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The mechanism of phosphate permeation in purified bean mitochondria

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The permeability properties and mechanism of Pi transport were investigated in purified bean mitochondria.

1. Purified bean mitochondria are impermeable to small molecules and ions. However, Pi, arsenate, acetate and formate can enter the osmotically active space of bean mitochondria.

2. Nigericin or the association of valinomycin and FCCP cause mitochondrial swelling in isoosmotic potassium phosphate.

3. The SH-blocking reagents mersalyl, pHMB and NEM inhibit various mitochondrial functions dependent on the translocation of Pi and arsenate across the membrane. These include the respiration stimulated by ADP, Ca^{2+}+Pi, and K^++valinomycin +Pi; the swelling in ammonium phosphate medium and, in the presence of nigericin, in potassium phosphate medium; the energy-linked valinomycin-induced swelling and the subsequent ClCCP-induced shrinking. The uncoupler-stimulated respiration, as well as the other processes when acetate is substituted for Pi, are not influenced by SH reagents.

4. Mersalyl and pHMB cause complete inhibition at about 20 nmoles/mg protein, whereas, NEM is effective at about 1 μmole/mg protein. The inhibition by mersalyl and pHMB, but not that by NEM, is sigmoidal and reversed by 2-mercaptoethanol. Non-inhibitory amounts of mersalyl protect the Pi transport from irreversible inhibition by NEM.

5. We concluded that a carrier-mediated transport system for Pi is present in bean mitochondria, and that some of its properties are similar to the Pi carrier of animal mitochondria.

In recent years, a great deal of work has been carried out with animal mitochondria to elucidate the mechanism of translocation of physiologically important metabolites across the mitochondrial membrane (*3*, *26*). Although the inner membrane of animal mitochondria appears to be impermeable to even small molecules and ions, specific carrier-mediated transport systems facilitate the permeation of those metabolites that must enter or leave the mitochondria. Furthermore, mitochondria from different tissues vary in their complement of carrier

Abbreviations: BSA, bovine serum albumine; pHMB, *p*-hydroxymercuribenzoate; NEM, N-ethylmaleimide; ClCCP, carbonylcyanide *m*-chloro-phenylhydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxy-phenylhydrazone.

systems (*3, 7, 14, 26, 27, 35, 36*). The question arises as to whether plant mitochondria contain specific transport systems similar to animal mitochondria.

The existence of carrier-mediated transport systems in plant mitochondria has been examined only in a few preliminary studies (*19, 25, 31, 32*). This may be due to the difficulty of preparing plant mitochondria which are intact and void of contaminants. Recently, Douce et al. (*6*) prepared highly purified plant mitochondria.

We studied the permeability properties of purified bean mitochondria toward different molecules and ions. Various reactions involving the transport of Pi and arsenate across the mitochondrial membrane were examined and their sensitivity to SH-blocking reagents investigated. Evidence indicates the presence of a carrier-mediated transport system which catalyzes an electroneutral translocation of Pi. The properties of this carrier in bean mitochondria were compared with those of the Pi carrier in animal mitochondria. A preliminary account of part of this work has been reported (*5*).

Materials and methods

Materials

Crystallized and lyophilized bovine serum albumin (BSA), cysteine, sodium deoxycholate, antimycin A, Tris (Trizma base), ADP and ATP (sodium salts), succinic acid, *l*-malic acid, sodium pyruvate, valinomycin, ClCCP, mersalyl {*o*-(3-hydroxymercuri-2-metoxypropyl)-carbamoylphenoxyacetate}, pHMB and NEM were purchased from Sigma. Nigericin and FCCP were gifts of Dr. Lardy and Dr. Heytler (du Pont), respectively. The other chemicals (analytical grade) were obtained from Merck, Darmstadt.

Preparation of purified mitochondria

Mitochondria were isolated from hypocotyls of 5-day-old etiolated bean (*Vigna sinensis* cv. Blue Lake) seedlings, grown in damp vermiculite in a dark-controlled environmental chamber at $27 \pm 1°C$ and 70% relative humidity.

The mitochondrial suspension was prepared using the method described by Ikuma (*24*) with minor modifications: for tissue disruption, a Braun mixer and a medium containing 0.5 M mannitol, 0.1% BSA, 5 mM Tris-Cl, 0.05% cysteine and 1 mM EDTA (pH 8.0) were used; the washing medium contained 0.4 M mannitol, 0.1% BSA and 5 mM Tris-Cl (pH 7.2).

Purification of the mitochondria was carried out on a discontinuous sucrose gradient according to Douce et al. (*6*), except that 5 mM Tris-Cl (pH 7.2) was used instead of 10 mM phosphate buffer. Purified mitochondria were suspended at a concentration of 30–40 mg protein/ml in a medium containing 0.4 M mannitol and 5 mM Tris-Cl (pH 7.2).

