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THE ACTION OF SERRATAMOLIDE ON ION MOVEMENT IN LIPID BILAYERS AND BIOMEMBRANES

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SUMMARY

The cyclicdepsipeptide serratamolide was isolated from *Serratia marcescens* in pure form and its action in ion movement examined. At a concentration of 10 μg/ml, serratamolide increased significantly the rate of movement of K^+ and H^+ across the membrane of *Staphylococcus aureus* without causing leakage of "260 nm-absorbing products". At this concentration, serratamolide neither altered membrane permeability nor inhibited cell growth. It also exhibited similar behaviour on "black" lipid bilayer membranes.

Many naturally occurring cyclicdepsipeptides and some synthetic analogues exhibit an appreciable cation specificity in biomembrane transport[1–7]. Our preliminary investigations have indicated that a group of cyclicdepsipeptides from *Serratia marcescens*[8], markedly increases the loss of K^+ and the rate of penetration of protons across the bacterial membranes (*Staphyloccoccus aureus*) while at the same time having little effect on the leakage of "260 nm-absorbing materials". This possibility of a new natural K^+ carrier with a relative simple structure, prompted the attempt to purify completely the cyclicdepsipeptide, serratamolide and further confirm the results with a chemically defined material on intact bacterial cells and artificial membranes.

All attempts to purify the analogues of serratamolide occurring in strain NTCC 1377 from each other, resulted in extremely low yields[8]. However, a mutant was generated with a mutagen, ethyl methyl sulphonate[9] from strain NTCC 1377 which appeared to produce only one isomeric form of cyclicdepsipeptide. This product was isolated and characterized as outlined below, and examined for ionophoric activity as described in the latter part of this report.

S. marcescens (red pigmented strain NTCC 1377) was treated with mutagen ethyl methyl sulphonate[9]. A pale pink mutant (No. 7), whose pigment proved to be indistinguishable from prodigiosin, was isolated by repeated subculturing of a single colony. This mutant was maintained on peptone–glycerol agar slopes and grown on a large scale using the same medium in stainless steel trays (24 inches $\times$ 18 inches $\times$ 0.5 inch) with matching lids. Cells were harvested after 3 days by scraping the agar

gel surface with a spatula, washed with distilled water and lyophylised. The lipid containing cyclicdepsipeptide fraction was extracted with acetone and purified by passage through Sephadex as described earlier[8]. Thin-layer chromatographic analysis in chloroform–methanol–water (65:25:4, by vol.); chloroform–mehatnol–7 M ammonia (65:25:4, by vol.); and chloroform–methanol–acetic acid–water (65:25:1:3, by vol.) revealed only one cyclicdepsipeptide band in contrast to the multiple bands as observed previously[8]. This band was purified by preparative thin-layer chromatography on 40 cm × 20 cm plates, coated with Silica gel HR (E. Merck) using chloroform–methanol–7 M ammonia solvent and final filtration[12] through Sephadex G-25. Recrystallization from 95% ethanol gave white crystalline needles (m.p. 145–146 °C uncorrected, on Kofler hot stage).

The crystalline product was hydrolysed with 10 M HCl at 100 °C for 30 min (to liberate the hydroxy fatty acids). The hydrolysate was extracted with diethylether and lipid fraction was methylated with 14% boron trifluoride in methanol[10]. Gas–liquid chromatography on a Packard Model 7508 having an electronic integrator, on both polar (25% DEGS–2% phosphoric acid on 100–120 mesh Gas Chrom Q) and nonpolar (Apiezon-L) columns showed by comparison with authentic standards, that the material consisted of more than 98% β-hydroxydecanoic acid, whereas a purified cyclicdepsipeptide from strain NTCC 1377 contained 84% β-hydroxydecanoic and 14% β-hydroxydodecanoic fatty acids. Mass spectral analysis (A.E.I. MS-902) gave an ion of highest *m/e* 514 (calcd mol.wt for a cyclicdepsipeptide containing 2 serine + 2 β-hydroxydecanoic acid residues = 514). NMR (Varian HA-100) and infrared (Perkin–Elmer 221) spectra were characteristic of the cyclicdepsipeptide structure of serratamolide[11,13,14] (Fig. 1).

