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IONOPHOROUS PROPERTIES OF NARASIN, A NEW POLYETHER MONOCARBOXYLIC ACID ANTIBIOTIC, IN RAT LIVER MITOCHONDRIA

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Abstract—Narasin, a new polyether monocarboxylic acid antibiotic, inhibits ATPase and reverses swelling and H^+ release from liver mitochondria after induction of cation transport. Among alkali metal cations, narasin shows ionophorous activities with a selectivity of $Na^+ > K^+ = Rb^+ > Cs^+ > Li^+$. However, narasin also induces permeability of mitochondria to the non-noble gas cation, Tl^+ and the polyatomic cation, NH_4^+. In fact, the polyether monocarboxylic acid ionophores, narasin and monensin, are more appropriate ionophores for NH_4^+ than for alkali cations. Narasin inhibits 50 per cent of the ATP hydrolysis activated by dinitrophenol, valinomycin-K^+ and monazomycin-K^+-NH_4^+ with IC_{50} values of 2.5, 200 and 13 nM respectively. The corresponding IC_{50} values for monensin are 7, 750 and 45 nM. The polyether monocarboxylic acid ionophores may play an important role in ionophore-producing organisms as NH_4^+ carriers.

Narasin is isolated from *Streptomyces aureofaciens* and has an empirical formula of $C_{43}H_{72}O_{11}$, mol. wt 764. Narasin [1, 2] and salinomycin [3] are similar in chemical structure except that narasin has an additional methyl group on the 6-member cyclic ether at the carboxylic terminal. The molecular features of narasin and salinomycin are typical of those of other known polyether monocarboxylic acid ionophores such as monensin [4], nigericin [5], A204 [6] and X537A [7]. These ionophores are capable of forming uncharged complexes with alkali metal cations [8].

Owing to a high lipid solubility of the ionophore–cation complexes, the polyether monocarboxylic acid ionophores are capable of inducing cation permeability in biological membranes. In liver mitochondria, these ionophores induce cation exchange leading to an inhibition of ATP hydrolysis and the reversal of swelling induced by active uptake of alkali metal cations [9, 10]. Narasin has been reported to be effective in treating coccidiosis in poultry and in improving feed efficiency in ruminants [11]. In this paper, we describe the ionophorous properties of narasin in comparison with monensin and X537A in liver mitochondria.

METHODS AND MATERIALS

Mitochondria were reported from the liver of male albino rats weighing 150 g, according to the described procedure [10]. Adenosine triphosphatase (ATPase) was determined by the liberation of inorganic phosphate [10]. Mitochondria swelling was monitored at 520 nm by means of an Aminco–Chance dual wavelength spectrophotometer. The release of H^+ was measured by a pH electrode connected to a pH meter (The London Co., Denmark).

Narasin, monensin, X537A, valinomycin and monzomycin were isolated in the Lilly Research Laboratories. All other biochemicals were purchased from Sigma Co.

RESULTS

In the presence of the classical uncoupling agent of oxidative phosphorylation, 2,4-dinitrophenol, mitochondria of rat liver catalyzed the hydrolysis of ATP (DNP–ATPase). Narasin at 6.6×10^{-8} M reduced the DNP–ATPase activity from 3.4 μmoles/mg of protein/10 min to 0.24 μmole/mg of protein/10 min in terms of inorganic phosphate formed (Table 1). On the other hand, the hydrolysis of ATP induced by the cyclododecadepsipeptide ionophorus antibiotic, valinomycin [12] and potassium (Val + K^+–ATPase) [13] was blocked by a 10-fold greater concentration of narasin (6.6×10^{-7} M).

The inhibition of the DNP–ATPase was further studied at various concentrations of narasin and two other polyether ionophores, monensin and X537A

Table 1. Effect of narasin on ATPases of rat liver mitochondria*

Narasin (M)	Hydrolysis of ATP: 2,4-Dinitrophenol induced (μmoles Pi formed/mg protein)	Hydrolysis of ATP: K^+-valinomycin induced (μmoles Pi formed/mg protein)
None	3.40	4.06
6.6×10^{-8}	0.24	3.09
2.7×10^{-7}	0.32	2.00
6.6×10^{-7}	0.28	0.33

* Mitochondria of 1 mg protein in duplicate samples were incubated in a reaction mixture of a 1-ml volume containing 0.25 M sucrose, 30 mM Tris–chloride (pH 7.4), 20 mM Tris–acetate (pH 7.4) 1 mM $MgCl_2$, 30 mM KCl and 6 mM Tris–ATP (pH 7.4) at 37°. Samples also contained 0.1 mM 2,4-dinitrophenol, 0.1 μM valinomycin and various concentrations of narasin as indicated. The reaction mixture was incubated at 37° for 10 min and trichloroacetic acid (TCA) was then added at a final concentration of 5%. Inorganic phosphate was assayed for the TCA extract.

