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Effects of Monazomycin on Ion Transport and Oxidative Phosphorylation in Liver Mitochondria*

Sergio Estrada-O and Carlos Gómez-Lojero

ABSTRACT: The antibiotic monazomycin stimulates an energy-linked uptake of Li^+, Na^+, K^+, Rb^+, or Cs^+ in exchange for protons in liver mitochondria. Simultaneous with the induced ion movements it also stimulates ATP hydrolysis and substrate oxidation in the absence of P_i acceptor. In the presence of K^+, the ATPase activity induced by monazomycin varies exponentially with the third power of the antibiotic concentration. The rate of ATP hydrolysis is also dependent on the concentration and radius of the alkali metal cations in the order: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$. In medium free of added alkali metal cations, the antibiotic stimulates ATP hydrolysis, succinate but not glutamate or α-ketoglutarate oxidation, and a rapid efflux of K^+ from the mitochondria. Substrate oxidation induced by the antibiotic in the absence of added cations, P_i, or acetate, is inhibited by low concentrations of Li^+, K^+, Rb^+, Cs^+, or Mg^{2+} but not Na^+. This latter cation actually opposes the effect of added Mg^{2+}. The inhibited state caused by alkali metal cation is overcome by phosphate, acetate, or nigericin. Inorganic phosphate stimulates oxygen uptake according to the sequence Cs^+, Rb^+, K^+, and Li^+. The inhibited state mediated by Mg^{2+} is reversed by P_i, ATP, or EDTA. Monazomycin appears to stimulate Mg^{2+} uptake in the presence of P_i. It does not affect the respiratory control induced by rutamycin in submitochondrial sonic particles. It is suggested that cations, protons, or anions influence the cooperative aggregation of monomers of the antibiotic, to form a conducting oligomer which translocates ions acting either in parallel or in series with cation carriers existing in the mitochondrial membrane.

Monazomycin is an antibiotic produced by *Streptomyces mashiuensis* (Akasaki *et al.*, 1963), which is known to contain 16 OH^- groups, 1 galactose, 1 dissociable amino group, and no amino acids (Mitscher *et al.*, 1967).

Lardy *et al.* (1967) and Ferguson and Lardy (1968) reported that the antibiotic induces ion transport, uncouples respiration and stimulates ATP hydrolysis in liver mitochondria. Recent observations by Mueller and Rudin (1969) indicate that monazomycin translocates K^+, Na^+, or H^+ across lipid bilayers. This compound was found to be capable of transporting ions as a function of its concentration and of applied electric potentials. From these results, Mueller and Rudin (1969) suggested that voltage or chemically controlled changes may cause the monomers of monazomycin to rearrange in conducting oligomers which translocate ions across lipid membranes by acting more like a channel than like a mobile carrier.

The present results in liver mitochondria indicate that the stimulation of ion movements as well as uncoupling of oxidative phosphorylation mediated by monazomycin are also subject to cooperative transitions. The possibility is proposed that such cooperative behavior could be explained by the assembly of several monomers of the antibiotic into one conducting oligomer controlled in its formation by cations, protons, anions, and membrane potential changes.

Materials and Methods

Mitochondria were prepared from livers of male rats weighing 150 g as described by Johnson and Lardy (1967). A continuous recording of oxygen consumption, light-scattering changes, and variations in the extramitochondrial concentration of alkali metal cations and protons was carried out by means of an apparatus designed, developed, and constructed by Chance, Mayer, and Pressman (Pressman, 1965, 1967; Graven *et al.*, 1966). ATPase activity was measured by the method of Lardy and Wellman (1953) and inorganic phosphate was determined by the method of Sumner (1944). The submitochondrial sonic particles were prepared and isolated according to Graven *et al.* (1967). Monazomycin was a kind gift from Dr. Henry A. Lardy. The antibiotic LLA491, which is very similar, if not identical with monazomycin (Mueller and Rudin, 1969), was a kind gift from Dr. E. L. Patterson.