The actions of serratamolide on suspensions of *S. aureus* strain Duncan were studied by methods previously described: the organism was grown, washed and made up at an initial density of 10.0 mg dry weight/ml in 0.01 M Tris–HCl or phosphate buffer, pH 7.5 (ref. 15); the release of K^+ was followed by means of a K^+-sensitive electrode with the suspension diluted to 2.0 mg dry weight/ml in 0.03 M Tris–HCl, pH 7.5 (ref. 16); the uptake of H^+ was followed with a pH microelectrode with cells suspended at 2.0 mg/ml in 2 mM sodium phosphate initially adjusted to pH 5.0 and containing 0.03 M NaCl and 6 mM iodoacetamide[16]; the uptake of ^{14}C-labelled glutamate (2 mM, specific activity 1.93 Ci/mole) or lysine (2 mM, specific activity

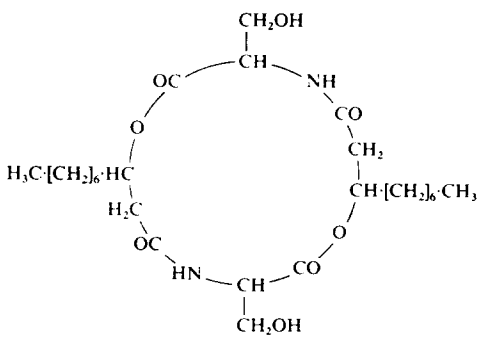

Fig. 1. Structure of serratamolide.

TABLE I

ACTIONS OF SERRATAMOLIDE ON *S. AUREUS* AT 20 °C

	μg serratamolide/ml				*Cells held 15 min at 100 °C*
	0	*5*	*10*	*15*	
K^+ release (nequiv/min per 2.0 mg cells)	4.5	11.5	51	- -	- -
H^+ uptake (nequiv/min per 2.0 mg cells)	8	10	20	- -	-
260 nm-absorbing release per 30 min per 2.0 mg cells	0.68	- -	0.65	0.72	2.27
Glutamate uptake (cpm/15 min per 0.2 mg cells)	1644	- -	2008	1364	- -
Lysin uptake (cpm/15 min per 0.2 mg cells)	712	- -	1031	1120	- -

3.84 Ci/mole) was studied with cells supended at 0.4 mg dry weight/ml in 0.1 M phosphate buffer, pH 7.5, containing 0.5% (final concentration, w/v) glucose and 30 μg chloramphenicol/ml[17]. The release of "260 nm-absorbing substances" was studied by suspending cells at 2.0 mg dry weight/ml in 0.01 M phosphate buffer, pH 7.5; after 30 min, the cels were centrifuged and the absorbance of the supernatant measured at 260 nm. The total available "260 nm-absorbing material" was obtained by holding one sample of suspension at 100 °C for 15 min before centrifuging the cells. All experiments were carried out at room temperature (20 °C). Serratamolide was made up at 1 mg/ml in ethanol and diluted as shown in Table I; an equivalent volume of ethanol was added to each control.

Table I summarises the results obtained. At a final concentration of 10 μg/ml, serratamolide markedly increased the rate of movement of K^+ and H^+ across the membrane of cells suspended at 2.0 mg/ml. At this concentration serratamolide had no effect on the rate of leakage of "260 nm-absorbing substances" from the cells nor any inhibitory action on the uptake of glutamate or lysine by cells at a density of 0.4 mg/ml. Serratamolide at 30 μg/ml inhibited the growth of *S. aureus* Duncan from an inoculum of 10^6 cells/ml. The action on cation transport therefore occurred at a concentration well below that which affected growth, or inhibited active transport of amino acids or produced such alteration of membrane permeability as would allow loss of nucleic acid precursors from inside the cells.

The influence of serratamolide on the conductance of "black" lipid bilayer membranes was also examined. The apparatus and techniques used were essentially those described previously[18]. The bilayer membranes were formed from solutions in *n*-decane of egg yolk phosphatidyl choline, phosphatidyl choline + cholesterol, glyceryl monooleate, and from a solution in *n*-hexadecane of glyceryl monooleate[19]. The serratamolide was added in ethanol solution (approx. 16 mg/ml) to either the the lipid phase or to the aqueous solution and, in some instances, to both. For all experiments the temperature was 20 ± 1 °C.

