EFFECT OF GRAMICIDIN S AND ITS DERIVATIVES ON THE MITOCHONDRIAL MEMBRANE

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1. Introduction

Gramicidin S was earlier shown to be an effective uncoupler of oxidative phosphorylation in mitochondria [1–3]. Our recent data [4,5] show that the uncoupling effect of the compound is apparently due to increased permeability of the mitochondrial membrane to alkali metal cations. The present paper reports the comparative study of gramicidin S, *N,N'*-diacetyl gramicidin S*, enantio gramicidin S and all-L-gramicidin S undertaken with the aim of understanding the mechanism of uncoupling as effected by these compounds in mitochondrial membranes. Some additional data which support our suggestion on the mechanism of gramicidin S action [4] has been obtained. It has been shown that the presence of the positive charges in the gramicidin S molecule are of no principal significance for its uncoupling effect, the conformation of gramicidin S molecule being much more important.

2. Material and methods

Gramicidin S was a gift of Professor G. F. Gause from the Institute of New Antibiotics. DA-gramicidin S was prepared by acetylation of gramicidin S [6], E-gramicidin S and L-gramicidin S were synthesized as described earlier [7]. Synthetic valinomycin was prepared according to Shemyakin et al. [8], biosynthetic valinomycin was obtained from Calbiochem. The rate of mitochondrial respiration was measured using an amperometric cell with Clark electrodes [9]. Swelling of the mitochondria was measured turbidimetrically at 610 nm. pH was measured with a glass electrode and an Ag/AgCl reference cell. The cell volume was 1 ml, measurements were made at 25°C, the amount of mitochondrial protein was 0.5 mg per ml in all the experiments. Protein was estimated according to Lowry et al. [10].

Rat liver mitochondria were prepared by a modification of Weinbach technique [11]. The respiratory control ratio according to Chance ranged from 3.4 to 6 with succinate. Mitochondria showed a high respiratory control ratio for 2–3 days. The incubation medium contained: sucrose (150 mM), EDTA (2.5 mM), $MgCl_2$ (6 mM), KCl (25 mM), Tris (25 mM, pH 7.3), phosphate (10 mM), succinate (10 mM), rotenone (1.5 μM). Cytochrome *c* (0.3 mg/ml) and DNP (0.2 mM) were added in some experiments.

To study the influence of particular cations, all cations in the media except Mg^{2+} were replaced by cations under study to a concentration of 40 mM. For anions, all anions except succinate were replaced by anions under study to a concentration of 20 mM except for phosphate when 10 mM was used.

* *Abbreviations:* DA-gramicidin S - *N,N'*-diacetyl gramicidin S; E-gramicidin S - enantio gramicidin S; L-gramicidin S - all-L-gramicidin S; DNP - 2,4-dinitrophenol.

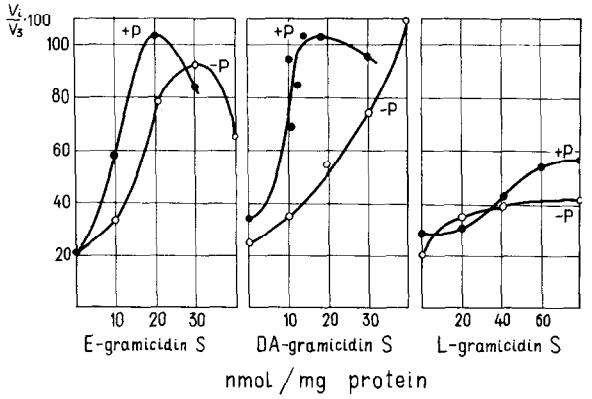

Fig.1. Effect of E-gramicidin S, DA-gramicidin S and L-gramicidin S on the rate of mitochondrial respiration in state 4′ with succinate in the presence (+ P) or in the absence (– P) of phosphate. The rate of state 3 respiration (V_3) is taken as 100 per cent; V_i is the rate of respiration in the presence of gramicidin S derivatives.