Mitochondrial swelling

The rate of mitochondrial swelling was monitored by recording the decrease in the absorbance at 546 nm with an Eppendorf photometer model 1101 M.

O_2 *uptake*

Oxygen uptake was measured polarographically with a Clark electrode

(Yellow Spring Instrument, Co.) in a 2-ml closed stirred cell, using a reaction medium containing 0.4 M mannitol, 5 mM Tris-Cl (pH 7.4), 5 mM $MgCl_2$ and 1–2 mg mitochondrial protein at 27°C. The oxygen concentration in the air-saturated medium was taken as 250 μM (*8*).

Determination of mitochondrial protein

Protein was determined by the biuret method (*15*) after being dissolved with sodium deoxycholate, using BSA as standard.

Results

Permeability properties of purified bean mitochondria

The intactness of the purified bean mitochondrial membrane was examined by studying the effect of changing the osmolarity of the medium on the mitochondrial volume (*2*, *37*). Fig. 1A shows that the reciprocal of the apparent optical density (the volume of the mitochondrial water) decreased linearly on increasing the concentration of sucrose in the medium, i.e., the mitochondria were osmotically active, behaving as osmometers. The same results were obtained using mannitol, D-glucose and galactose instead of sucrose, indicating that all these sugars, like in rat liver mitochondria (*1*), do not permeate purified bean mitochondria.

The properties of ion permeation into mitochondria can be studied using the method described by Chappell and Crofts (*2*). Isoosmotic solutions of different salts lead to swelling of mitochondria when both anion and cation can penetrate the mitochondria. As illustrated in Fig. 1B, in the presence of NH_4^+, Pi and

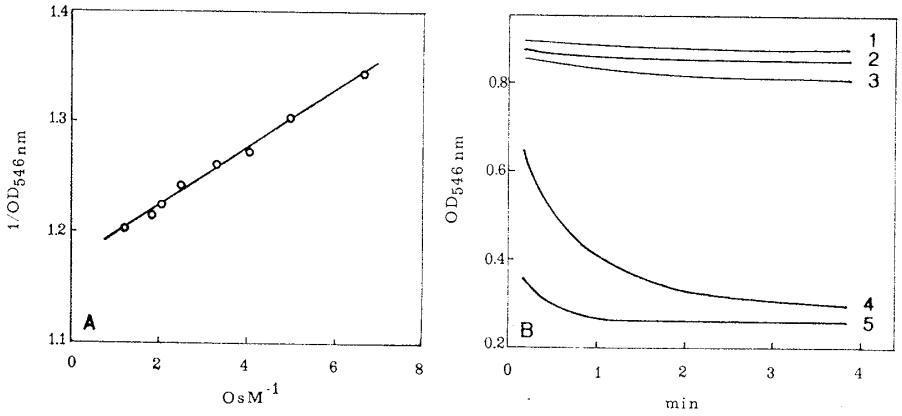


Fig. 1. *The osmotic behaviour of purified bean mitochondria.* A) Effect of changing the osmolarity of the medium on mitochondrial volume. Purified bean mitochondria (0.9 mg protein) were suspended in the indicated sucrose solutions containing 0.3 μg antimycin A, 1 mM EDTA and 5 mM Tris-Cl (pH 7.2). Final volume 2 ml, temperature 20°C. The optical density at 546 nm was read 2 min after addition of the mitochondria. B) Swelling of the mitochondria in isoosmotic solutions of various salts. Purified bean mitochondria (1 mg protein) were added to the indicated salt solutions containing 0.3 μg antimycin A, 1 mM EDTA and 5 mM Tris-Cl (pH 7.4). Final volume 2 ml, temperature 24°C. Curve 1, 200 mM NH_4Cl or 200 mM KCl; curve 2, 130 mM potassium phosphate; curve 3, 200 mM potassium acetate; curve 4, 130 mM ammonium phosphate; curve 5, 200 mM ammonium acetate.

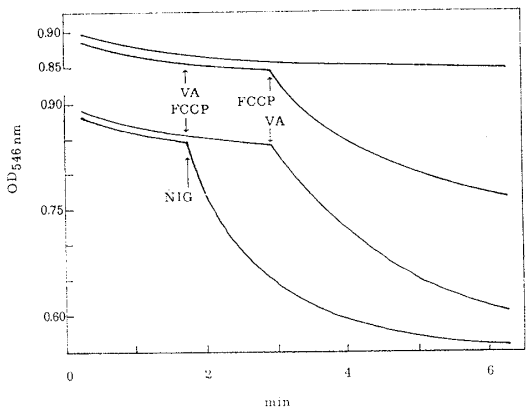


Fig. 2. *Effects of valinomycin, FCCP and nigericin on the volume of the mitochondria suspended in isotonic potassium phosphate.* Purified bean mitochondria (0.9 mg protein) were suspended in 130 mM potassium phosphate (pH 7.4) containing 0.3 μg antimycin A and 1 mM EDTA. Where indicated, 0.5 μg valinomycin (VA), 2.5 μM FCCP or 0.35 μg nigericin (NIG) was added. Final volume 2 ml, temperature 24°C.

