Activation of Energy-Linked K^+ Accumulation in Isolated Heart Mitochondria by Non-Ionic Detergents

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Summary: Sub-lytic concentrations of a non-ionic detergent such as Triton X-100 or Lubrol WX markedly activate energy-dependent ion accumulation and osmotic swelling of beef heart mitochondria suspended in media containing acetate or phosphate salts. Both the efficiency and the absolute amount of ion accumulation are increased by the detergent. The detergent-dependent increment in acetate or phosphate uptake is specific for K^+ and, like the spontaneous ion uptake reaction, is sensitive to and reversed by dinitrophenol and other uncouplers. These results suggest that mitochondrial ion accumulation is normally limited by the low permeability of the membrane to K^+, and that in the presence of the detergent the membrane is altered so that this restriction is decreased.

Energy-dependent accumulation of ions and osmotic swelling has been shown to occur spontaneously when isolated heart mitochondria are suspended in 0.1 M salts of a monovalent cation and acetate or phosphate (1-5). In media containing sucrose the osmotic pressure of this non-penetrating solute reduces the spontaneous swelling and ion uptake, although the reaction can still be observed by conventional procedures. A number of different chemical modifications of the coupling membrane have been shown to result in increases in the magnitude of this energy-dependent ion uptake. The modifications include (a) the addition of an ionophore or ion carrier such as valinomycin or gramicidin (6-8), (b) removal of a portion of the Mg^{+2} of the membrane (9-10), (c) addition of a heavy metal cation such as Zn^{+2} (1, 11), (d) addition of parathyroid hormone and certain histones (12-13), (e) addition of organic mercurials and other thiol-group reagents (14-15). In most of these cases an enhanced passive permeability to one or more of the ionic species in the suspending medium can also be demonstrated.

In addition to these modifications it is established in the present communication that the presence of sub-lytic concentrations of a non-ionic detergent such as Triton X-100 (Rohm and Haas) or Lubrol WX (ICI America, Inc.) markedly activates the energy-dependent ion uptake and swelling. The effect of the detergents is rather similar to that of low concentrations of valinomycin.

Results - The study shown in Fig. 1 establishes that low concentrations of Triton X-100 activate the succinate-supported swelling and ion uptake which occurs in a medium of sucrose (100 mM) and K^+ acetate (10 mM). Optimal response is obtained in the range from 0.002 to 0.005% (v/v) Triton. Higher concentrations of detergent result in an immediate decrease in absorbance and little effect on the subsequent succinate-dependent swelling.

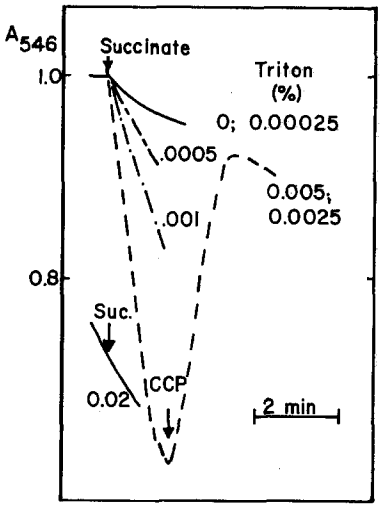

Fig 1 - Activation of succinate-dependent K^+ acetate accumulation and swelling by Triton X-100. Beef heart mitochondria (10) (5 mg of protein) were treated with rotenone and suspended in 10 ml of sucrose (100 mM) and K^+ acetate (10 mM) containing Tris acetate (2 mM, pH 7.0) at 25° in a chamber mounted on an Eppendorf photometer. The absorbance at 546 mμ was recorded. Respiration and pH were monitored simultaneously (records not reproduced). The indicated amount of Triton X-100 (per cent, v/v) was added before the mitochondria and the reaction was started by addition of K^+ succinate (2 mM). M-chlorocarbonylcyanidephenylhydrazone (CCP, 5×10^{-7}M) was added at the point indicated. Higher levels of detergent (0.02% as shown) result in an immediate partial clarification of the suspension and little activation of the succinate-dependent swelling reaction.

The activated reaction retains the properties of the spontaneous ion accumulation in that (a) it is supported by all substrates tested and by exogenous ATP, (b) no swelling occurs when respiration is blocked with an appropriate inhibitor, (c) the reaction is insensitive to oligomycin when supported by respiration and sensitive to this reagent when supported by ATP, and (d) uncouplers prevent the ion accumulation and result in release of accumulated ions and contraction when added to the swollen mitochondria (Fig. 1).

