A COMPARISON OF BEAUVERICIN, ENNIATIN AND VALINOMYCIN AS CALCIUM TRANSPORTING AGENTS IN LIPOSOMES AND CHROMATOPHORES

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SUMMARY

The ion transporting antibiotic beauvericin is compared with enniatin and valinomycin for ability to transport monovalent and divalent metal cations in phospholipid liposomes and bacterial chromatophores. Valinomycin would transport potassium and caesium; enniatin potassium, caesium, sodium and lithium; and beauvericin calcium, potassium, caesium, lithium and sodium. For beauvericin the cation specificity is found to be Ca>K>Cs>Li>Na.

INTRODUCTION

In an earlier paper, Roeske, Isaac, King and Steinrauf (1) have studied the cation specificity of the ion transporting antibiotic beauvericin (2) in the presence of the anion picrate. These studies included the ease of crystallisation with various cations, ability of the antibiotic to partition various picrate salts between aqueous and organic solvents, and the ability of the antibiotic to transport ions through a U-tube. They concluded that the cation specificity in the presence of picrate was Rb>Ba>K>Na>>Ca>>>Li. However, Dorschner and Lardy (3) had previously found a different sequence of alkali ions for the beauvericin stimulated swelling of rat liver mitochondria, and Estrado-O et al. (4) found that the selectivity of alkali ions by mitochondria in the presence of beauvericin depended on the anion present. Roeske et al. (1) have suggested that the active form of beauvericin in ion-transport may well be a dimer or a tetramer, containing two (or four) cations, and two (or four) beauvericin molecules. Such a complex might have binding sites for anions, and different anions might change the cation specificity.

In the present paper the cation specificity of beauvericin is studied in

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two simple membrane systems in which the anion picrate is not required or present, thus allowing the possibility of a different specificity than that found by Roeske et al. (1). A comparison of the very similar antibiotic enniatin-A as well as valinomycin has also been included.

MATERIALS AND METHODS

Beauvericin was synthesized in the laboratory of Dr. Roger Roeske of Indiana University (1), and crystal structure determinations are in progress (1). Valinomycin was a gift from the Eli Lilly Laboratories, Indianapolis, and has been the subject of a molecular structure determination by X-ray crystallography as the potassium complex by Pinkerton, Steinrauf and Dawkins (5). Enniatin-A was a gift from Dr. V. T. Ivanov, Shimyakin Institute for the Chemistry of Natural Products, U.S.S.R. Academy of Sciences, Moscow. A molecular structure for the potassium complex was proposed by Dobler, Dunitz and Krajewski (6).

Liposomes were prepared from acetone washed soya-bean phospholipid (Sigma) plus 2 mole % bacteriochlorophyll from Rhodopseudomonas capsulata as previously described (7). The buffer enclosed inside the liposomes was 50 mM MES, pH 6.0, and the liposomes were suspended in 50 mM Tricine, pH 8.0. A pH gradient was thus established across the liposome membrane, with a greater concentration of hydrogen ions inside than out. When a salt of a metal cation was then added to the external volume, a cation gradient was also established across the liposome membrane, in the opposite direction to the hydrogen ion gradient. The addition of FCCP would allow protons to leave the internal volume of the liposomes, but since FCCP is an electrophoretic proton carrier this collapse of the pH gradient would be opposed by a membrane potential, negative inside. The addition of a metal cation-carrying antibiotic would collapse this membrane potential by allowing metal cations to enter the liposomes in exchange for the protons leaving via FCCP. In the presence of excess FCCP, the rate of collapse of the transmem-

MES, 2(N-morpholino) ethane sulfonic acid;

Tricine, N-tris (hydroxymethyl) methyl glycine;

FCCP. carbonyl cyanide p-trifluoromethoxy phenylhydrazone.

brane pH gradient would depend on the rate of cation transport induced by the other antibiotic.

The decay of the pH gradient was measured by the quenching of fluorescence of 0.4 μM 9-amino acridine as previously described (7).