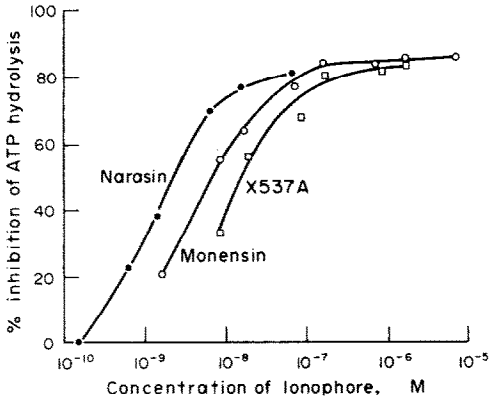

Fig. 1. Inhibition of 2,4-dinitrophenol–ATPase by various concentrations of narasin, monensin and X537A. The 2,4-dinitrophenol–ATPase in liver mitochondria was determined under the conditions shown in Table 1.

(Fig. 1). Narasin was most effective with an IC_{50} value of 2.5×10^{-9} M, followed by monensin and X537A with IC_{50} values of 8×10^{-9} M and 1.5×10^{-8} M respectively. Thus, the polyether ionophores share a common property, an inhibition of the DNP–ATPase.

Another antibiotic, monazomycin, is also known to induce cation-dependent ATPase activity in mitochondria [14]. Rat liver mitochondria in the presence of monazomycin and alkali metal cations hydrolyzed ATP at 3–4 μmoles/mg of protein/10 min (Table 2). Dependent upon the alkali metal cations, narasin at 2.6×10^{-7} M inhibited the monazomycin–ATPase activity to different degrees. Narasin was most effective in blocking the Na^+-dependent monazomycin–ATPase activity (85 per cent inhibition), followed by the Rb^+- and K^+-dependent activities with 74 and 54 per cent inhibition. With Li^+ and Cs^+ as cations, narasin reduced only 27 and 20 per cent of the ATPase activities.

The cation selectivity of narasin was observed only at concentrations of the ionophore below 5×10^{-6} M (Fig. 2). At 8×10^{-6} M, narasin inhibited over 90 per cent of the ATPase activities with all alkali metal cations.

Based on earlier studies [10] the concentration of alkali chloride was chosen at 30 mM for the measurement of ATPase activity in rat liver mitochondria. At this concentration of NaCl, KCl and RbCl, narasin exerted maximum inhibition (Fig. 3). Narasin inhibited the ATPase activities to the same levels even at a higher salt concentration of 50 mM.

Mitochondria swell upon induction of ion transport by monazomycin in the presence of alkali cations, as indicated by the downward tracings in Fig. 4. The first addition of 0.4 nmole narasin caused a 50 per cent contraction (upward tracing) of the mitochondrial volume when Na^+ was the cation. The second addition of 0.4 nmole narasin led to the shrinkage of the mitochondrial volume beyond the original level. With Li^+ or Rb^+ as cation, a total addition of 0.8 nmole narasin was required to initiate the contraction of mitochondrial volume. Narasin had no effect when Cs^+ was the alkali metal cation.

When mitochondria were induced to take up alkali metal cations by monazomycin, protons were released into the medium (upward tracings in Fig. 5). Monazomycin released about $\frac{1}{3}$ to $\frac{1}{2}$ the amount of H^+ ions from mitochondria when the alkali metal cations were Li^+ and Na^+ in comparison with the amount of H^+ ions released in the presence of K^+, Rb^+, Cs^+ and Tl^+. Upon the addition of narasin, protons were taken up from the medium by the mitochondria due to the reversed movement of the monovalent cations,

