6. Effect of Cl^- and NO_3^- on $\Delta\tilde{\mu}_H$ in presence and absence of NH_4^+. Experimental conditions as in Fig. 5. When indicated 5 mM NH_4^+ was added as acetate salt and Cl^- and NO_3^- added as strontium salt. Each point is the average of at least three experiments.

NO_3^-, $\Delta\psi$ was between 36 and 48 mV in the absence, and between 65 and 100 mV in the presence of NH_4^+. (ii) ΔpH was inhibited by NH_4^+. 10 mM NH_4^+ decreased ΔpH from 48 mV to zero in the absence, and from 76 (range 60—95) to 20 mV in the presence, of Cl^-. (iii) $\Delta\psi$ was inhibited by Cl^- and NO_3^-. 60 mM Cl^- decreased $\Delta\psi$ from 48 to 8 mV in the absence and from 66 to 35 mV in the presence of NH_4^+. 15 mM NO_3^- decreased $\Delta\psi$ to 15 mV in the absence and to 22 mV in the presence of NH_4^+. (iv) ΔpH was stimulated by Cl^- and NO_3^-. 60 mM Cl^- increased ΔpH from 50 to 76 mV. 15 mM NO_3^- increased ΔpH from 50 to 74 mV. In the presence of 60 mM Cl^- and 15 mM NO_3^- there was a ΔpH of about 20 mV in the presence of 5 mM NH_4^+.

The inhibition of ΔpH and stimulation of $\Delta\psi$ by NH_4^+, and the inhibition of $\Delta\psi$ and stimulation of ΔpH by anions have been interpreted as due to interchangeability of ΔpH with $\Delta\psi$ [7]. Figs. 5 and 6 show that such interchangeability occurs in a small range of concentrations and thus the values of $\Delta\tilde{\mu}_H$ remain maximal only at low concentrations of either NH_4^+ or anions. $\Delta\tilde{\mu}_H$ begins to decline above 2 mM NH_4^+, 5 mM NO_3^- or 10 mM Cl^-. In the presence of 10 mM NH_4^+, 15 mM NO_3^- or 60 mM Cl^- the $\Delta\tilde{\mu}_H$ was partially decreased. As

expected the combined addition of strong acids and weak bases resulted in a more marked depression of $\Delta\tilde{\mu}_H$.

It is possible to make a comparison of the concentrations of weak bases and strong acids required to cause a 50% inhibition of the uptake of organic and inorganic ions and the releative ΔpH and $\Delta\psi$. In the case of the organic ions an apparent discrepancy exists between the concentration either of strong acids of of weak bases required to inhibit the uptake on one side and $\Delta\psi$ or ΔpH on the other. For example, Cl^- caused 50% inhibition of 8-anilino-1-naphthalene sulfonate uptake at concentrations of 7 and 13 mM, in the absence and presence of 10 mM NH_4^+, respectively, and 50% inhibition of $\Delta\psi$ at more than 100 mM. NO_3^- caused 50% inhibition of 8-anilino-1-naphthalene sulfonate uptake at 1 and 2 mM, in the absence and presence of 10 mM NH_4^+, and 50% inhibition of $\Delta\psi$ at 10 and 100 mM, respectively. Furthermore NH_4^+ caused 50% inhibition of 9-aminoacridine uptake at 1 mM and of ΔpH at 8 mM. The discrepancy is due to the logarithmic nature of the $\Delta\psi$ and ΔpH scale. As the uptake of inorganic ions is about one order of magnitude smaller, the discrepancy between uptake and ΔpH and $\Delta\psi$ is much reduced.

Effect of weak bases and strong acids on ATP synthesis and ΔGp

In a previous paper [20], it was shown that a concentration of NH_4Cl higher than 10 mM has a negligible effect on ATP synthesis. Since at these concentrations of weak bases the magnitude of ΔpH is reduced to negligible values (cf. Fig. 6) it appears that ATP synthesis is independent of the presence of significant values of ΔpH. The effects of 8 mM NH_4Cl and of 40 mM KCl + nigericin are compared in Fig. 7A. It is seen that ATP synthesis was about 30% lower with 40 mM KCl + nigericin than with 8 mM NH_4Cl. Since ΔpH is negligible in both cases the difference is presumably due to an effect of Cl^- on $\Delta\psi$. Addition of increasing concentrations of valinomycin, which permits the extrusion of K^+ and NH_4^+, led to uncoupling. This is in accord with the concept that, due to the partial interchangeability of ΔpH with $\Delta\psi$, it is necessary to abolish both ΔpH and $\Delta\psi$ to determine uncoupling.

