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Inhibition of the mitochondrial ATP synthesis by polygodial, a naturally occurring dialdehyde unsaturated sesquiterpene

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Abstract

Polygodial is a naturally occurring sesquiterpene dialdehyde that exhibits several pharmacologically interesting activities. Among them, its antifungal properties have been more thoroughly studied. The mitochondrial ATPase has been suggested as one of the possible targets for polygodial action. However, its mechanism of action is not well defined yet. The effect of polygodial on the mitochondrial energy metabolism is described in this paper. Polygodial inhibited ATP synthesis coupled to succinate oxidation in beef-heart submitochondrial particles at concentrations ($IC_{50} = 2.4 \pm 0.1$ μM) which marginally affected electron transport and ATPase activity ($IC_{50} = 97 \pm 4$ μM). A transitory stimulation of the electron transport in intact rat liver mitochondria in state 4 was also obtained at low polygodial concentrations ($EC_{50} = 20 \pm 4$ μM). These results suggest that polygodial uncouples ATP synthesis from electron transport at low concentrations. Similar concentrations of polygodial partially abolished the ANS fluorescence enhancement ($IC_{50} = 2.2 \pm 0.4$ μM) induced by succinate oxidation in submitochondrial particles but did not collapse the ΔpH. We postulate that polygodial uncouples mitochondrial ATP synthesis by affecting the electrical properties of the membrane surface and consequently collapsing the membrane potential ($\Delta\psi$) and/or the localized transmembrane pH difference (ΔpH^S) without affecting the ΔpH between the two bulk aqueous phases (ΔpH^B). The relevance of these findings for the understanding of the biochemical basis of the antifungal activity of polygodial and the evaluation of its potentiality as a therapeutic agent are discussed.

Keywords: Polygodial; ATP synthesis; Mitochondria; Uncoupler; Antifungal; Mechanism

1. Introduction

Polygodial (Fig. 1) is a sesquiterpene dialdehyde isolated from plants belonging to Polygonaceae, Canellaceae and Winteraceae families. Plants from these families have been used in the folk medicine of several countries – such as Brazil and Eastern African ones – to treat skin infections, rheumatism, pains, affections of the respiratory tract among other diseases (see [1,2] and references quoted in there).

It has been shown that polygodial exerts various biological activities such as: (i) anti-hyperalgesia when assessed in a chemical model of nociception in mice and rats [3,4]; (ii) gastromucosal protection against necrotizing agents [5]; (iii) inhibition of the binding of resiniferatoxin to vanilloid receptors [6]—which is possibly involved in the above mentioned antinociception [7]; (iv) inhibition of the carbachol-induced muscarinic acetylcholine receptor signal transduction [8]; (v) inhibition of the contractile responses caused by inflammatory mediators in the guinea-pig ileum and trachea in vitro [9], (vi) inhibition of paw and ear edemas induced by irritants and inflammatory mediators in rats and mice [10]; (vii) vasorelaxation of blood vessels from rabbit and guinea-pig in vitro [11]; and (viii) antimicrobial and antifungal activities [12,13]. The latter was one of the more thoroughly studied. Several mechan-

Abbreviations: SMP, phosphorylating submitochondrial Mg^+-ATP particles; RLM, rat liver mitochondria; ANS, 8-anilino-1-naphtalene sulfonate; 9-AA, 9-aminoacridine; ACMA, 9-amino-6-chloro-2-methoxyacridine; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone

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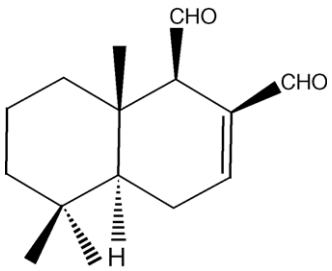

Fig. 1. Polygodial.

isms have been proposed to explain the mode of action of polygodial as antifungal, viz.: (i) structural disruption of the cell membrane that results in an increase in its permeability and in leakage of intracellular components [14], (ii) disruption of the hydrogen bonds at the lipid bilayer interface with the plasma membrane H^+-ATPase (an integral membrane protein) [15]; and (iii) depletion of mitochondrial glutathione with the consequent increase in the production of reactive oxygen species [16]. Lunde and Kubo have shown that the ATPase activity of crude mitochondrial membranes from *Saccharomyces cerevisiae* is inhibited by polygodial. On that grounds they have suggested that the mitochondrial ATPase could be a target for polygodial and that the inhibition of the mitochondrial ATP synthesis could be responsible for its antifungal activity [17].