Results

At a concentration of approximately 2.3×10^{-7} M, monazomycin causes an oscillatory uptake of Li^+, Na^+, K^+, Rb^+, or Cs^+ supported by ATP hydrolysis in liver mito-

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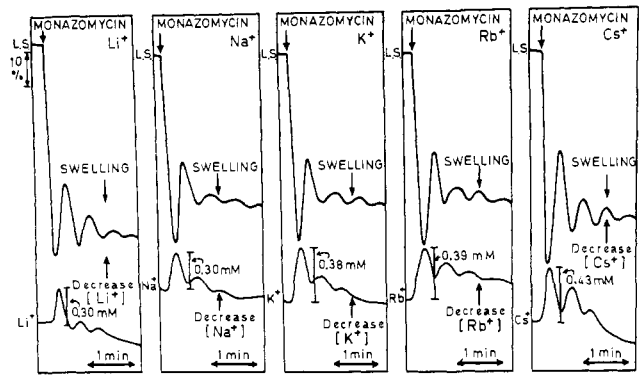

FIGURE 1: Effect of monazomycin on the movements of Li^+, Na^+ K^+, Rb^+, or Cs^+ and light-scattering changes supported by ATP hydrolysis in liver mitochondria. The reaction mixture contained: 6 mM Tris–ATP, 10 mM triethanolamine-Cl^-, 10 mM acetate–triethanolamine, 180 mM sucrose, 3 mM $MgCl_2$, 7.5 mM of the indicated alkali metal cations, 2.3×10^{-7} M monazomycin, and mitochondria equivalent to 1.0–1.2 mg of N in a 5-ml volume. Final pH 7.4; temperature 28°.

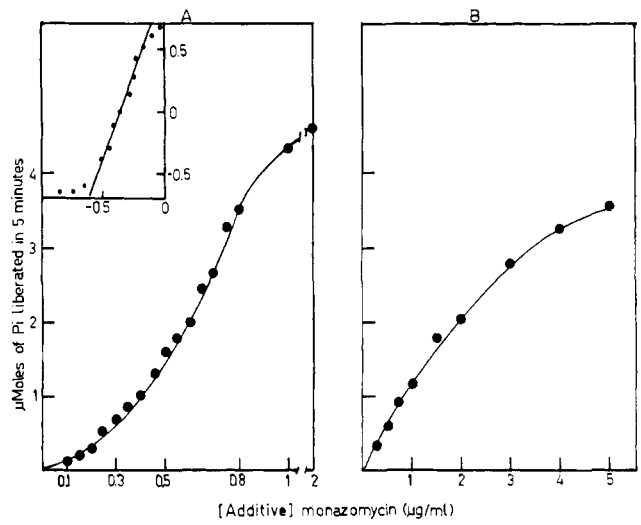

FIGURE 2: The effect of the concentration of monazomycin on the activity of mitochondrial ATPase in the presence (panel A) and in the absence (panel B) of added KCl. The reaction mixture contained: 12 mM Tris–ATP, 10 mM triethanolamine-Cl^-, 180 mM sucrose, mitochondria from 0.06 g of liver in 0.3 ml of 0.25 M sucrose, and the indicated concentrations of monazomycin in a 1.0-ml final volume at pH 7.4. The reaction mixture from panel A also contained 24 mM KCl. Insert: log – log plot of antibiotic concentration against P_i liberated in the presence of K^+. The experiment from panel B does not contain added alkali metal cations. Time of incubation, 5 min at 30°.

chondria. This is shown in Figure 1 which indicates that the uptake of alkali metal cations is simultaneously accompanied by oscillations in the light-scatter trace, presumably associated to swelling–contraction cycles of the mitochondria. Cation dependence shows that the uptake of Cs^+ and Rb^+ is slightly higher than that of Li^+ and Na^+.

As shown in Table I, the period and amplitude of oscillations of the light scatter trace induced by monazomycin, K^+, and ATP is dependent on the concentration of Mg^{2+} ion. Maximal number of discrete oscillations occur at 5 mM Mg^{2+}, while 10 mM of divalent cation block the oscillatory state. Such oscillations are also dependent on the concentration of hydrogen ions in the medium. Table II indicates that rates of swelling caused by monazomycin increase on going from pH 6.0 to 6.7. Oscillations occur in the vicinity of pH 7.0 with maximal frequency and amplitude at pH 7.4. The antibiotic does not cause a significant change of the light-scatter trace above pH 8.0.