acetate induced mitochondrial swelling, whereas no swelling occurred with impermeable anions such as Cl^- and (not shown) Br^- and I^-. With both Pi and acetate, swelling did not occur when K^+ was substituted for the permeant cation NH_4^+. Since ammonia probably enters the mitochondria as NH_4 (*2*), these results indicate that Pi and acetate can permeate the mitochondrial membrane together with protons or in exchange for hydroxyl ions, i.e., in an electroneutral manner.

The question of the mechanism of Pi permeation in bean mitochondria can also be approached by examining the effect of ionophores on mitochondrial swelling in isotonic potassium phosphate (*29*). Fig. 2 shows that nigericin, which catalyzes a K^+/H^+ exchange, or the association of the K^+-conductor valinomycin and the H^+-conductor FCCP, induced swelling of the mitochondria suspended in potassium phosphate. Neither valinomycin nor FCCP had any effect when added alone.

Similar results were obtained when the effect of ionophores was tested in the presence of potassium salts of arsenate, acetate and formate (not shown). As a contrast, we found (unpublished results) that valinomycin alone caused swelling of bean mitochondria in isotonic KSCN, indicating that SCN^- can permeate through

Table 1 *Effect of mersalyl, pHMB and NEM on the swelling of bean mitochondria in various ammonium salts*

Suspending medium	Rate of swelling [a] (ΔOD/30 sec)			
	No inhibitor	Mersalyl	pHMB	NEM
NH_4 phosphate (130 mM)	0.47	0.09	0.08	0.09
NH_4 acetate (200 mM)	0.73	0.72	0.72	0.71
NH_4 arsenate (130 mM)	0.45	0.07	0.08	0.09
NH_4 formate (200 mM)	0.56	0.54	0.55	0.55

Thirty μl of purified bean mitochondria (1.2 mg protein) was preincubated with 20 μl of a solution containing 0.4 M mannitol, 5 mM Tris-Cl (pH 7.4) and mersalyl (0.16 μmoles/mg protein), pHMB (0.16 μmoles/mg protein) or NEM (2.5 μmoles/mg protein). After incubation, the mitochondria were added to the indicated ammonium salts containing 0.3 μg antimycin A, 1 mM EDTA and 5 mM Tris-Cl (pH 7.4). Final volume 2 ml, temperature 24°C.

[a] The rate of swelling is shown as ΔOD_{546nm}/30 sec after the addition of the mitochondria.