In order to obtain direct evidence that polygodial inhibits the mitochondrial ATP synthesis and to determine the mechanism of such inhibition, we studied the effect of polygodial on the energy metabolism using purified mitochondrial preparations from mammals. Our results show that polygodial is a potent uncoupler of mitochondrial ATP synthesis that exerts its effect without increasing the proton permeability of the inner mitochondrial membrane. We discuss the mechanism of its antifungal activity and also the relevance of the mitochondrial ATP synthesis inhibition for the evaluation of its potential as a therapeutic agent.

2. Materials and methods

Rat liver [18] and heavy bovine heart [19] mitochondria were prepared as described. Phosphorylating submitochondrial Mg^+-ATP particles (SMP) were prepared from heavy bovine heart mitochondria according to Löw and Vallin [20].

2.1. Electron transport

Electron transport in rat liver mitochondria (RLM) was measured following oxygen consumption with a Clark electrode connected to a YSI Model 5300 Biological Oxygen Monitor. The reaction medium (2 ml) contained 250 mM sucrose, 30 mM KCl, 6 mM $MgCl_2$, 1 mM EDTA, 10 mM KH_2PO_4, 25 mM Tris-HCl (pH 7.4). Succinate (10 mM) or malate + glutamate (5 mM each) were used as oxidizable substrates. The reaction was started by the addition of RLM.

Electron transport in SMP was measured as described above in a medium (2 ml) containing 180 mM sucrose and 50 mM Tris-HCl (pH 7.5). Before the assay, SMP were thawed and incubated as described by Kotlyar and Vinogradov [21] to activate the succinate dehydrogenase.

2.2. ATPase activity

ATPase activity of SMP (0.07 mg protein) was determined spectrophotometrically essentially as described by Pullman et al. [22]. The reaction medium (1 ml) was: 180 mM sucrose, 1.5 mM $MgCl_2$, 40 mM Tris-HCl (pH 8.0), 4 mM potassium phosphoenolpyruvate, 0.35 mM NADH, 0.5 mM ATP, 30 I.U. pyruvate kinase (EC.2.7.1.40), 25 I.U lactate dehydrogenase (EC.1.1.1.27) and 3 μM rotenone.

2.3. ATP synthesis

The reaction was measured in a medium containing 180 mM sucrose, 1 mM $MgCl_2$, 10 mM succinate, 0.5 mM EDTA, 3 μM rotenone, 50 mM glucose, 0.01 mM ADP, 5 I.U. of yeast hexokinase (EC 2.7.1.1) and 50 mM Tris-HCl (pH 7.5). SMP (0.08 mg) were added to this medium and incubated for 5 min. ATP synthesis was started by adding 0.1 μmol Pi, 2×10^6 cpm of carrier-free ^{32}Pi and 3 μmoles $MgCl_2$. The final volume was 1 ml. The pre-incubation and the reaction were carried out under aerobiosis obtained with a gyratory water bath shaker. After 5 min the reaction was stopped and inorganic phosphate was quantitatively precipitated by the procedure of Sugino and Miyoshi [23] as modified by Roveri et al. [24]. Tubes were centrifuged for 10 min at 3000 rpm. Aliquots were analyzed for [^{32}P]glucose-6-phosphate by Cerenkov counting in a Beckman 8100 liquid scintillation counter.

2.4. Energy-linked 8-anilino-1-naphtalene sulfonate fluorescence (ANS) enhancement

Measurements were carried out in a Kontron fluorescence spectrofluorometer SFM25. SMP (0.5 mg) were added to a medium (1.5 ml) containing 180 mM sucrose, 40 mM Tris-HCl (pH 7.5), $MgCl_2$ 1 mM and ANS 5 μM. ANS fluorescence was excited at 380 nm and measured at 480 nm as described by Ferguson et al. [25].

2.5. Succinate-driven H^+ translocation in SMP

Succinate-driven H^+ translocation was measured following the quenching of 9-aminoacridine (9-AA) and 9-amino-6-chloro-2-methoxyacridine (ACMA) fluorescence according to Rottenberg and Moreno-Sanchez [26].