Figure 2A shows the effect of increasing concentrations of monazomycin on the ATPase activity of liver mitochondria in the presence of KCl. The rate of ATP hydrolysis varies exponentially with the third power of the antibiotic concen-

TABLE I: Effect of Added Mg^{2+} on the Oscillations of the Light-Scattering Trace Induced by Monazomycin ATP and K^+ in Liver Mitochondria.[a]

Mg^{2+} Added (mM)	Swelling–Shrinkage Cycle (% Scattering Change)				
	I	II	III	IV	V
None	64	25	28	26	27
1	64	22	32	24	26
3	71	23	38	26	32
5	83	25	47	30	42
7	81	34	65	52	
10	72	44	63		

[a] The reaction mixture contained: 10 mM triethanolamine-HCl (pH 7.4), 10 mM acetate–triethanolamine (pH 7.4), 15 mM KCl, 6 mM ATP–Tris (pH 7.4), 180 mM sucrose, the indicated concentrations of $MgCl_2$, and mitochondria equivalent to 1.5 mg of N in 5-ml volume at 25°. Volume change were induced by the addition of 2.3×10^{-7} M monazomycin and measured as described in Methods. Phases of oscillations are shown by I–V with data taken at the peak amplitudes for swelling cycles. The average values from three individual experiments which agreed closely are presented.

TABLE II: The Effect of pH on the Oscillations of the Light-Scatter Trace Caused by Monazomycin, Mg^{2+}, and Alkali Metal Cations in Mitochondria.[a]

pH	Light-Scatter Changes (%) Time in sec after Monazomycin Addition				
	30	60	90	120	150
6.0	10	20	24	27	28
6.4	20	35	44	49	49
6.7	22	45	58	63	63
7.2	54	37	45	33	33
7.4	46	21	36	29	32
7.9	29	4	17	11	11

[a] The reaction mixture contained: 10 mM acetate–triethanolamine, 10 mM triethanolamine–HCl, 8 mM histidine (Cl^-), 6 mM Tris-ATP, 15 mM KCl, 5 mM $MgCl_2$, and 180 mM sucrose. The vessel contents were adjusted to the indicated pH values with HCl and 2.3×10^{-7} M monazomycin was added to the corresponding media. Mitochondria equivalent to 1.4 mg of N were added in a final volume of 5 ml at 25°. Data were taken at the indicated times after monazomycin addition. Other conditions are similar to those of Table I.

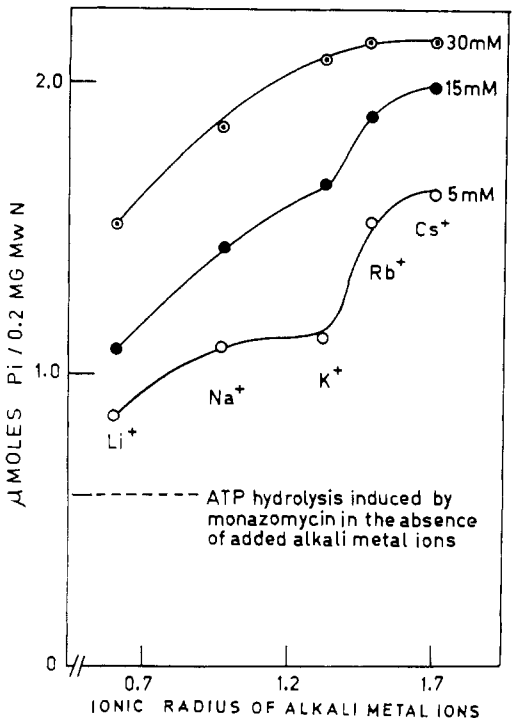

FIGURE 3: The effect of the concentration of alkalimetal cations on the hydrolysis of ATP stimulated by monazomycin in liver mitochondria. The media contained 6 mM Tris–ATP, 10 mM triethanolamine-Cl^-, 10 mM acetate–triethanolamine, sucrose at varying concentrations to maintain the osmolarity of the media at 280 mosm, 1×10^{-7} M monazomycin, 1.27 mitochondrial protein N/vessel, and the indicated concentrations of the chloride salts of Li^+, Na^+, K^+, Rb^+, or Cs^+. Final pH 7.4.