Addition of the detergent results in only slight activation of succinate respiration under the conditions of Fig. 1 so that the increased swelling and ion uptake represent a substantial increase in the efficiency of the reaction. The data of Fig. 2 establish that swelling efficiency is increased three fold in the optimum concentration range of Triton X-100. Responses similar to those obtained with Triton are found in the same concentration range with Lubrol WX and with IGEPAL®, CO-630 (GAF Corp).

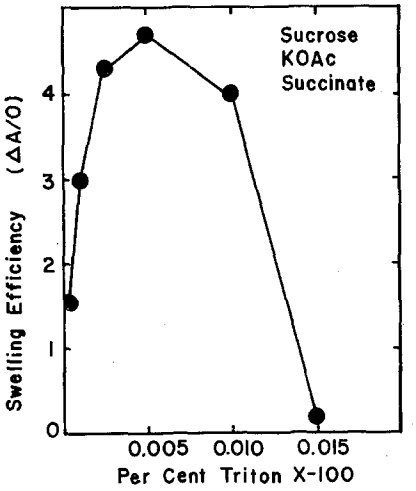

Fig 2 - Increase in efficiency of succinate-dependent K^+ acetate swelling with concentration of Triton X-100. The reaction was carried out as described in Fig 1. The initial rate of swelling (ΔA per min) was evaluated and the ΔA per μatom of O_2 consumed per mg of protein (ΔA/O) was calculated. At higher levels of Triton the ΔA in the absence of respiration was subtracted so that in all cases the respiration-dependent ΔA is compared.

Specificity for Ions - The Triton-activated swelling and ion accumulation shows nearly an absolute dependence on the presence of K^+. Replacing the K^+ in studies such as that shown in Fig. 1 with Na^+, Li^+, $Tris^+$, or tetramethylammonium$^+$ results in no activation by Triton in the indicated concentration range. The dependence of the Triton-induced increment in swelling on the concentration of K^+ is shown in Fig. 3.

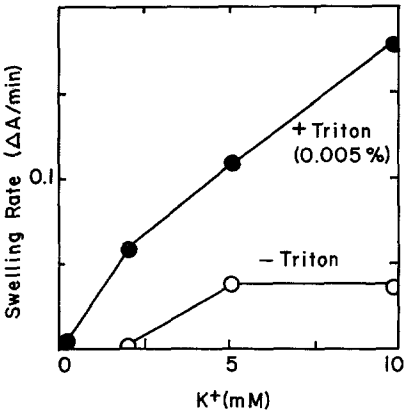

Fig 3 - Dependence of Triton-activated swelling on K^+ concentration. The experiment was carried out as described in Fig 1 using a medium of sucrose (100 mM), Tris acetate (2 mM, pH 7.0), and Na^+ acetate (10 mM). The indicated amount of K^+ acetate was added to replace Na^+ so that the acetate concentration remained constant (10 mM). Swelling was initiated by the addition of Tris succinate.

Like the spontaneous reaction (2, 16), the detergent-induced swelling depends on the presence of either phosphate or a weak acid anion such as acetate. Triton induces no energy-dependent swelling in the presence of the K^+ salt of a non-penetrating anion (Cl^-) or a permeant strong acid anion (NO_3^-). Triton (0.005%) does increase the amount of H^+ which appears when anaerobic heart mitochondria are subjected to a pulse of O_2 in a medium of KCl (data not shown). When the identical experiment is carried out in NaCl instead of KCl little response to Triton is observed. These results are in accord with those of Packer and Utsumi (17) using Triton-treated liver mitochondria.

Effects of Triton on Oxidative Phosphorylation and ATPase Activity -

Triton at the levels required for maximum activation of K^+ acetate or phosphate accumulation results in an increase in State 4 respiration, increased swelling, and a decline in the respiratory control ratio in a medium containing K^+ (Table I). The P/O ratio is decreased from 1.8 to 1.1 under these conditions. In the absence of K^+, but the presence of a corresponding concentration of Tris, these adverse effects of Triton on energy-coupling (P/O ratio) are still present but are much less striking than in a medium containing K^+ (Table I). Triton induces a high rate of ATPase activity in a KCl medium, but not in NaCl (Table II). The

Table I

Effect of Triton X-100 on Phosphorylation and Respiratory Control

Suspending Medium	Addition	Respiration Rate (μatoms min^{-1} mg^{-1}) State 3	State 4	Control Ratio (State 3/4)	P/O Ratio
Sucrose-KCl	None	0.12	0.035	3.4	1.8
"	Triton	0.075	0.050	1.5	1.1
Sucrose-Tris Cl	None	0.17	0.046	3.7	1.7
"	Triton	0.11	0.050	2.2	1.3

Mitochondria (5 mg of protein) were treated with rotenone and added to 8 ml of a medium of sucrose (100 mM), KCl or Tris Cl (10 mM), Tris succinate (2 mM), and Tris Pi (2 mM, pH 7.0) and the respiration rate at 25° recorded with a Clark electrode. Respiratory control and P/O ratios were estimated by adding a pulse of ADP as described by Chance and Williams (18). Swelling at a rate of 0.06 to 0.13 absorbance units per min was observed only in the presence of both KCl and Triton (0.005%).