The technique of following ion transport induced by antibiotics in chromatophores was that of Jackson and Crofts (8). The chromatophores were prepared in 10 mM HEPES buffer, pH 7.5, with 150 mM choline chloride, and washed twice in the same buffer. The bacteriochlorophyll content was determined spectrophotometrically after extraction into acetone-methanol (7:2v/v), assuming an extinction of 75 mM^{-1} cm^{-1} at 772 nm (9). The membrane potential generated by the addition of a salt of a metal cation and an ion transporting antibiotic was measured by the carotenoid shift (8) at 530-515 nm for Rps. capsulata St. Louis and 590-470 nm for Rps. spheroides Ga. None of the organic buffers used (MES, HEPES and Tricine) bind significant amounts of metal cations (10), and all were brought to the required pH with tetramethylammonium hydroxide.

RESULTS

Liposomes. A typical experiment is shown in Figure 1. Control experiments were performed by omitting in turn each of the reactants. The rate of transport of the different ions by the three antibiotics may be expressed as follows, where Val is used as an abbreviation for valinomycin, Bv for beauvericin and En for enniatin-A, and the metal cations are represented by their chemical symbols:
ValK = ValCs>BvCa>BvK>EK = ECs = BvCs>BvLi>BvNa>EnNa>EnLi.

Identical results were obtained with KCl and K_2SO_4, and with liposomes prepared in phosphate buffers instead of organic ones. In the presence of phosphate buffers, calcium chloride could not be tested, since calcium phosphate precipitated. None of the antibiotics would transport barium, strontium, magnesium, lanthanum or aluminum chlorides under the conditions described.

The ion selectivities of valinomycin and enniatin-A reported here are identical to those of Henderson et al. (11).

HEPES, N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid;

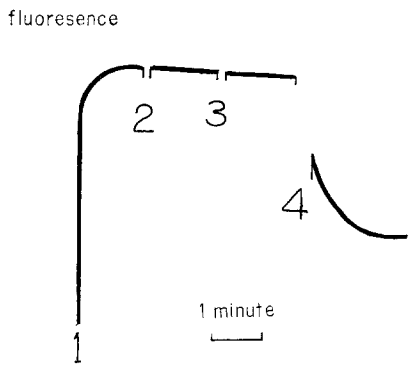

Figure 1. Ion transport mediated by antibiotics in liposomes. Liposomes (point 1) (internal pH = 6.0) were suspended in 2.5 ml of buffer at pH 8.0 in the presence of 0.4 μM 9-amino acridine. Lipid concentration = 0.08 mg/ml. FCCP (point 2) was added to 2 μM followed by the addition of 40 mg of beauvericin (point 3) and 4 mM $CaCl_2$ (point 4). Similar traces were obtained with beauvericin plus KCl, K_2SO_4, CsCl, LiCl and NaCl, with enniatin-A plus KCl, K_2SO_4, CsCl, NaCl and LiCl, and with valinomycin plus KCl or CsCl. All antibiotics were added at the same concentration. No effect was seen with any antibiotic plus $BaCl_2$, $SrCl_2$, $MgCl_2$, $AlCl_3$ or $LaCl_3$.

Chromatophores. The addition of a salt of a metal cation to a suspension of vesicles containing no metal cations produces a concentration gradient of the added cation. The addition of an antibiotic which is able to transport the metal cation into the vesicles partially dissipates this concentration gradient, but generates a membrane potential, positive inside, in its place. Chromatophores of photosynthetic bacteria contain carotenoids, and these undergo a shift in their absorption spectra when a membrane potential is generated across the chromatophore membrane (8). Jackson and Crofts (8) have demonstrated that the magnitude of the carotenoid shift of Rps. spheroides (measured as the difference in absorption at a maximum and a minimum of the difference spectrum) was proportional to the logarithm of the concentration of potassium ions added in the presence of valinomycin, and more recently similar results have been presented for Rps. capsulata (12).