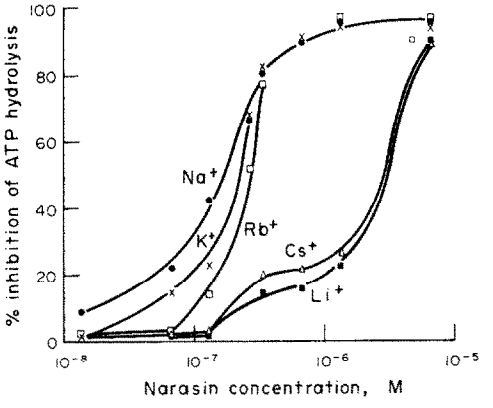

Fig. 2. Selectivity of alkali cations in the inhibition of monazomycin-induced ATPase by various concentrations of narasin. Liver mitochondria were incubated under the identical conditions given in Table 1 except that monazomycin at 15 μg/ml instead of valinomycin, alkali chloride at 30 mM instead of 30 mM KCl, and various concentrations of narasin were used.

Table 2. Alkali metal cations specificity for narasin to block the monazomycin-activated hydrolysis of ATP in rat liver mitochondria*

Alkali metal chloride	Radii (A)	Monazomycin alone (μmoles Pi/mg protein)	Monazomycin + Narasin (μmoles Pi/mg protein)	% Inhibition
Li	0.60	3.39	2.48	27
Na	0.96	2.94	0.43	85
K	1.33	3.19	1.45	54
Rb	1.46	3.98	1.05	74
Cs	1.69	3.77	3.01	20

* Mitochondria were incubated in the same reaction mixture as shown in Table 1 except that monazomycin at 15 μg/ml, instead of valinomycin, and 2.6×10^{-7} M narasin were used.

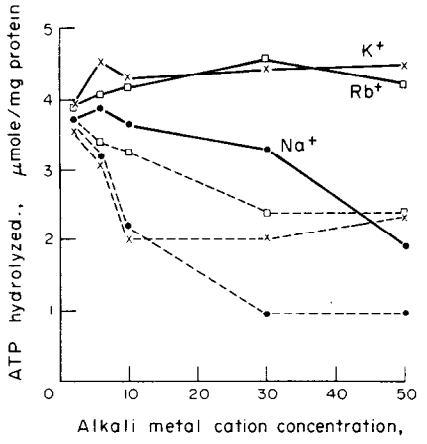

Fig. 3. Effects of narasin on the monazomycin–cation-dependent ATPase at various concentrations of NaCl, KCl and RbCl. Liver mitochondria were incubated in the identical manner as in Fig. 2 except that various concentrations of NaCl, KCl and RbCl were present in the control (solid lines) and in the 0.25 μM narasin-treated (dotted lines) samples.

except Li^+. Only 0.5 nmole of narasin in 5 ml of total volume was sufficient to induce the complete reuptake of protons by mitochondria when Na^+ or Tl^+ was the metal cation (traces b and d, Fig. 5); 1 nmole narasin was required with K^+ and Rb^+ as cations (traces c and e) and 1.5 nmoles with Cs^+ as cation (trace f).

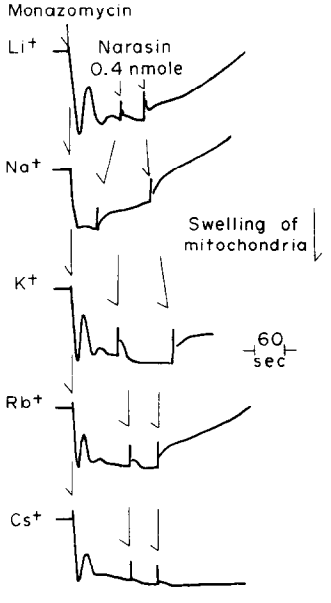

Fig. 4. Effects of narasin on mitochondrial swelling induced by monazomycin and alkali metal cations. Liver mitochondria at 0.16 mg/ml of protein were incubated at room temperature in a reaction mixture of 3-ml volume containing 225 mM manitol, 75 mM sucrose, 10 mM Tris–Cl, pH 7.4, 1.7 mM Tris–ATP, pH 7.4, and 10 mM alkali metal chloride in two absorption cells which were introduced into the cell holders of the Aminco–Chance dual wavelength spectrophotometer. Mitochondrial swelling was initiated upon addition of monazomycin at 0.3 μg/ml, and 0.4 nmole narasin was introduced at each addition as indicated.