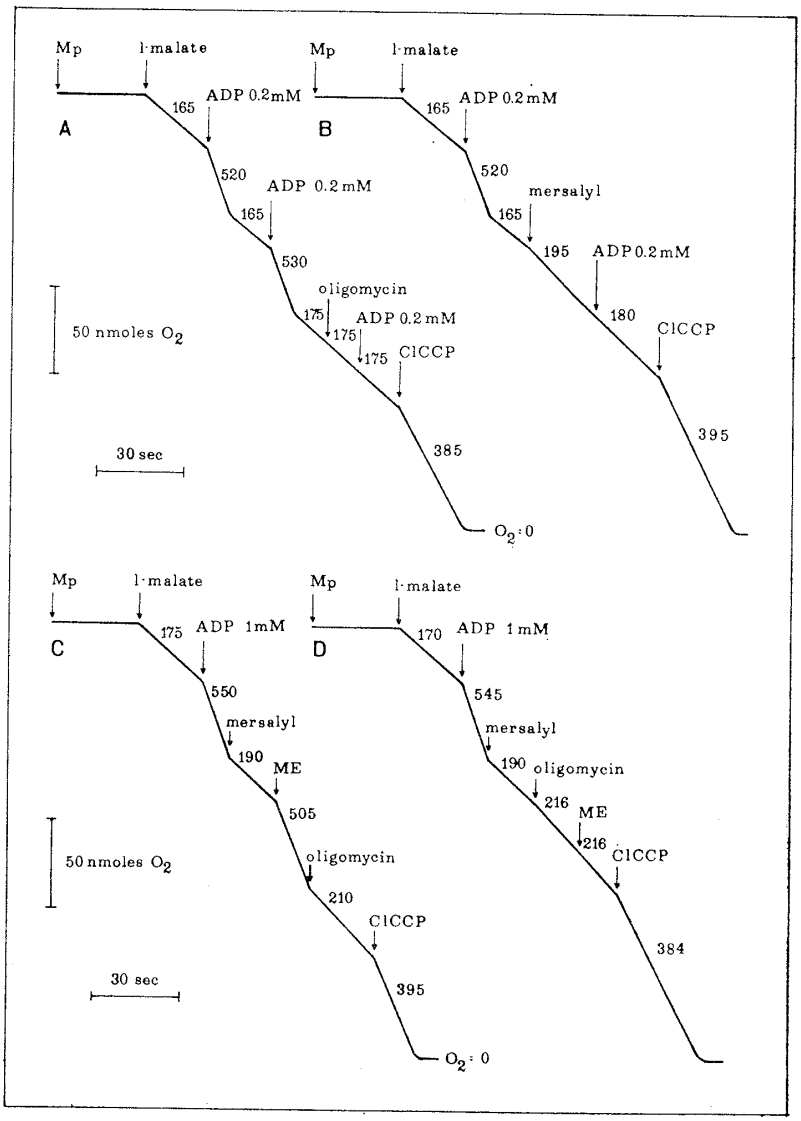


Fig. 3. *Effect of mersalyl on* l-*malate oxidation of bean mitochondria.* Conditions are as described in **Materials and methods** except that 10 mM Pi (Tris salt) was also included in the reaction medium. Where indicated, 1.1 mg protein of purified bean mitochondria, 30 mM Tris-*l*-malate, 200 μM or 1 mM ADP, 20 μg oligomycin, 7.5 μM ClCCP, 0.2 μmoles mersalyl or 5 mM 2-mercaptoethanol (ME) was added. The numerals on the slopes are nmoles O_2/mg protein per min.

the membrane in the anionic form, in accordance with its polarizability and lipid solubility (*22*).

Effect of SH reagents on swelling in isoosmotic salts

Since the transport of Pi in animal mitochondria is inhibited by low concentrations of SH-blocking reagents (*9*, *10*, *20*, *38*), we investigated the effect of these reagents on the swelling of purified bean mitochondria in isoosmotic salts of Pi. Table 1 shows that mersalyl, pHMB and NEM inhibited mitochondrial swelling in ammonium phosphate or ammonium arsenate medium. On the other hand, the swelling in ammonium acetate or ammonium formate medium was not

Table 2 *Effect of mersalyl on the respiration stimulated by calcium or valinomycin in the presence of different anions*

Exp.	Addition	Rate of oxygen uptake [a] (nmoles O_2 min^{-1} mg^{-1} protein)	
		No inhibitor	With mersalyl
1	None	190	192
	ADP+Pi	520	195
	2,4-Dinitrophenol	472	476
	Calcium+Pi	408	201
	Calcium+acetate	396	381
	Calcium+arsenate	385	197
2	None	180	180
	ADP+Pi	490	165
	ClCCP	370	367
	Valinomycin	220	210
	Valinomycin+Pi	330	220
	Valinomycin+acetate	300	300
	Valinomycin+arsenate	310	225

The reaction medium contained 0.4 M mannitol, 10 mM KCl, 5 mM $MgCl_2$, 5 mM Tris-Cl (pH 7.2) and purified bean mitochondria (1.9 mg protein in Exp. 1 and 1.2 mg in Exp. 2). The mitochondria were incubated in the reaction medium for 2 min, either in the absence or presence of mersalyl (0.2 μmoles/mg protein), before addition of 4 mM Tris-succinate in Exp. 1 or 8 mM Tris-*l*-malate and 8 mM sodium pyruvate in Exp. 2. One min after addition of the respiratory substrates, 1 mM ADP, 10 mM Pi, 30 μM 2,4-dinitrophenol, 100 μM $CaCl_2$, 10 mM Tris-acetate, 10 mM sodium arsenate, 7.5 μM ClCCP or 0.5 μg valinomycin was added as indicated. The final volume was 2 ml, the temperature was 27°C. Other conditions are described in **Materials and methods.**

[a] In agreement with Douce et al. purified bean mitochondria exhibited a very strong control by ADP and their respiratory activity was very fast (*6*).

affected by these SH reagents. As shown below (Fig. 6) mersalyl, pHMB and NEM also effectively inhibited bean mitochondria swelling in isoosmotic potassium phosphate, caused by the addition of nigericin.

Effect of SH reagents on respiration

Polarographic experiments using purified bean mitochondria confirmed the report of Hanson et al. (*19*) that mersalyl inhibits the stimulation of oxygen uptake by ADP but not that by the uncoupling agents (Fig. 3, trace B). Inhibition of the ADP-stimulated respiration by mersalyl was released by 2-mercaptoethanol (trace C) to approximately the rate of the control, indicating that no permanent alteration was caused by the SH reagent. Oligomycin abolished the effect of 2-mercaptoethanol (trace C) or prevented it when added before (trace D). Similar results were also found with 10 mM *l*-malate plus 10 mM pyruvate or with 5 mM succinate as respiratory substrates, and using pHMB instead of mersalyl.