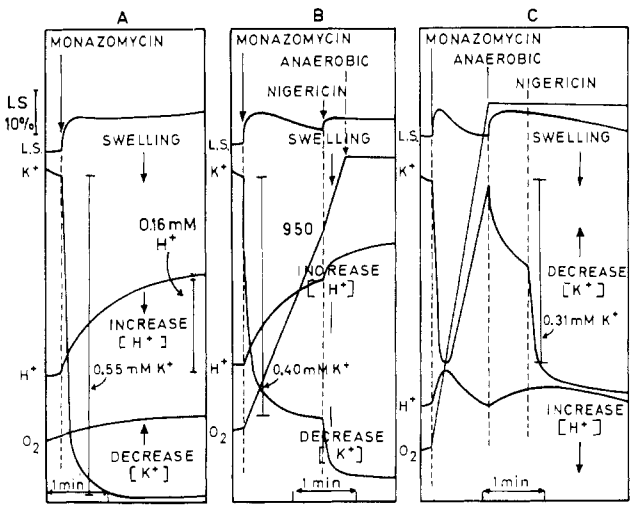

FIGURE 4: The effect of monazomycin and different oxidizable substrates on K^+ and H^+ movements, light-scattering changes, and oxygen uptake in mitochondria suspended in medium free of added alkali metal cations. The basic reaction mixture contained: 10 mM Triethanolamine-Cl^- (pH 7.4), 5 mM P_i–(triethanolamine), 190 mM sucrose, 2.3×10^{-7} M monazomycin, and mitochondria equivalent to 1.5 mg of N in a 5.0-ml volume. Final pH 7.4; temperature 28°. Other additions were as follows: panel A, 10 mM of the triethanolamine salts of either glutamate, ketoglutarate, pyruvate, L-malate, or citrate. Panel B, 10 mM succinate–triethanolamine and 2.3×10^{-7} M nigericin. Panel C, 5 mM glutamate + 5 mM L-malate (as triethanolamine salts).

tration (insert in Figure 2A). The omission of alkali metal ions from the media (Figure 2B) leads now to a hyperbolic dependence of ATPase activity on monazomycin concentration. It may also be observed that the presence of added cation greatly enhances the effectiveness of the antibiotic in eliciting ATPase activity.

As shown in Figure 3, both the concentration and the hydrated radius of the alkali metal cation determines monazomycin effects on ATPase activity. Alkali metal cations stimulate the hydrolysis of ATP over the values induced by monazomycin alone, with the sequence $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$.

Monazomycin causes K^+ uptake into mitochondria providing that ATP (Figure 1) or oxidizable substrates are present in the medium (Lardy *et al.*, 1967; Ferguson and Lardy, 1968). Figure 4 indicates that in the absence of added KCl (external concentration of K^+ of less than 0.7 mM) monazomycin causes the efflux of 0.55 mM K^+. This efflux is independent of added (10 mM) glutamate, pyruvate, α-ketoglutarate, citrate, or L-malate (panel A of Figure 4); none of the preceding substrates are oxidized. On the other hand, succinate oxidation does occur but now with the loss of only 0.40 mM K^+ (panel B of Figure 4). The addition of nigericin after monazomycin, further increases the release of K^+ from the mitochondria. Glutamate plus malate oxidation is also stimulated when monazomycin causes the efflux of mitochondrial K^+ (panel C of Figure 4). However, the loss of 0.31 mM K^+ induced by the antibiotic in the presence of this substrate pair, is rapidly followed by an active uptake of the previously extruded cation. Cation accumulation ceases when the preparation reaches the anerobic state. The loss of K^+ mediated by anerobiosis is further accelerated by nigericin. The reversal of the cation and proton movements supported by glutamate plus malate does not take place when this substrate pair is replaced by other oxidizable substrates. ATP, like succinate, partially prevents the loss of K^+ caused by monazomycin in medium free of added cations. The stimulation of succinate or glutamate plus malate oxidation caused by monazomycin in the absence of added alkali metal ions parallels the uncoupling of ATPase activity induced by the antibiotic in K^+-free media. Alkali ions inhibit the oxidation of succinate or glutamate plus malate induced by monazomycin in medium free of added K^+, acetate, or phosphate (Figure 5). This is in contrast with the effect of the cations on ATPase activity (Figure 3). In Figure 5, tracing A indicates that in the presence of 10 mM triethanolamine-Cl (pH 7.4) and 180 mM sucrose, the addition of 5 mM KCl completely inhibits the monazomycin-stimulated oxidation of 10 mM succinate. The inhibition by K^+ is overcome by the subsequent addition of 10 mM acetate. This stimulation of respiration induced by acetate is in turn inhibited by the further addition of 3 mM $MgCl_2$, which is released by 5 mM inorganic phosphate or by 1.3×10^{-7} M nigericin. Substitution of KCl by LiCl or CsCl in the above experiment, abolishes the ability of nigericin but not that of phosphate to stimulate respiration.