In Fig. 7B are shown the effects of increasing concentrations of a hydrophilic anion Cl^-, and a lipophilic anion NO_3^-, on ATP synthesis in the presence and absence of NH_4^+. The inhibition of ATP synthesis was about 15% at 96 mM Cl and 40% at 32 mM NO_3^-. When Cl^- and NO_3^- were added as NH_4^+ salts the inhibition was increased by 20%. Since these concentrations of Cl^-, NO_3^- and NH_4^+ cause a marked inhibition of $\Delta\tilde{\mu}_H$ (cf. Figs. 5 and 6) the experiment indicates that ATP synthesis, in the presence of hexokinase, occurs also in the presence of relatively low levels of $\Delta\tilde{\mu}_H$.

Fig. 8 shows the relation between ATP and $\Delta\tilde{\mu}_H$. ATP synthesis was measured in the absence of hexokinase. The experiment was carried out in the absence of sucrose in order to obtain a lower osmolarity and therefore a lower $\Delta\mu_H$. Also, the values of $\Delta\tilde{\mu}_H$ were calculated on the basis of Eqns. 2 and 3, i.e. the internal concentrations of NH_4^+ and NO_3^- are the maximal compatible with electrochemical and osmotic equilibrium. The assumptions of osmotic and electrochemical equilibrium lead to a decrease of $\Delta\tilde{\mu}_H$ parallel to the increase of the NH_4NO_3 concentration. The values of $\Delta\tilde{\mu}_H$ calculated from Eqns. 2 and 3 are close to those obtained on the basis of the organic ion distribution and

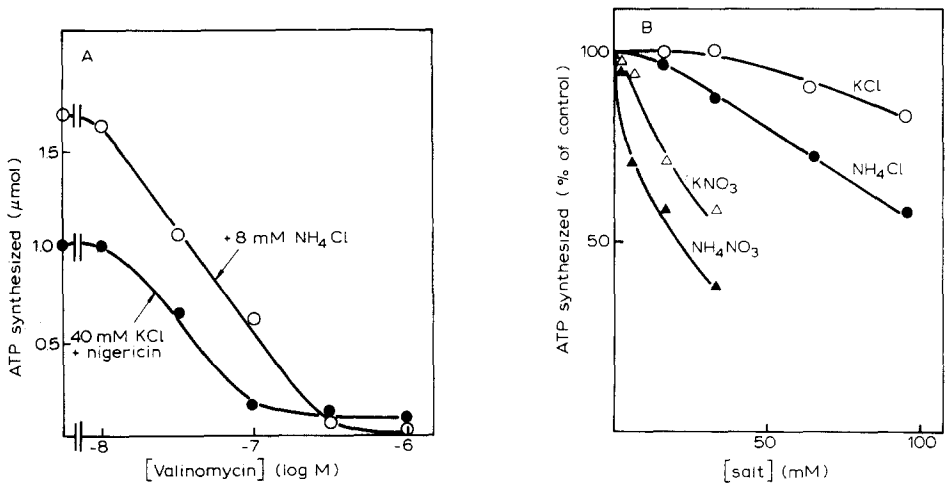

Fig. 7. (A and B). Effect of electrolytes on ATP synthesis. The medium for ATP synthesis contained 0.1 M sucrose, 20 mM P_i-Tris, pH 7.0, 10 mM $MgSO_4$, 200 μM ADP, 0.05% bovine serum albumin, 3 mM succinate-Tris, 1 mg hexokinase and 10 mM glucose. Other additions as indicated in the Figure. 1.8 mg protein of Mn^{2+} submitochondrial particles. Time of incubation 15 min.

about twice those obtained on the inorganic ion distribution (not shown, cf. however Figs. 5 and 6). It is seen that the synthesis of ATP declined parallel to the decline of $\Delta\tilde{\mu}_H$. However ATP synthesis fell to negligible values only when $\Delta\tilde{\mu}_H$ was reduced below 20 mV. In Fig. 8 are also reported the $\Delta Gp/\Delta\tilde{\mu}_H$ ratios at the various $\Delta\tilde{\mu}_H$ values. The ratio increased from values of 2.3 at very high $\Delta\tilde{\mu}_H$ to values as high as 37 at values of $\Delta\tilde{\mu}_H$ of 12 mV. The $\Delta Gp/\Delta\tilde{\mu}_H$ ratio