In a wide variety of plant mitochondria, Chen and Lehninger (*4*) have shown that Ca^{2+} is not taken up and does not stimulate respiration unless a permeant anion is also present. Table 2 compares (Exp. 1) the effect of mersalyl on the stimulation of succinate oxidation by Ca^{2+} in the presence of different anions. The SH reagent

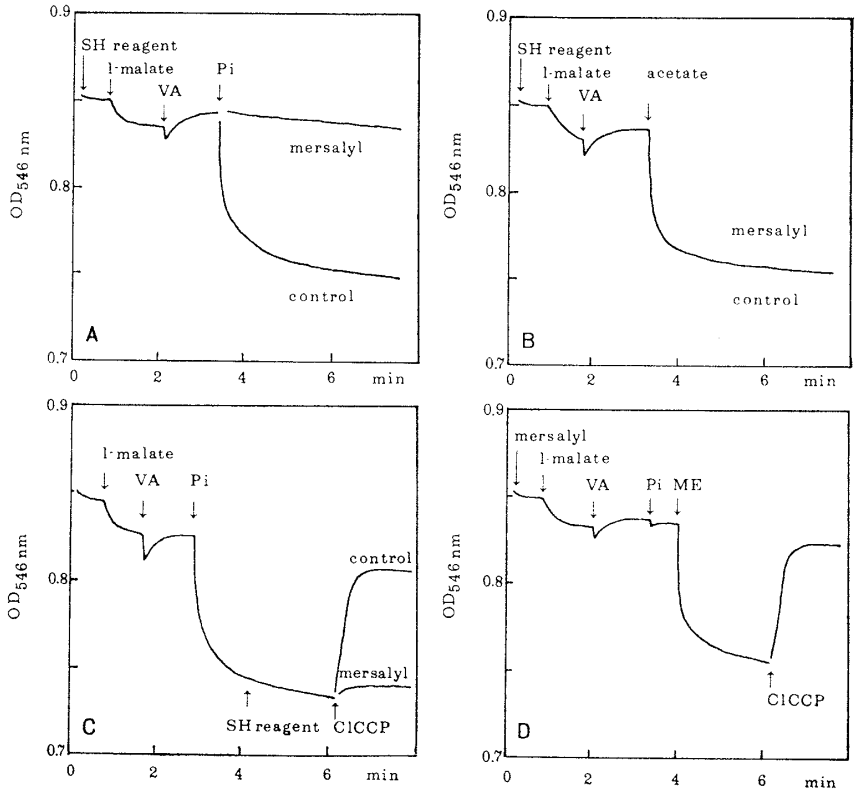


Fig. 4. *Effect of mersalyl on the energy-linked valinomycin-induced swelling.* Purified bean mitochondria (1 mg protein) were suspended in a medium containing 0.4 M mannitol, 1 mM EDTA, 10 mM KCl and 5 mM Tris-Cl (pH 7.4). Where indicated, 100 nmoles of the SH reagent mersalyl, 30 mM Tris-*l*-malate, 0.5 μg valinomycin (VA), 1 mM Tris-phosphate, 5 mM Tris-acetate, 7.5 μM ClCCP and 5 mM 2-mercaptoethanol (ME) were added. Final volume 2 ml, temperature 22°C.

markedly inhibited succinate oxidation stimulated by calcium ions, when Pi or arsenate was added as the permeant anion. On the other hand, when acetate was substituted for Pi, stimulation of succinate oxidation by Ca^{2+} was unaffected by mersalyl.

Mitochondrial respiration is also stimulated by K^+ uptake in the presence of valinomycin and a suitable permeant anion (*19*, *30*, *40*). The data in Table 2 (Exp. 2) show that mersalyl abolished the increase in oxygen uptake induced by Pi or arsenate, in the presence of valinomycin and K^+, but had no effect on the stimulation induced by acetate.