Magnesium does not require the presence of added K^+ to inhibit the monazomycin-stimulated succinate oxidation. This is displayed in tracing A of Figure 6 which also indicates again that P_i but not acetate reverses the effect of added Mg^{2+}. Tracing B of Figure 6 indicates that 3 mM ATP added prior to monazomycin and Mg^{2+} completely prevents the inhibitory effect of the divalent cation on substrate oxidation. This action of ATP is not due to a direct effect on succinate

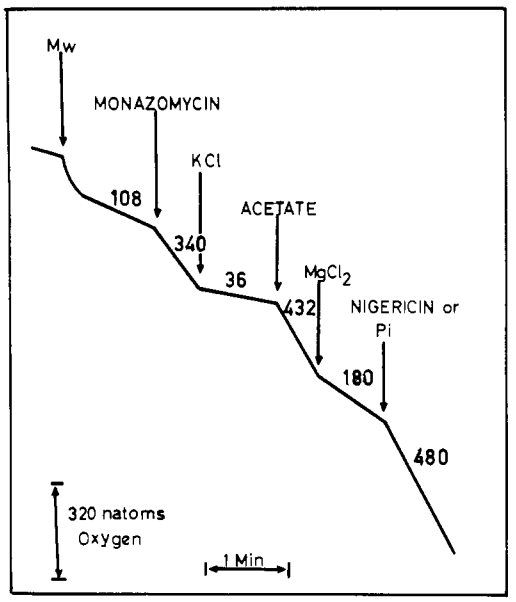

FIGURE 5: The effect of K^+, Mg^{2+}, acetate, P_i, and nigericin on the oxidation of succinate stimulated by monazomycin in mitochondria suspended in medium initially free of added K^+. The reaction mixture contained 10 mM triethanolamine-Cl^- (pH 7.4), 180 mM sucrose, 6 mM succinate–triethanolamine, and mitochondria equivalent to 1.2 mg of N. Where indicated other additions were: 1×10^{-7} M monazomycin, 12 mM KCl, 6 mM $MgCl_2$, 4.8 mM acetate–triethanolamine, 2.4 mM phosphate triethanolamine, and 2.3×10^{-7} M nigericin.

oxidation since similar results are found when succinate is replaced by glutamate plus malate. It is also different from the effects of ATP exerted on the transport of alkali metal cations catalyzed by carboxylic antibiotics in mitochondrial membranes (Estrada-O *et al.*, 1968, 1970), since rutamycin (Lardy *et al.*, 1965) does not prevent the ATP effect (Tracing B of Figure 6). Moreover, 3 mM EDTA replaces ATP in preventing the inhibition of oxygen uptake caused by Mg^{2+} in tracing B of Figure 6. Therefore, it is likely that by interfering with the binding of Mg^{2+} with the antibiotic or the mitochondrial membrane, EDTA, or ATP prevent its inhibitory effect mediated in the absence of added P_i.