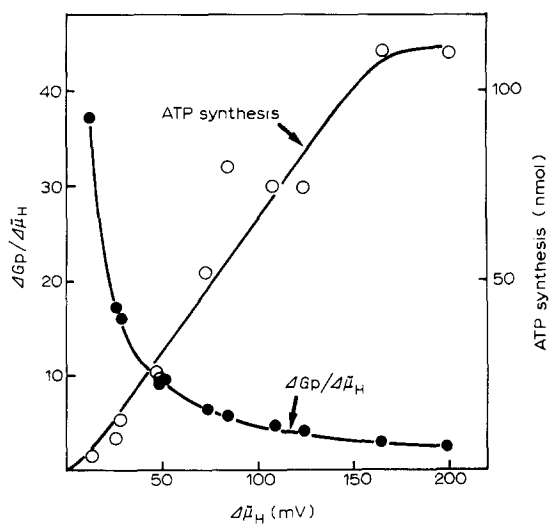

Fig. 8. Relationship between $\Delta\tilde{\mu}_H$ and ΔGp. The values for $\Delta\tilde{\mu}_H$ were calculated as described in Experimental. Synthesis of ATP was measured in the presence of variable concentrations of NH_4NO_3 in a medium containing, 20 mM P_i-Tris, pH 7.4, 10 mM $MgSO_4$, 3 mM succinate-Tris, 200 μM ADP, 0.05% bovine serum albumin and 2.4 mg protein of Mn^{2+}-particles. The samples were incubated for 5 min before addition of perchloric acid. The content of ATP and ADP was determined by enzymatic analysis.

thus tended to infinity when $\Delta\tilde{\mu}_H$ tended to zero. When the values of $\Delta\tilde{\mu}_H$, calculated from Eqns. 2 and 3, were replaced with those obtained on the basis of the inorganic ion distributions, the pattern of the relationship $\Delta Gp/\Delta\tilde{\mu}_H$ vs. $\Delta\tilde{\mu}_H$ was unchanged. However the $\Delta Gp/\Delta\tilde{\mu}_H$ ratios were twice as high.

Discussion

ΔpH and $\Delta\psi$ can be calculated by determination of the inorganic ion distribution or titration of intrinsic or extrinsic probe response, i.e. carotenoid shift or acridines. The probe response procedure relies on the titration against the inorganic ion distribution [2]. The calculation of ΔpH and $\Delta\psi$ rests on the assumption that the activity coefficient for the inorganic and the organic ions in the inner space is very close to 1. Since this assumption is difficult to prove it might be better to denote ΔpH and $\Delta\psi$ as ΔpH_{app} and $\Delta\psi_{app}$. Furthermore this assumption is less likely to be correct for the organic than for the inorganic ions because of the stronger interaction with the membrane of 8-anilino-1-naphthalene sulphonate and acridines as compared to I^- and K^+. This may lead to discrepancies between the two sets of values.

In submitochondrial particles Azzi et al. [21] calculated a $\Delta\psi$ of 165 mV on 8-anilino-1-naphthalene sulfonate response. Rottenberg and Lee [22] have titrated in submitochondrial particles the acridine response against NH_4^+ uptake and concluded that particles possess a ΔpH of 3.5 units and a negligible $\Delta\psi$. In the present study the uptake of I^- was slightly larger than that of SCN^- found by Papa et al. [31] while the uptake of Rb^+ was smaller than that of NH_4^+ found by Rottenberg and Lee [22]. One possibility is that energy coupling is more tight in Rottenberg and Lee particles. However, no essential difference exists between the two preparations in respect to the uptake of acridines. Furthermore the dependence of the ion gradients on the extent of uptake can be diminished by extrapolation to infinitely low ion concentrations. When this is done for I^-, a larger $\Delta\psi$ is not found. The alternative explanation is that the uptake of acridines, although driven primarily by a pH gradient, leads to binding to charged phospholipid. This is in accord with the fact that the extent of dye uptake is proportional to the lipophilicity of the dye. Further evidence for acridine binding has been discussed elsewhere [20,23,36]. A similar explanation would account for the fact that 8-anilino-1-naphthalene sulfonate, which interacts mostly with the phospholipid head groups at the outer surface, accumulates more than I^-. The lower values for Rb^- uptake in respect to NH_4^+ uptake may be due to our using Mn-particles instead of EDTA-particles.