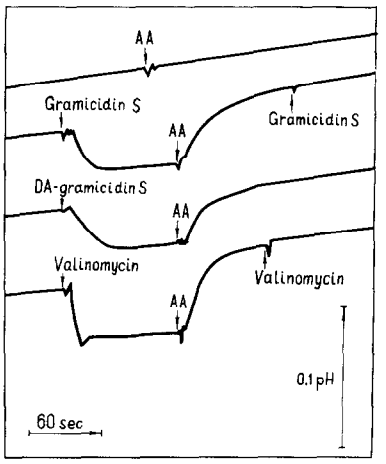

Fig.2. Effect of gramicidin S (5 nmol/mg protein), DA-gramicidin S (20 nmol/mg protein) and Valinomycin (10 pmol/mg protein) on the proton release from mitochondria in the presence of substrate and K^+ (in the absence of phosphate). (AA) antimycin A.

3. Results

The presence of a permeant anion (for example phosphate) enhanced the effect of gramicidin S, so that the maximal activation of mitochondrial respiration in state 4′, that is before addition of ADP (cf. Chance, [12]), is observed at lower concentrations of the antibiotic [4]. Thus the maximal activity of gramicidin S in the absence of phosphate is observed at 16 nmol/mg protein, and in its presence at 10 nmol/mg protein.

The analogous effect was found for E-gramicidin S and DA-gramicidin S (fig.1), only it was about two times lower than for gramicidin S (table 1). Unlike these 3 compounds, L-gramicidin S does not stimulate state 4′ mitochondrial respiration to the level of state 3 respiration. Phosphate also did not affect the system in the presence of L-gramicidin S.

Table 1
Concentration of gramicidin S and its derivatives required to double the mitochondrial respiration in state 4′ (nmol/mg protein)

Compounds	In the presence of phosphate	In the absence of phosphate
Gramicidin S	5	8
DA-gramicidin S	10	19
E-gramicidin S	10	16
L-gramicidin S	–	–

It should be noted that in the experiments with DA-gramicidin S and L-gramicidin S the incubation media were supplemented with cytochrome *c*, since in the absence of additional quantities of cytochrome *c* DA-gramicidin S induced only short stimulation of respiration. Gramicidin S and E-gramicidin S did not require cytochrome *c* addition [13]. It seems that similarly to valinomycin, gramicidin S, E-gramicidin S and DA-gramicidin S also induce proton release from the respiring mitochondria (fig.2) [14]. The proton release was measured in a medium containing K^+ and not containing any permeant anions. Antimycin A inhibition resulted in a decrease of protons in the medium (fig.2). In such cases L-gramicidin S did not stimulate the output of protons.

In the presence of some cations (K^+, Na^+, $Tris^+$) gramicidin S induces swelling of respiring mitochondria [4]. K^+ and Na^+ seemed to induce approximately similar swelling rates. The process is inhibited by antimycin or DNP. DA-gramicidin S and E-gramicidin S also induce mitochondrial swelling, however DA-gramicidin S in low concentrations can do this effectively enough only in the presence of K^+. In the presence of Na^+ or $Tris^+$ the swelling proceeds more

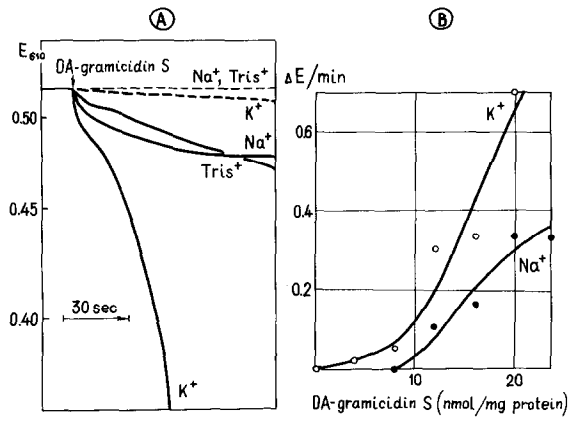

Fig.3. Effect of DA-gramicidin S on the swelling of mitochondria in the presence of succinate, phosphate and K^+, Na^+, $Tris^+$: (A) Concentration of DA-gramicidin S 8 nmol/mg protein. (B) Under various concentrations of DA-gramicidin S. The broken lines show the control curves in the absence of DA-gramicidin S.