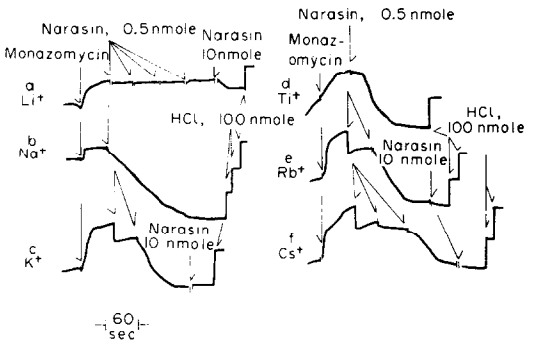

Fig. 5. Effects of narasin on the release of H^+ from liver mitochondria. Liver mitochondria at 0.9 mg/ml of protein were incubated at 32° in a reaction mixture of 5-ml volume containing 0.25 M sucrose, 2 mM Tris–HCl, pH 7.4, 30 mM alkali metal chloride, and 1 mM Tris-succinate, pH 7.4. Monazomycin at 1 μg/ml and narasin at 0.5 nmole/ml/addition were introduced as indicated. The addition of a HCl standard solution at the end of each experiment was used to calibrate the proton movements.

We found that liver mitochondria catalyzed ATP hydrolysis with an activity of 4.81 ± 0.33 μmoles/mg of protein/10 min when the reaction mixture contained 30 mM NH_4Cl, 30 mM KCl and 1 μg/ml of monazomycin. Narasin and monensin inhibited 50 per cent of the activity at 13 and 45 nM respectively (Fig. 6).

DISCUSSION

Narasin like other polyether monocarboxylic ionophores, monensin, X537A and A204, inhibited the mitochondrial ATPase induced by active transport of alkali metal cations. Narasin also reversed the mitochondrial swelling and proton release associated with the activation of cation uptake by the addition of monazomycin. The effectiveness of narasin in reversing these three expressions of cation transport in liver mitochondria varies with the alkali metal cation present. Narasin is most effective with Na^+, intermediate with K^+ and Rb^+, and less active with

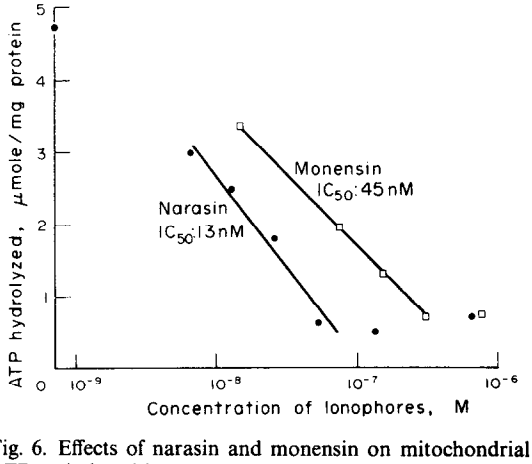

Fig. 6. Effects of narasin and monensin on mitochondrial ATPase induced by monazomycin + KCl + NH_4Cl. Liver mitochondria were incubated in the reaction mixture as shown in Table 1 except that monazomycin (1 μg/ml), instead of valinomycin, and 30 mM NH_4Cl were included.

Cs^+ and Li^+. The cation selectivity of polyether ionophores is largely due to the size of the cavity which has the oxygen atoms as ligands facing inside. Thus, the cavity provided by narasin can best accommodate the Na^+ ion.

It is most interesting to point out that narasin shows differential inhibition of the ATPase activities induced under three different conditions: (1) 2,4-dinitrophenol activated ATPase; (2) valinomycin-K^+ activated ATPase and (3) monazomycin-KCl-NH_4Cl activated ATPase, with IC_{50} values of 2.5, 200 and 13 nM respectively. The corresponding IC_{50} values for monensin are 7, 750 and 45 nM. Narasin is about three to four times more effective than monensin under each condition of ATPase measurement.

The presence of NH_4Cl lowers the IC_{50} values of both narasin and monensin required to inhibit the monazomycin plus cation-induced ATPase. This is in agreement with the finding that polyether ionophores form complexes with the polyatomic cation, NH_4^+ [15]. In fact, narasin and monensin prefer NH_4^+ over the alkali metal cations, as indicated by the smaller IC_{50} values. Hydrogen bonding occurs between the oxygen atoms of the ionophores and the protons of NH_4^+ and result in a complex with a tetrahedral coordination. Since these ionophorous agents are isolated from micro-organisms, the microbial metabolites may serve an important function as carriers of NH_4^+, a nutrient for bacterial growth, in the ionophore-producing organisms.

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