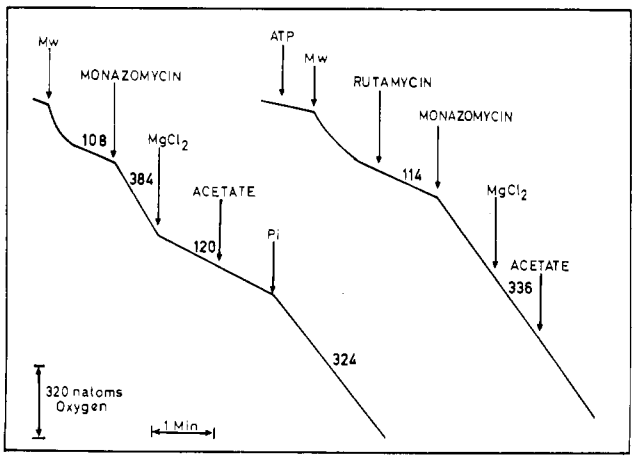

FIGURE 6: The effect of ATP, rutamycin, Mg^{2+}, and acetate on the oxidation of succinate stimulated by monazomycin in mitochondria suspended in medium initially free of added K^+. The basic reaction mixture was essentially the same to that described in Figure 5. Other additions were: 5 mM Tris–ATP, 3×10^{-7} M rutamycin, 2×10^{-7} M monazomycin, 6 mM $MgCl_2$, 4.8 mM acetate–triethanolamine, 12 mM KCl, and mitochondria equivalent to 1.2 mg of N in a 5-ml volume at 28°. Final pH 7.4.

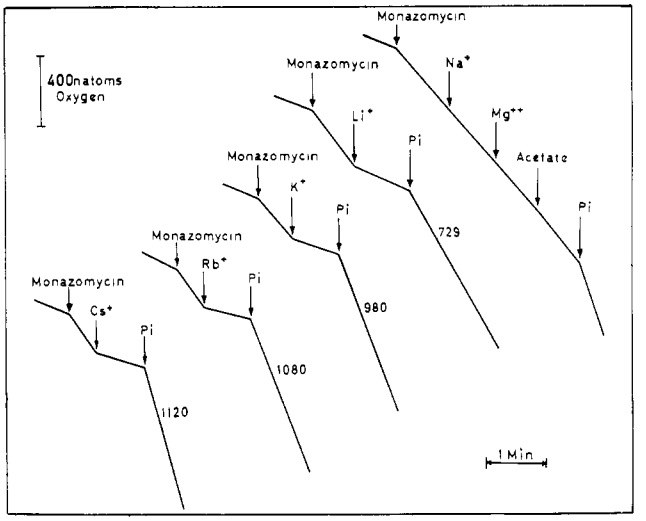

FIGURE 7: The effect of different alkali metal cations, Mg^{2+}, and P_i on the oxidation of succinate stimulated by monazomycin in mitochondria suspended in medium initially free of added cations and anions. The reaction mixture was essentially the same to that described in Figure 5. The chloride salts of Li^+, Na^+, K^+, Rb^+, or Cs^+ were added at a concentration of 12 mM, $MgCl_2$ at 6 mM, monazomycin at 2×10^{-7} M, and P_i (triethanolamine) at 2.4 mM. Mitochondria equivalent to 1.2 mg of N were present in a 5-ml volume at 28°.

Alkali metal cations show different effects on the oxidation of succinate stimulated by monazomycin in media free of P_i or acetate. Figure 7 shows that the inhibition of succinate oxidation by 5 mM CsCl is comparable to that of equal concentrations of the chloride salts of Rb^+, K^+, or Li^+. However

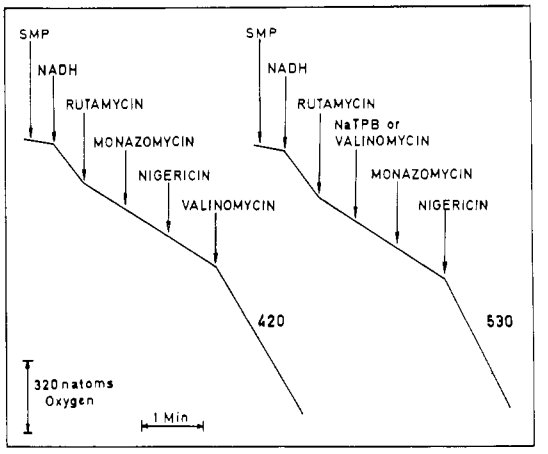

FIGURE 8: The effect of monazomycin, nigericin, valinomycin, and Na^+-tetraphenylboron on the respiratory control induced by rutamycin in submitochondrial sonic particles. Tracing A, combined effect of monazomycin, nigericin, and valinomycin on the respiratory control induced by rutamycin. Tracing B, combined effect of Na^+-tetraphenylboron, monazomycin and nigericin on the respiratory control induced by rutamycin. The reaction mixture contained: 200 mM sucrose, 50 mM Tris–acetate (pH 7.4), 15 mM KCl, 1.5 mM DPNH, 1.0 μg/ml of rutamycin and, where indicated, 1×10^{-6} M monazomycin, 2.3×10^{-7} M nigericin, 1.3×10^{-7} M valinomycin, 10 μM Na^+-tetraphenylboron, and submitochondrial sonic particles from rat liver equivalent to 1.5 mg of N in a 3.0-ml volume at 25°.