Measurements were carried out in a Kontron spectrofluorometer SFM25. Excitation was performed at 415 nm and measurements at 520 nm. Succinate 10 mM was added to a medium (1.5 ml) containing 200 mM sucrose, 10 mM KCl, 5 mM $MgCl_2$, 2 μM rotenone, 20 mM Tris-HCl (pH 7.5), 2 μM 9-AA, 0.4 μM ACMA and 1 mg SMP.

2.6. General

Protein determinations were carried out by a modified biuret procedure [27] using BSA as standard, whose concentration was determined spectrophotometrically (A_{279} = 6.67 cm^{-1} for 1% solution [28]).

Experimental values reported are average of determinations in duplicate, which agreed within 10%.

Polygodial was isolated from the barks of *Drymis winteri* as described previously [2] and dissolved in DMSO for the assays. Controls with the solvent (less than 2%) were performed for all the reactions studied. All other chemicals were of analytical grade.

Measurements were carried out at 30 °C.

3. Results

Polygodial inhibited the oxidation of malate + glutamate and succinate by RLM in state 3. The inhibition was progressive with time (Fig. 2A). A similar inhibition could be observed in the uncoupled state.

A slowly developed inhibition was also observed when the effect of polygodial on succinate oxidation by SMP was studied (Fig. 2C). Therefore, in order to titrate the effect of polygodial on electron transport from succinate to oxygen, SMP were pre-incubated with polygodial before the oxidizable substrate was added. Linear rates of oxygen uptake were obtained after 10 min pre-incubation, showing that polygodial had already exerted its maximal effect at that particular concentration (Fig. 2D). From the titration curve it could be estimated that 50% of the maximal effect was obtained with 145 μM polygodial (Fig. 3).

The ATPase activity of SMP was also inhibited by polygodial. The IC_{50} estimated was 97 ± 4 μM (Fig. 3). However, a much more powerful effect of polygodial was observed on ATP synthesis coupled to succinate oxidation (Fig. 3). The estimated IC_{50} value (2.4 ± 0.1 μM) was almost two orders of magnitude lower that those estimated for ATPase activity and for succinate oxidation.

On the other hand, when the effect of polygodial on oxygen consumption by RLM in state 4 was determined, a transient stimulatory effect – which was followed by inhibition – was observed immediately after the addition of polygodial (Fig. 2B). Maximal stimuli were 8.9 and 5.1 times for malate + glutamate and succinate oxidation respectively. The relative stimuli for both substrates (calculated as described in the legend to Fig. 4) could be fitted to a unique hyperbola on polygodial concentration: 50% of the maximal effect was attained at 20 ± 4 μM polygodial (see Fig. 4).

The results shown above strongly suggest that low concentrations of polygodial uncouple ATP synthesis from electron transport. In order to test such hypothesis, we studied the effect of polygodial on the ANS fluorescence enhancement that follows the generation of an electrochemical potential difference across the inner mitochondrial membrane induced by succinate oxidation. Such enhancement is completely and rapidly collapsed by the addition of carbonyl cyanide *p*-trifluoromethoxyphenyl hydrazone (FCCP), a well-known protonophore (see Fig. 5A). Similarly, polygodial collapsed the ANS fluorescence enhancement induced by succinate oxidation. However, this effect was only partial and it was slower than that exerted by FCCP (Fig. 5). Upon addition of polygodial, a short induction phase followed by a mono-exponential decay of the enhanced-fluorescence to a fluorescence value ($F_{polygodial}$) higher than that obtained with FCCP (F_{FCCP}) could be observed (Fig. 5B). The amplitude of the decay relative to that produced by FCCP depended hyperbolically on polygodial concentration. The maximal effect produced by polygodial was only 81% of that produced by FCCP. Half of the maximal effect was exerted by 2.2 ± 0.4 μM (Fig. 6, closed circles). The first order rate constant *k* also depended hyperbolically on polygodial concentration. Half of the maximal rate constant (0.012 ± 0.002 s^{-1}) was obtained with 4.5 ± 1.1 μM polygodial (Fig. 6, open circles).