Figure 2 presents results obtained with valinomycin, enniatin-A and beauvericin. The results obtained with valinomycin plus potassium and caesium, caesium and lithium, and enniatin-A with potassium are very similar to those ob-

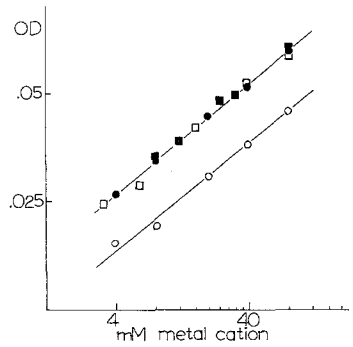

Figure 2. Ion transport mediated by antibiotics in chromatophores from Rps. capsulata. Chromatophores (24.3 µM BChl) were suspended in 2.5 ml of 10 mM HEPES, 150 mM choline chloride, pH 7.5. Ionophores were added at the following concentrations: beauvericin and enniatin-A, 4 µg, valinomycin, 0.4 µg, and the chloride salts of the metal cations added as indicated. • -• Valinomycin plus KCl, ■ - ■ enniatin-A plus KCl, □ - □ beauvericin plus $CaCl_2$, O - O beauvericin plus KCl. Similar results to the valinomycin plus KCl line were obtained with valinomycin plus CsCl, and enniatin-A plus CsCl, LiCl and NaCl. Beauvericin plus CsCl, LiCl or NaCl gave similar lines to that shown with KCl. In the absence of choline chloride, K_2SO_4 gave identical results to KCl. Similar results were obtained with chromatophores from Rps. spheroides, Ga.

tained by Jackson and Crofts (8) for valinomycin plus potassium. They showed that the extent of the membrane potential generated would vary with the gradient of chemical activity of the transported ion according to the equation.

$$\Delta\psi_{I-O} = \frac{RT}{ZF} \ln \frac{(C)_O}{(C)_I}$$

where $\Delta\psi$ is the difference in potential in mV between (I) the inside and (O) th outside of the chromatophore, and (C) is the activity of the transported ion. For monovalent cations the equation simplifies to approximately

$$\Delta\psi_{I-O} = 60 \log_{10} \frac{(C^+)_O}{(C^+)_I}$$

and for divalent cations to

$$\Delta\psi_{I-O} = 30 \log_{10} \frac{(C^{++})_O}{(C^{++})_I}$$

Hence, antibiotics transporting divalent cations would be expected to give a line of half the slope obtained with monovalent cations when plotted as in Figure 2.

It is therefore noteworthy that beauvericin plus calcium ions gives a slope identical to that for valinomycin plus potassium ions (Fig. 1). The displacement of the lines for beauvericin plus monovalent alkali cations may be due to the fact that the membrane potential generated by the entry of the alkali ions caused calcium ions bound to phospholipids to leave the chromatophores, thereby partially dissipating the membrane potential. This is supported by the observation that the calcium plus beauvericin line is not displaced from the valinomycin plus potassium line. Nevertheless, the relative rates of transport were the same as found in the liposome experiments.

CONCLUSIONS

We have shown that beauvericin will transport calcium ions in systems where enniatin will not. Since beauvericin is an alternating sequence of three D-α-hydroxyisovaleryl and three N-methyl-L-phenylanalyl residues, and enniatin-A is the same sequence of D-α-hydroxyisovaleryl and N-methyl-L-valyl residues, it is not obvious why this selectivity of beauvericin, but not enniatin, for calcium should exist. It is also intriguing that calcium appears to be transported as a single charged moiety. Perhaps the answers to these questions will be provided by the X-ray crystal structures of beauvericin rubidium picrate and of beauvericin barium picrate which are now in progress.

The specificity of beauvericin for monovalent cations in liposomes and chromatophores is rather similar to that found by Dorschner and Lardy (3) in mitochondria. Unfortunately, they did not test divalent cations. The specificity found by Roeske *et al.* (1) is quite different; however, their systems all had picrate anions present. If the picrate anion were modifying the ion binding of beauvericin, then the anion should appear as an integral part of the complex as determined by X-ray crystallography.

The use of beauvericin as a cation transporting agent in biological systems will have to be carefully considered in light of the ability of the antibiotic

to transport calcium as well as alkali metal ions. However, we might suggest that observations on the difference between the effects of beauvericin and enniatin on a biological system may be a way of isolating the consequences of calcium transport.

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