UNCOUPLING OF SUBMITOCHONDRIAL PARTICLES BY GRAMICIDIN

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1. Introduction

The gramicidins A, B, and C are linear polypeptide units with the following generic formula: *N*-formyl-pentadecapeptide ethanolamine [5]. These ion-transporting antibiotics show little ionic discrimination, the spectrum of selectivity being K^+, Rb^+, Cs^+, NH_4^+, Na^+, Li^+, $MeNH_3^+$, as well as H^+ [5, 6]. Its ion transporting properties have been studied in mitochondria [7, 8], chloroplasts [9, 10], chromatophores [11, 12, 13], erythrocytes [6, 7, 14] phospholid micelles [6, 7, 14], black lipid membranes [15], and *Streptococcus faecalis* [16].

Previous studies from this laboratory [2, 3, 4] have explored the effects of ion-transporting antibiotics [cf. 1] on the energy-linked functions and ion-translocation properties of submitochondrial particles (SMP)*. As a continuation of these studies, in the present communication we wish to report the effects of gramicidin on SMP and discuss the results in terms of the permeability-modifier action of gramicidin.

2. Materials and methods

EDTA [17] and Mg-ATP [18] particles derived by sonic disruption of beef-heart mitochondria were prepared as previously described. Oxygen consumption was measured polarographically with a Clark oxygen electrode. Esterification of inorganic phosphate was estimated by the isotope distribution method of Lindberg and Ernster [19]. The energy-linked color change of the chromophore, bromthymol blue (BTB) was followed as described by Chance and Mela [20]. Changes in the concentration of H^+ and K^+ were monitored with the A.H.Thomas 4858-L15 combination electrode and the Beckman electrode 39047 respectively, in conjunction with a Radiometer No. 22 pH meter connected to standard potentiometric recorders [21]. The electrode response was calibrated by the addition of known standards of HCl and KCl at the end of each experiment. Gramicidin was kindly supplied by Dr. B.C.Pressman.

3. Results

Harris, Hoffer and Pressman [8] first reported that gramicidin behaved like dinitrophenol in SMP, its effect being independent of the presence of Na^+ or K^+ in the medium. Table 1 shows that the P/O ratio of Mg-ATP "phosphorylating" SMP is inhibited about 80% by gramicidin; this inhibitory effect is not markedly affected by the presence of either K^+ or Na^+ in the medium.

Lee and Ernster [22] have shown that oligomycin induces an inhibition of respiration in EDTA particles (ESMP), which is released by uncouplers. Fig. 1 (right hand trace) shows that 1.5 μg/ml gramicidin released the oligomycin-inhibited respiration of ESMP with NADH as substrate, giving a respiratory control ratio of 3.3 in this particular case. The left hand trace of fig. 1 shows the energy-linked BTB response. Upon initia-

* Abbreviations used: (E-) SMP, (EDTA) Submitochondrial particles. FCCP, carbonyl cyanide, *p*-trifluoromethoxyphenylhydrazone, BTB, bromthymol blue (3,3-dibromothymol sulfonphtaleine.

Table 1
Effect of gramicidin on the P/O ratio of Mg-ATP particles.

	P/O	% inhibition
Control	0.76	
FCCP	0.15	80
K^+	0.43	42
Na^+	0.47	36
Gramicidin	0.19	75
Gramicidin + K^+	0.13	82
Gramicidin + Na^+	0.15	80

The reaction mixture consisted of 180 mM sucrose, 50 mM tris-Cl, pH 7.4, 3 mM ^{32}P (P_i) (1.2×10^6 cpm/μmole), 10 mM $MgSO_4$, 2 mM ADP, 60 mM glucose, 150 μg hexokinase, 0.9 mg of Mg-ATP SMP protein, and when indicated, 1 μM FCCP, 30 mM KCl, 30 mM NaCl and 1 μg of gramicidin. Final volume: 2.8 ml. Temperature: 30°C. The reaction was started by addition of 1.5 mM NADH, and stopped by addition of 0.3 ml of 5 M H_2SO_4 after about 80% of the oxygen was consumed.

tion of electron transport reactions by addition of 200 μM NADH, a decrease in the absorbance (indicated by an upward deflection) of the dye at 618 nm is observed. If gramicidin is added during the steady-state of NADH oxidation, the signal reverses and comes to the original baseline in about 10 sec.

The effect of gramicidin on ion-movements in SMP is shown in fig. 2. In *A*, the particles have been pretreated with 85 μM tris succinate and 143 ng/ml nigericin in order to stimulate K^+ uptake [4]. Upon addition of oligomycin, a significant uptake of K^+ is observed, reaching a steady-state in about 2 min [4]. Subsequent addition of gramicidin results in efflux of the K^+ taken up, returning the trace to the original baseline. If ESMP are preincubated with gramicidin, the nigericin-stimulated and oligomycin-dependent accumulation of K^+ by ESMP is prevented, as illustrated in *B*. In *C*, ESMP are pretreated with 85 μM tris succinate, and upon addition of oligomycin the respiration dependent H^+ translocation is monitored [4]. When gramicidin is added after the H^+ uptake has reached a steady-state a fast collapse of the pH gradient is observed. As shown in *D*, pretreatment of ESMP with gramicidin prevents the respiration-linked H^+ movements.