slowly (fig.3). The rate of the swelling markedly depended on the anion present (fig.4). Thus in the presence of chloride DA-gramicidin S induced only a slight decrease in light-scattering, which moreover quickly ceased. Substitution of acetate for chloride resulted in intensive swelling. Phosphate anions in concentrations twice as low as that for acetate (to

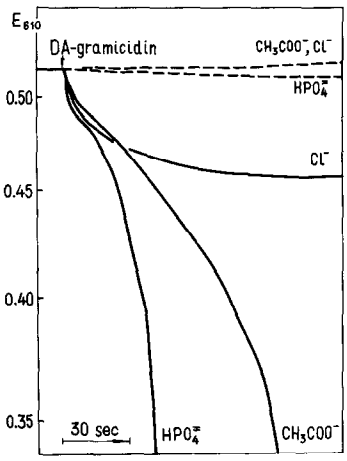

Fig.4. Effect of DA-gramicidin S (8 nmol/mg protein) on the swelling of mitochondria in the presence of succinate, K^+ and various anions. The broken lines – as in fig.3.

equilibrate anion charge) resulted in even higher swelling. Under the same conditions L-gramicidin S did not cause any swelling of the mitochondria.

It should be noted that gramicidin S when used in concentration exceeding 20–25 nmol/mg protein caused an intensive swelling of mitochondria also in the presence of respiratory inhibitors. This effect which is due to mitochondrial lysis, is not observed if the concentration of gramicidin S is below 20 nmol/mg protein. DA-gramicidin S and L-gramicidin S do not induce any mitochondrial lysis even at concentrations exceeding 50 nmol/mg protein.

4. Discussion

An increase of the gramicidin S effect on mitochondrial respiration and swelling caused by permeant anions, and inhibition of swelling as caused by respiratory inhibitors or DNP has led us to conclude that the uncoupling effect of gramicidin S is due to stimulation of the cation transport into mitochondria [4]. This conclusion was supported by the well known enhancement of K^+-transport into mitochondria by anions in the presence of valinomycin [15].

This fact can possibly be attributed to higher rates of K^+ and phosphate symport in comparison with the rate of K^+-uniport. The process is accompanied by a release of protons from the mitochondria [14] which is due to the functioning respiratory chain [16]. The release of protons induced by gramicidin S and its derivatives (fig.2) is indicative of a strong similarity between gramicidin S and valinomycin activity. We also know that on lipid bilayers (Goodall, [17]) gramicidin S considerably increased cation permeability of the membranes. The data obtained on the effect of gramicidin S derivatives allow us to make some suggestions on the molecular mechanism of action. The twice lower stimulation of mitochondrial respiration by E-gramicidin S, a compound with fully reversed configuration, suggests that proteins are also possibly involved in induced cation transport. However the present data are insufficient for a final conclusion to be reached on the role of the protein components. It is interesting that L-gramicidin S, a compound with only two inverted asymmetric centres did not induce any swelling at all. It activated respiration very poorly, even at much higher concentrations than gramicidin S

(fig.1). This could be due to the disturbance of 'β-pleated sheet' conformation [18,19] essential for the gramicidin S effect. Acetylation makes gramicidin S less active but more specific (DA-gramicidin S) and also shows that positive charges in the molecule are of no great significance for the uncoupling effect.

As to the mechanism of cation transport in the presence of gramicidin S, we suggest transmembrane ion transport without formation of intermediate complexes with this uncoupler, as unlike complexing ionphores [20] gramicidin S cannot form complexes with cations [19]. The gramicidin S effect seems to be due to a change in the membrane structure which results in formation of specific ionic channels. These channels are not accessible to chloride-ions (fig.4) and in the case of DA-gramicidin S they have different permeabilities for K^+ and Na^+. The experiments with E-gramicidin S show that protein components of the membrane could take part in the formation of these channels.

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