Effect of SH reagents on energy-linked valinomycin-induced swelling

Fig. 4 (traces A and B) shows that mersalyl inhibited the energy-linked valinomycin-induced swelling of bean mitochondria when Pi was the permeant anion, but no effect was observed when acetate was used instead of Pi. In the experiment illustrated in trace C of Fig. 4, the mitochondria were first swollen by the energy-linked K^+ uptake in the presence of valinomycin and Pi. When ClCCP was added, a fast contraction occurred. However, if mersalyl was added before ClCCP, the shrinking (i.e., the efflux of Pi and K^+) was abolished. Addition of 2-mercaptoethanol (trace D) to mitochondria incubated with mersalyl, valinomycin

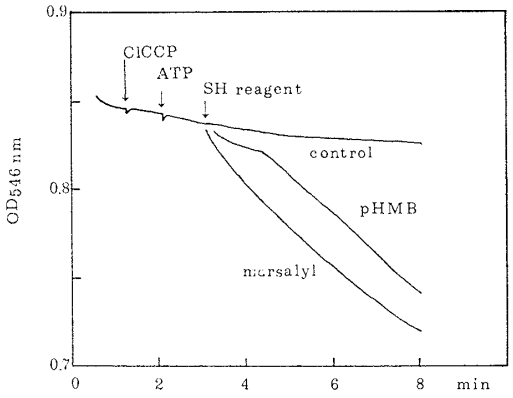


Fig. 5. *SH reagent-induced swelling during ClCCP-stimulated ATP hydrolysis.* Purified bean mitochondria (1 mg protein) were suspended in a medium similar to that reported in Fig. 4. Where indicated, 2 mM ATP, 7.5 μM ClCCP, 100 nmoles mersalyl or 500 nmoles pHMB were added. Final volume 2 ml, temperature 24°C.

and Pi, in the presence of a respiratory substrate, released the mersalyl inhibition of both the valinomycin-induced swelling and the ClCCP-induced shrinking. Similar results were found using 0.1 mM pHMB instead of mersalyl, and arsenate instead of Pi (not shown). NEM (1 mM) also inhibited the energy-linked valinomycin-induced swelling when Pi or arsenate was the permeant anion. In this case, however, further addition of 2-mercaptoethanol did not release the inhibition.

SH reagent-induced swelling during ClCCP-stimulated ATP hydrolysis

Another way of testing the effect of SH reagents on the Pi transport across the mitochondrial membrane is to generate Pi in the matrix by the uncoupler-stimulated ATP hydrolysis. Thus, if the efflux of Pi is inhibited under these conditions, Pi builds up in the matrix and the mitochondria swell (*16*, *39*). Fig. 5 shows that the addition of mersalyl or pHMB to bean mitochondria, incubated with ClCCP and ATP, caused mitochondrial swelling.

Titration of Pi-dependent mitochondrial swelling with SH reagents

Fig. 6A shows the effect of increasing concentrations of mersalyl on the energy-linked valinomycin-induced swelling and on the nigericin-induced swelling in potassium phosphate medium. Inhibition of the two Pi-dependent processes by any given mersalyl concentration was similar. With both systems, mersalyl had virtually no effect at a concentration of 3 nmoles/mg protein. Above this concentration, it exerted its inhibitory effect giving half-maximal inhibition at about 7 nmoles/mg protein. A sigmoidal type of inhibition by mersalyl has also been found when Pi-dependent mitochondrial functions were studied in rat liver mitochondria (*11*, *13*, *28*, *34*).

Titration of Pi-dependent mitochondrial swelling with mersalyl was compared with that obtained in the presence of pHMB (Fig. 6B) or NEM (Fig. 6C). pHMB was approximately as effective as mersalyl and its curve of inhibition was also sigmoidal. The other SH reagent, NEM, on the other hand, inhibited the Pi-dependent swelling in an hyperbolic way and a much higher concentration was required for half-maximal inhibition (about 350 nmoles/mg protein).

As recently found by Fonyo in rat liver mitochondria (*13*), Fig. 7 shows that non-inhibitory amounts of mersalyl protected the Pi transport from irreversible inhibition by NEM. In this experiment, the valinomycin-induced swelling was used to assay Pi transport. Trace B demonstrates that the inhibition of Pi uptake