this effect is not shared by Na^+; moreover, when added prior to Mg^{2+}, it completely prevents the inhibition of oxygen uptake caused by the divalent cation. If now P_i is added, in the presence of Na^+, it leads to a stimulation of succinate oxidation. P_i also reverses the block in oxygen uptake caused by alkali metal cations other than Na^+, the order of its effectiveness being $Cs^+ > Rb^+ > K^+ > Li^+$, resembling the ionic selectivity of cations to stimulate ATPase activity in the presence of the antibiotic. These data indicate that the stimulation by alkali metal ions of ATP hydrolysis and substrate oxidation induced by monazomycin are dependent on the presence of P_i. The fact that ATP hydrolysis induced by monazomycin is not inhibited by Mg^{2+} or K^+ (Figures 2–3) in a medium devoid of added P_i could be explained by the fact that the stimulated ATPase releases P_i which by facilitating cation uptake, prevents the inhibited state.

In conditions where both nigericin plus K^+ plus tetraphenylboron, or valinomycin plus nigericin uncouple oxidative phosphorylation in submitochondrial particles (Montal *et al.*, 1969, 1970; Cockrell and Racker, 1969), monazomycin does not affect the respiratory control induced by rutamycin (tracings A and B of Figure 8). These results indicate that the transport of ions across lipid membranes, induced by monazomycin is due to a different mechanism from that due to nigericin or valinomycin.

Discussion

The antibiotic monazomycin has been found in the present work to catalyze the influx or the efflux of cations in mitochondria, as a function of the intra- to extramitochondrial concentration ratio of protons (Table II) and cations (Figures 1 and 4) as well as the presence of anions (Figures 5–7) and a suitable energy source for supporting active cation movements (Figure 4). One of several questions which arise from the described results is whether monazomycin transports ions by acting as a mobile carrier, in series (Pressman, 1968; Massari and Azzone, 1970) or in parallel with K^+ or Na^+ carriers existing in the mitochondrial membrane (Mitchell 1966a,b; Mitchell and Moyle, 1967), or else by some other mechanism. Recent data from Mueller and Rudin (1969) indicate that the conductance of lipid bilayers shows an exponential sixth power dependence on monazomycin concentration. These data suggested that in the presence of an applied electrical potential, several monomers of the antibiotic assembled in the membrane interior to give a conducting oligomer. Such oligomers would transport ions by acting more like a channel rather than acting like mobile carriers. In line with the above proposal, the antibiotic may also translocate ions in mitochondria by several protomers of the antibiotic (an average 3) acquiring a stable oligomeric form. This suggestion is mainly derived from Figure 2 which indicates that the stimulation of ATP hydrolysis in the presence of K^+ is a third power function of the concentration of the antibiotic. No clear evidence of cooperative effects of monazomycin have been found so far in our experiments in media devoid of added cations. Thus it is likely that a higher affinity of the antibiotic for cations with respect to protons, determines a more stable form of the oligomeric carrier in the presence of the former ionic species. In fact, the selectivity of $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ to enhance the hydrolysis of ATP induced by the antibiotic (Figure 3) could indicate their relative effectiveness to stabilize a hypothetical conducting oligomer. The monazomycin carrier could operate in parallel with alkali metal cation/H^+ translocators (Mitchell, 1968a,b) existing in the mitochondrial membrane. However, the possibility that the oligomeric form of the antibiotic is arranged in series with cation carriers present in the mitochondrial membrane, cannot be discarded at the present moment.