In the presence of serratamolide the lipid membranes tended to become unstable and, for this reason, the concentrations given below were the highest that

could be used. The background specific conductance of the membranes in 0.1 M KCl (pH approx. 5.8) and in the ethanol concentrations produced by the addition of the serratamolide, was approx. $10^{-8}\Omega^{-1}\cdot cm^{-2}$. With serratamolide initially at 0.6 mg/ml in the lipid and 23 μg/ml in the aqueous solution the specific conductance often rose to approx. $10^{-5}\Omega^{-1}\cdot cm^{-2}$ but it is possible that the ion leakages which sometimes develop just prior to the breakage of the membrane may have contributed to this increase. The results were very similar for the various types of membrane and were not significantly dependent on the hydrogen ion concentration between pH 7.0 and pH 3.0. Lower concentrations of serratamolide gave proportionately lower conductances. Due to the uncertain origin of the conductance, and to the instability of the relatively highly conducting membranes, it was not considered profitable to attempt ion selectivity measurements. The data suggest nevertheless that in KCl solution, serratamolide does induce some conductance in lipid membranes which is not entirely a consequence of impending breakage. The activity of serratamolide seems, however, to be at least 10^3 times smaller than that of nonactin.

REFERENCES

1 Shemyakin, M. M., Ovchinnikov, Yu. A., Ivanov, V. T., Antonov, V. K., Shkrob, A. M., Mikhaleva, I. I., Evstratov, A. V. and Malenkov G. G. (1967) *Biochem. Biophys. Res. Commun.* 29, 834–840
2 Pinkerton, M., Steinrauf, L. K. and Dawkins, P. (1969) *Biochem. Biophys. Res. Commun.* 35, 512–518
3 Pressman, B. C. (1968) *Fed. Proc.* 27, 1283–1288
4 Khokhlov, A. S. and Blinov, N. O. (1970) *FEBS Lett.* 11, 1–7
5 Dobler, M., Dunitz, J. D. and Krajewski, J. (1969) *J. Mol. Biol.* 42, 603–606
6 Taylor, A. (1970) *Adv. Appl. Microbiol.* 12, 189–276
7 Gromet-Elhanan, Z. (1970) *Biochim. Biophys. Acta* 223, 174–182
8 Bermingham, M. A. C., Deol, B. S. and Still, J. L. (1970) *Biochem. J.* 116, 759–761
9 Fargie, B. and Holloway, B. W. (1965) *Genet. Res.* 6, 284–299
10 Hancock, I. C. and Meadow, P. M. (1969) *Biochim. Biophys. Acta* 187, 366–379
11 Hassall, C. H., Martin, T. G., Schofield, J. A. and Thomas, J. O. (1967) *J. Chem. Soc. C* 977, 997–1003
12 Wuthier, R. E. (1966) *J. Lipid Res.* 7, 558–561
13 Wasserman, H. H., Keggi, J. J. and McKeon, J. E. (1962) *J. Am. Chem. Soc.* 84, 2978–2982
14 Shemyakin, M. M., Ovchinnikov, Yu. A., Antonov, V. K., Kiryushkin, A. A., Ivanov, V. T. Shchelokov, V. I. and Shkrob, A. M. (1964) *Tetrahedron Lett.* 1, 47–54
15 Gale, E. F. and Folkes, J. P. (1967) *Biochim. Biophys. Acta* 144, 452–460
16 Gale, E. F. and Llewellin, J. M. (1972) *Biochim. Biophys. Acta* 266, 182–205
17 Gale, E. F. and Folkes, J. P. (1967) *Biochim. Biophys. Acta* 144, 461–466
18 Hanai, T., Haydon, D. A. and Taylor, J. (1964) *Proc. R. Soc. A* 281, 377–391
19 Fettiplace, R., Andrews, D. M. and Haydon, D. A. (1971) *J. Membrane Biol.* 5, 277–296