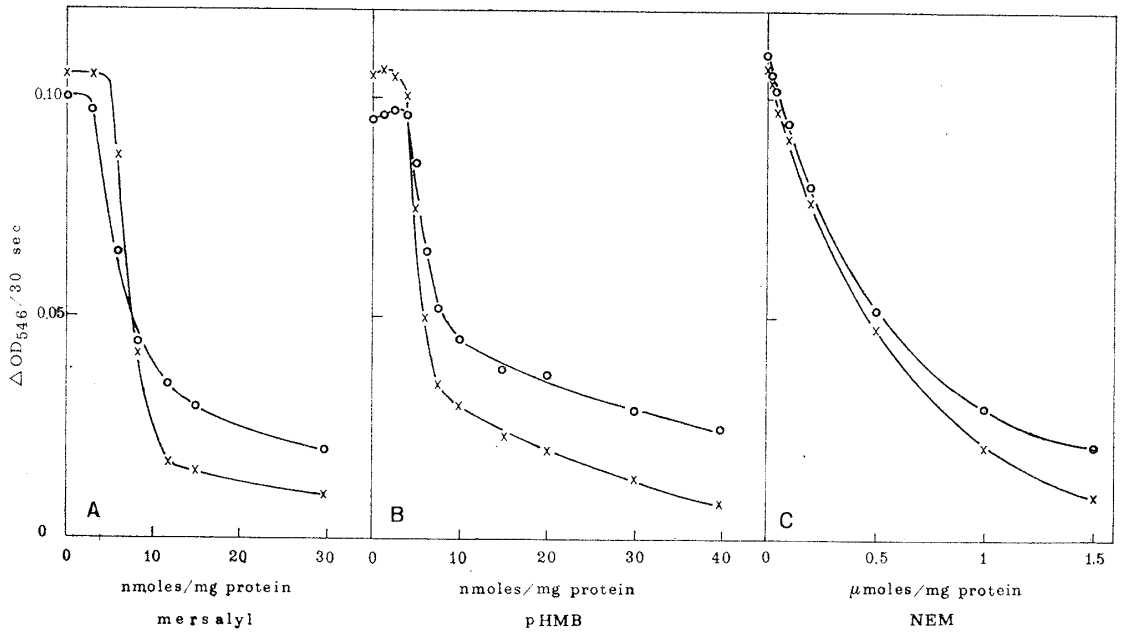


Fig. 6. *Effects of increasing concentrations of mersalyl, pHMB and NEM on the energy-linked valinomycin-induced swelling and nigericin-induced swelling in potassium phosphate.* To assay the energy-linked valinomycin-induced swelling (×–×), purified bean mitochondria (1.2 mg protein) were incubated for 2 min in the same medium described in Fig. 4 in the presence of 30 mM Tris-*l*-malate, 1 mM Tris-Pi and the indicated concentrations of mersalyl, pHMB or NEM. To initiate the assay, 0.5 μg valinomycin was added. To assay the nigericin-induced swelling (○–○), purified bean mitochondria (1.1 mg protein) were incubated for 2 min in a medium containing 130 mM potassium phosphate buffer (pH 7.4), 5 mM $MgCl_2$, 0.3 μg antimycin A, and the indicated concentrations of mersalyl, pHMB or NEM. To initiate the assay, 0.35 μg nigericin was added. In both assays, the total volume was 2 ml and the temperature 22°C. The rate of swelling is shown as ΔOD_{546nm}/30 sec.

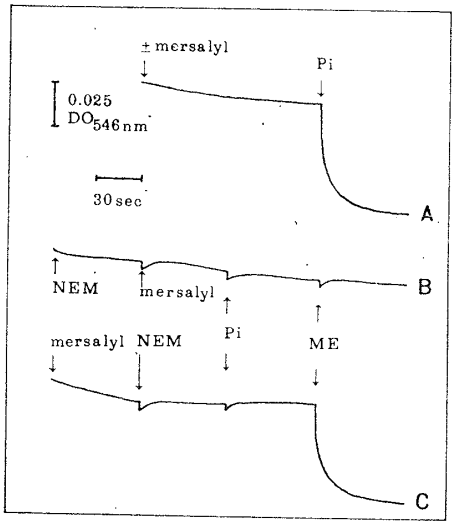


Fig. 7. *Protection of Pi transport activity by a non-inhibitory amount of mersalyl.* Purified bean mitochondria (0.8 mg protein) were incubated for 1 min in a medium containing 0.4 M mannitol, 1 mM EDTA, 10 mM KCl, 5 mM Tris-Cl (pH 7.4), 30 mM Tris-*l*-malate and 0.5 μg valinomycin. Where indicated, 3.8 nmoles/mg protein mersalyl, 2.5 mM NEM, 5 mM 2-mercapto-ethanol (ME) or 5 mM Pi was added. The control (Trace A) was virtually the same in the presence or absence of mersalyl. Final volume 2 ml, temperature 22°C.

by NEM was not reversed by 2-mercaptoethanol. However, when a non-inhibitory amount of mersalyl (3.8 nmoles/mg protein) was added to the reaction mixture before a large excess of NEM, Pi-dependent swelling occurred, provided 2-mercaptoethanol removed mersalyl and the unreacted NEM (trace C). The addition of 2-mercaptoethanol, in the absence of Pi, did not cause swelling (not shown). These data indicate that non-inhibitory amounts of mersalyl react with SH-groups, which are able to elicit Pi transport activity when they are liberated and the rest of the SH-groups are irreversibly blocked by NEM.