Anions such as inorganic phosphate or acetate are required for monazomycin to stimulate respiration in the presence of alkali metal cations or Mg^{2+}. In fact, when those anions are omitted from the reaction mixture, the cations completely eliminate the stimulation of oxygen uptake stimulated by the antibiotic (Figures 5–7). It is likely that the penetration of anions through the mitochondrial membrane by means of a uniport (Chappell and Haarhoff, 1967; Mitchell, 1968) or symport mechanism with H^+ (Mitchell and Moyle, 1969) abates the chemical gradient of protons (Mitchell, 1966b). In turn, the subsequent extrusion of H^+ by the ATPase or the respiratory chain could facilitate the formation of a negative membrane potential, which allows monazomycin to acquire the oligomeric form and to translocate K^+ into mitochondria. Thus by dissipating the electrogenic component of the membrane potential (Mitchell, 1966b, 1968), the antibiotic-induced uptake of K^+ could stimulate ATPase and oxygen uptake in the absence of P_i acceptor.

The substrate specificity of oxygen-uptake inhibition mediated by monazomycin in media free of added alkali metal cations (Figure 4) is identical with that induced by nigericin and similar carboxylic antibiotics in liver mitochondria (Lardy *et al.*, 1958, 1967; Graven *et al.*, 1966). The effect of nigericin is accounted for by the inhibition of substrate penetration (Harris *et al.*, 1967) secondary to the efflux of alkali metal cations (Graven *et al.*, 1966; Harris *et al.*, 1967) or P_i from the mitochondria (Cockrell and Racker, 1969; Henderson *et al.*, 1969; Estrada-O and Calderon, 1970). Thus it is likely that the inhibition of substrate accumulation is also the reason for the specific block of oxygen uptake caused by monazomycin in medium free of added cations (Figure 4). It is unlikely that alkali ions and Mg^{2+} inhibit the stimulated oxidation of 6 mM succinate by preventing substrate translocation in the absence of acetate or P_i (Figures 5–7). The fact that nigericin reverses the inhibited respiratory state caused by Mg^{2+} and K^+ (Figure 5), while causing K^+ and substrate loss from the mitochondria, supports this suggestion. This inhibited state may be accounted for by a possible competition existing between cations and protons for complexing with the antibiotic in medium free of acetate or P_i. Since the affinity of monazomycin for cations may be higher than for H^+, the latter ionic species is displaced from the antibiotic by cations, which in turn fail to stimulate respiration by the lack of an appropriate anion to allow their being transported into the mitochondria. The fact that the hydrolysis of ATP induced by monazomycin, with or without added cations, is not inhibited by Mg^{2+} or K^+ (Figures 2–3) may be accounted for by the continuous liberation of P_i by the stimulated ATPase which by facilitating cation uptake prevents the inhibited state. Moreover the oxygen uptake block induced by alkali metal cations is reversed by P_i, its effectiveness being $Cs^+ > Rb^+ > K^+ > Li^+$ (Figure 7). P_i also reverses the oxygen uptake block caused by Mg^{2+} (Figures 5–6). This suggests that the antibiotic translocates not only alkali metal cations (Figures 1 and 4) but also Mg^{2+} into mitochondria. Maximal frequency and amplitude of light-scattering oscillations induced by the antibiotic in media which contains ATP and alkali metal cations are dependent on the concentration of Mg^{2+} (Table I). Moreover, maximal number

of oscillations associated to the presence of Mg^{2+} occur at a pH of 7.4. That the oscillations induced by monazomycin, K^+, and Mg^{2+} are primarily unrelated to a transport of alkali metal cations independent from monazomycin is supported by the fact that the oscillatory swelling linked to the alkali metal ion uptake induced by EDTA, P_i, and substrates has an optimum pH of 8.2 and is completely inhibited by the addition of Mg^{2+} to the medium (Packer *et al.*, 1966).

It is not immediately apparent why Na^+ does not inhibit the oxidation of succinate stimulated by monazomycin, nor why it prevents the oxygen uptake block caused by Mg^{2+} (Figure 7). It is likely that the existence of a Na^+/H^+ antiport carrier more active than a K^+/H^+ translocator (Mitchell, 1966a; Mitchell and Moyle, 1967, 1969), arranged in parallel with monazomycin in the mitochondrial membrane could modify the competition between Na^+ and H^+ for the antibiotic so as to facilitate the uptake of H^+ into mitochondria.

Finally, it is of interest that monazomycin does not affect the oxidative phosphorylation of submitochondrial sonic particles (Figure 8). These observations suggests that membrane sidedness or the membrane polarity linked to the integrity of the mitochondria, is necessary for the antibiotic to stimulate ion movements and uncoupling of oxidative phosphorylation.

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