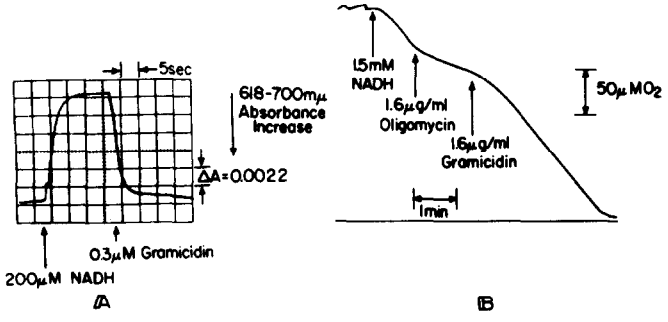

Fig. 1. Effect of gramicidin on the energy-linked functions of SMP:
A. *Effect of gramicidin on the energy-linked BTB response:* 0.7 mg/ml of ESMP protein, 0.25 M mannitol-sucrose, 0.02 M tris Cl, pH 7.4, 0.5 μg/ml oligomycin, 10 μM BTB. Final volume: 1.1 ml. Temperature, 20°C.
B. *Effect of gramicidin on the oligomycin-induced respiratory control:* 0.16 mg/ml of SMP protein, 0.25 M sucrose, 0.05 M tris acetate, pH 7.5. Final volume: 2.8 ml. Temperature, 30°C.

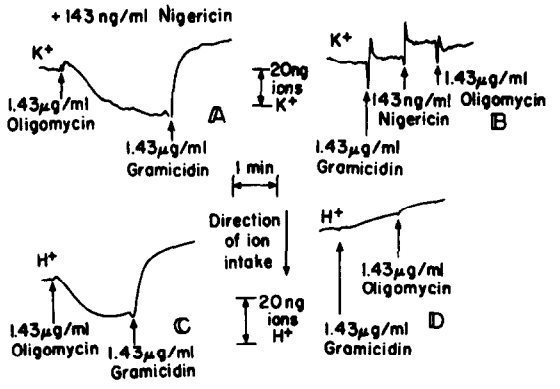

Fig. 2. Effect of gramicidin on ion movements in SMP: 0.43 mg/ml of ESMP protein, 100 mM choline chloride, 0.57 mM tris Cl, pH 7.5, 85 μM tris succinate, pH 7.5, 0.285 mM KCl. *A* and *B* are K^+-specific glass-electrode traces. *C* and *D* are H^+-specific glass-electrode traces.

4. Discussion

The effect of gramicidin in SMP is to uncouple oxidoreduction from phosphorylation [8]. Gramicidin causes an 80% inhibition of phosphorylation, a release of the oligomycin-induced respiratory control [22], and inhibition and dissipation of the energy-linked BTB response [20], K^+ uptake [4], and respiration-dependent H^+ uptake [20, 23]. Its uncoupling effect can be related to its ion-transport inducing properties. Harris, et al. [8], Chappell and Crofts [7], Chappell and Haarhoff [14], Henderson, et al. [6], Meuller and Rudin [15] and Skulachev, et al. [24] have concluded that gramicidin has the property of rendering the membranes studied permeable to monovalent cations including H^+.

It is now well established that those membrane systems associated with the energy-conservation process (mitochondria, SMP, chloroplasts and bacterial chromatophores) can reversibly transform 3 types of energy-sources: redox, phosphate bond (ATP) and electrochemical, i.e., they can perform oxidative phosphorylation [28], or ionic phosphorylation [29, 30] ionic-oxidoreduction [31, 32] or reversal of electron transfer (phosphorylative and nonphosphorylative oxidoreduction) [28], oxidoreductive ion-pumping [33] or phosphorylative ion-pumping [33]. Thus, either electron transfer or ATP hydrolysis may establish an electrochemical gradient of an ionic species.

If the high-energy state of SMP is associated with the build-up of a gradient of the electrochemical activity of H^+ [25], then H^+ conduction via gramicidin would allow electrolytic equilibration across the membrane with the consequent dissipation of the energy of the system.

Analogies can be drawn between the effects in SMP and observations that have been reported in bacterial chromatophores and chloroplasts. Gramicidin inhibits photophosphorylation in chromatophores [11, 13] and in chloroplasts [9]. Gramicidin inhibits the light-induced H^+ uptake [12, 13] and carotenoid shifts (which have been associated with the membrane potential) [27] in chromatophores [26] as well as the light-induced H^+ uptake and the light-induced absorption changes at 515 nm [10] (which have been associated with the membrane potential) in chloroplasts.

By mediating H^+ and K^+ equilibration across the coupling membrane thus collapsing the electrical and chemical components of the gradient, gramicidin uncouples oxidoreduction from phosphorylation in SMP, chromatophores and chloroplasts, as well as in mitochondria [7, 8].

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