Table II

Effect of Triton X-100 on ATPase Activity

Additions	ATPase Activity (μmoles Pi/mg/3 min) KCl (100 mM)	NaCl (100 mM)
None	0.08	0.06
Triton (0.005%)	0.59	0.09
Dinitrophenol (50 μM)	0.66	0.63
CMS (100 μM)	1.01	0.41

Mitochondria (0.5 mg of protein) were treated with rotenone and added to 0.6 ml of the indicated medium containing 3.3 mM ATP ($diNa^+$ salt neutralized with Tris). ATPase activity was estimated from the release of Pi in 3 min at 25° in the presence of the indicated additions (cf. Ref. 15).

marked specificity for K^+ in activation of ATPase activity is similar to the effect of mercurials such as p-chloromercuriphenyl sulfonate (CMS) on ATPase activity (cf. Table II and Ref. 15).

Effects of Triton on Passive Permeability to Ions - Heart mitochondria which accumulate ions and swell spontaneously to a limited extent in the sucrose-K^+ acetate (100 and 10 mM respectively) medium contract slowly following addition of an uncoupler (Fig. 4). This contraction depends on the rate of passive K^+ loss in response to the sucrose osmotic pressure and can be markedly accelerated by the addition of valinomycin to increase the permeability of the membrane to K^+ (Fig. 4A). Triton mimics the ionophore in this regard. Addition of Triton after CCP accelerates the uncoupler-dependent contraction and no response can then be detected to valinomycin (Fig. 4B). If Triton is added before CCP the contraction following addition of uncoupler is accelerated (Fig. 4C).

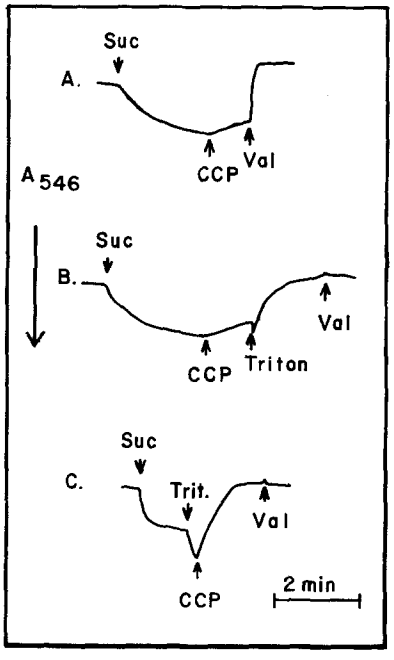

Fig 4 - Triton-dependent increase in outflow of accumulated K^+. The experiments were carried out as described in Fig 1 with the addition of CCP ($5x10^{-7}M$), valinomycin ($2x10^{-7}M$), and Triton (0.005% v/v) as indicated.

Discussion - It has been suggested that valinomycin accelerates mitochondrial ion accumulation by increasing the availability of K^+ to an endogenous ionophore in the mitochondrion which meshes with the energy-transfer system in such a way as to result in energy-linked ion transport (6). Alternatively, the effect of valinomycin has been visualized as increasing the transmembrane permeability to K^+ so that increased fluxes of this cation can occur in response to electrochemical gradients (3, 19). The present studies establish that non-ionic detergents such as Triton and Lubrol can produce the same effects as those of the ionophore. Triton accelerates the energy-dependent accumulation of K^+ salts of phosphate or weak acids such as acetate; it increases the H^+ pulse seen during an anaerobic-aerobic transition in KCl media; it activates K^+ dependent ATPase activity; and it results in an apparent increase in passive permeability to K^+. Triton dependent decreases in respiratory control, and P/O ratio with succinate are also accentuated by the presence of K^+.

The present studies do not permit a choice between a carrier-mediated K^+ transport mechanism (cf. Ref. 6, for example) as opposed to transmembrane movement of K^+ in response to a potential gradient (cf. Ref. 19). However, the data do suggest that regardless of which of these models is correct, a barrier to K^+ movement which restricts the rate of the reaction can be markedly decreased by the interaction of the membrane with a non-ionic detergent. It is clear from these studies (cf. Fig. 1 and 4) that the detergent in the range of concentrations which promote K^+ permeability and energy-dependent K^+ accumulation does not duplicate the effects of an uncoupler such as CCP. This result implies that the detergent does not promote permeability to H^+. A more complete account of these studies will be presented elsewhere.

Acknowledgements

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