The effect of weak bases and strong acids on ΔpH and in submitochondrial particles, are qualitatively similar to that found in chloroplasts and chromatophores in that weak bases enhance $\Delta\psi$ and depress ΔpH, while strong acids enhance ΔpH and depress $\Delta\psi$. This is observed with both organic and inorganic ions, in spite of the differences on the extent of uptake. This result supports the view that the nature of the process monitored by inorganic and organic ions is identical both in respect to ΔpH and $\Delta\psi$. The effect of weak bases and strong acids on $\Delta\psi$ and ΔpH have been interpreted as due to interchangeability of $\Delta\psi$ and ΔpH [7]. This suggestion is consistent with the present data at low concentrations of bases and acids. At higher concentrations the extent of

depression of ΔpH by weak bases is not compensated by the increase of $\Delta\psi$ and conversely the extent of depression of $\Delta\psi$ by acids is not compensated by increase of ΔpH. As a consequence addition of weak bases or strong acids, either alone or in combination, leads, above a certain concentration, to a depression of $\Delta\tilde{\mu}_H$ in respect to the values observed in sucrose. Cl^-, a hydrophilic anion, depresses $\Delta\psi$, in the same manner as the more lipophilic NO_3^-, although at higher concentrations. This is in accord with the stimulation induced by Cl^- of both K^+ and acridine uptake. Whether this be due to the fact that Cl^- acts as a penetrant in the leaky particle membrane, or that the acridine responses are located at the surface, is not resolved [23,36].

In the case of chromatophores, where ATP synthesis is completely insensitive to abolition of ΔpH, Gromet-Elhanan [6] has suggested that $\Delta\tilde{\mu}_H$ is not depressed by NH_4Cl because of the interchangeability of ΔpH with $\Delta\psi$ [7]. In the case of submitochondrial particles Montal et al. [18] found release of respiratory control when NH_4Cl or KCl + nigericin were added in the presence of valinomycin or permeant anions. Azzone et al. [20] found that NH_4Cl is very inefficient as uncoupler either in the absence or in the presence of anions, and becomes very efficient in the presence of valinomycin.

The present data indicate that abolition of either ΔpH or $\Delta\psi$ is not accompanied by abolition of ATP synthesis. As in the case of chloroplasts, concentrations of anions capable of abolishing almost completely $\Delta\psi$ have only a slight effect on ATP synthesis. Furthermore, as in the case of chromatophores, concentrations of NH_4Cl or KCl + nigericin capable of abolishing completely ΔpH have a negligible effect on ATP synthesis. The insensitivity of ATP synthesis to abolition of either ΔpH or $\Delta\psi$ may be attributed to the interchangeability of ΔpH with $\Delta\psi$. However in the experiment of Fig. 7 a high rate of ATP synthesis is observed also in the presence of concentrations of NH_4Cl capable of inducing a marked decrease of both ΔpH and Δw (cf. Figs. 5 and 6). A similar conclusion holds also in the case of NH_4NO_3. In principle, the resistance of ATP synthesis to depression of $\Delta\tilde{\mu}_H$ may be explained by the fact that the experiments of Fig. 7 are carried out in the presence of hexokinase where hexokinase induces a decrease of the phosphate potential from 16 to 6 kcal/mol [36]. This is not the case in the experiment of Fig. 8, where hexokinase is absent. In this experiment depression of $\Delta\tilde{\mu}_H$ is accompanied by inhibition of ATP synthesis. However ATP synthesis is abolished only when $\Delta\tilde{\mu}_H$ is reduced below 20 mV. Furthermore, the $\Delta Gp/\Delta\tilde{\mu}_H$ ratio increases with the decline of $\Delta\tilde{\mu}_H$ tending to infinite while $\Delta\tilde{\mu}_H$ tends to zero. Fig. 8 is similar to Fig. 6 of the preceding paper [29]. This suggests that the occurrence of ATP synthesis at low $\Delta\tilde{\mu}_H$ and the tendency of the $\Delta Gp/\Delta\tilde{\mu}_H$ ratio to increase with the depression of $\Delta\tilde{\mu}_H$, be a property independent of the operation of the adenine nucleotide carrier. The observations reported in the present and preceding papers [28,29] are taken as evidence that energy transduction within, and energy transfer between, energy transducing units involve events which are microscopic in nature.

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