Discussion

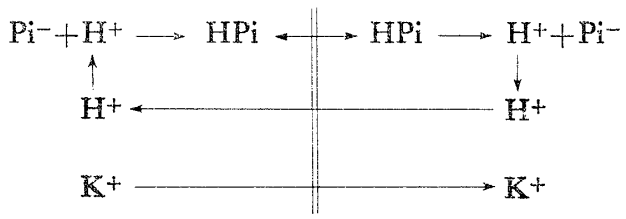
The data of Fig. 1 demonstrate that Cl^- and K^+ cannot enter the osmotically active space of purified bean mitochondria. Thus, these mitochondria do not swell in KCl, and even in NH_4Cl, potassium acetate and potassium phosphate. These results are in contrast with the passive swelling in KCl, K acetate and K phosphate, which has been observed in plant mitochondria (see *17*, *18*, *40* for refs.). Conceivably the reported differences in ion permeability properties between plant and animal mitochondria may reflect membrane damage suffered during isolation by a great proportion of plant mitochondria (*33*). Our findings indicate that mitochondria carefully prepared and purified from bean hypocotyls, are impermeable not only to Cl^-, K^+, Br^- and I^- ions, but also to small uncharged molecules such as several monosaccharides and disaccharides, as already shown in rat liver mitochondria (*1*).

Among the anions tested, Pi, arsenate, acetate and formate are able to enter the mitochondria. Our data show that they permeate the membrane of bean mitochondria in an electroneutral manner as undissociated acids or in the ionized state in exchange for OH^-. The following evidence supports this:

a) Mitochondria swell in ammonium salts. In this case, the free diffusion of NH_3 into the matrix creates a pH gradient across the membrane, alkaline inside, which allows influx of the above-mentioned anions in exchange for OH^- (or together with H^+).

b) Valinomycin, which catalyzes an electrogenic translocation of K^+ (*21*), does not cause swelling of mitochondria suspended in potassium salts. This rules out the possibility of an electrogenic translocation of Pi, arsenate, acetate and formate.

c) The requirement for swelling in K^+ salts of high permeability for both K^+ and H^+ can be accounted for by an electroneutral transport of acids according to the following scheme:



In this scheme, the permeation of Pi together with H^+ is represented for the sake of convenience and does not imply that an exchange of Pi with OH^- cannot occur.

As found in animal mitochondria (*9*, *10*, *20*, *38*), the transport of Pi and arsenate in purified bean mitochondria is inhibited by SH-blocking reagents. Thus mersalyl,

pHMB and NEM inhibit various mitochondrial functions dependent on the translocation of Pi across the membrane. That the point of inhibition is the specific transport of Pi is supported by the observation that all processes in which acetate can be used instead of Pi are not affected by SH reagents. Furthermore, the uncoupler-stimulated respiration which is not dependent on the entry of Pi into the mitochondria, is not inhibited by the thiol reagents, in contrast to the respiration stimulated by ADP, Ca^{2+}+Pi, K^++valinomycin+Pi. Note that the presence of an uncoupler per se does not abolish the effect of the SH reagents on the Pi transport. Thus, these reagents inhibit the ClCCP-induced shrinking of mitochondria previously swollen by the energy-linked K^+ uptake, in the presence of valinomycin and Pi, and vice versa cause mitochondrial swelling during ClCCP-stimulated ATP hydrolysis.

The sensitivity to SH reagents is the main indication that the Pi transport is a carrier-mediated process. As in animal mitochondria, arsenate seems to be a substrate for this carrier, since its transport is sensitive to the same SH reagents. Several properties of the inhibition by SH reagents of the Pi transport in bean mitochondria are similar to those observed in rat liver mitochondria: a) The inhibitory effect of mersalyl and pHMB is reversed by 2-mercaptoethanol, whereas that of NEM is not. b) Pi transport in bean mitochondria is inhibited almost completely by about 20 nmoles/mg protein mersalyl and pHMB. This value compares well with the observations with rat liver mitochondria (*9*, *11*, *13*, *28*, *34*). c) The curve representing inhibition as a function of the concentration of mersalyl and pHMB is sigmoidal. d) Low, non-inhibitory amounts of mersalyl react with SH-groups of the Pi carrier. The transport system for Pi in bean mitochondria is, however, different from that of animal mitochondria with respect to its inhibition by NEM. Thus, NEM completely inhibits Pi transport in bean mitochondria only at a concentration of about 1 μmole/mg protein, whereas 15–60 nmoles/mg protein cause total inhibition in rat liver mitochondria (*12*, *13*, *20*, *23*, *28*). Furthermore, the dependence of the inhibition on NEM concentration is not sigmoidal in bean mitochondria, in contrast to that observed in rat liver mitochondria (*23*, *28*). According to Fonyo (*13*), the sigmoidal dependence of the inhibition of Pi transport on the concentration of SH reagents in liver mitochondria may be accounted for by the presence of two functionally equivalent SH groups per unit of Pi carrier with largely different affinities for the inhibitors. Possibly, in bean mitochondria, the proposed two SH groups on the Pi carrier have lower and approximately equal affinity for NEM.

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