H^+ translocation coupled to succinate oxidation induces a pH difference (ΔpH) between the phases separated by the inner mitochondrial membrane, which has low proton permeability. This succinate-driven ΔpH, assessed by monitoring the fluorescence quenching of a mixture of 9-AA and ACMA, is completely collapsed by FCCP (Fig. 7A). Conversely, the ΔpH was not collapsed by polygodial (Fig. 7B). Instead, it was increased approximately 50% in a dose dependent manner: half of the maximal effect was observed with 15 μM polygodial (Fig. 8).

4. Discussion

4.1. Effect of polygodial on the mitochondrial energy metabolism

Polygodial inhibits the electron transport from succinate to oxygen, the ATPase activity and the ATP synthesis in mitochondrial preparations from mammals. The latter is inhibited at concentrations (IC_{50} = 2.4 μM) that only marginally affect electron transport and ATPase activity (Fig. 3). Thus, the most likely explanation is that the strong inhibition of mitochondrial ATP synthesis by polygodial is due to uncoupling between electron transport and ATP synthesis and not to inhibition of the mitochondrial ATPase

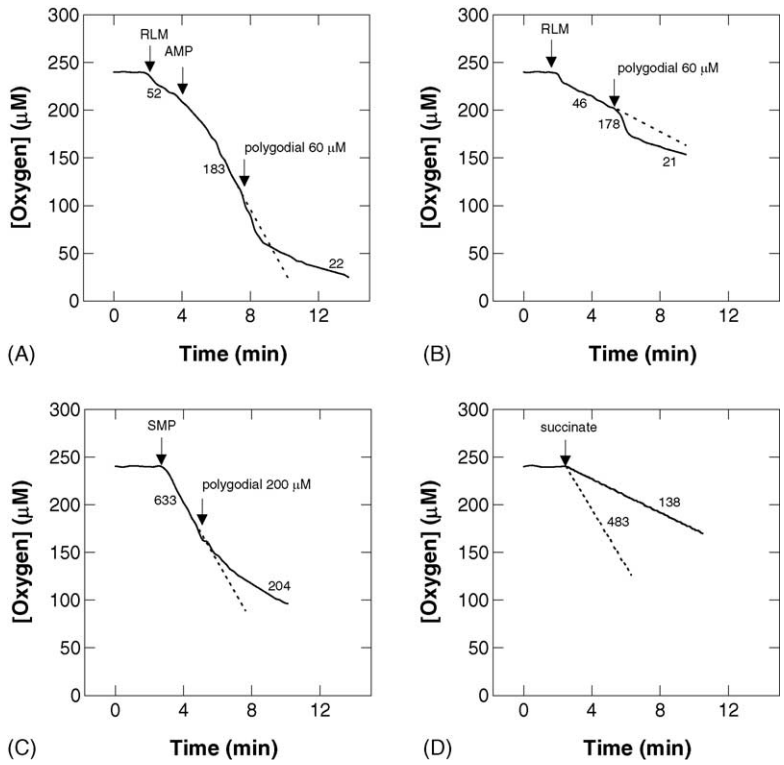

Fig. 2. Effect of polygodial on mitochondrial electron transport. (A) and (B) Succinate oxidation by RLM (0.76 mg protein) was determined as described under Section 2. When indicated, 1 mM AMP was added. (C) and (D) Electron transport in SMP was measured as described in Section 2. (C) The reaction was started by adding 0.2 mg SMP to a medium containing 10 mM succinate. (D) SMP (0.2 mg) were pre-incubated during 10 min in the presence of 200 μM polygodial before the oxygen consumption was started by adding succinate (10 mM final concentration). The negative controls obtained when DMSO was added instead of polygodial are shown in dotted lines. Numerals at the slopes represent oxygen consumption in nat O_2/min/mg protein.

as it has been suggested by Lunde and Kubo [17]. The transient stimulation of the electron transport in RLM in state 4 observed at low polygodial concentrations (Fig. 4) is in agreement with this proposal.

4.2. Mechanism of the uncoupling of the mitochondrial ATP synthesis by polygodial

The driving force for the ATP synthesis by the mitochondrial F_1F_o ATP synthase (reversible H^+-ATPase) is a proton electrochemical gradient ($\Delta\mu_H{}^+$) generated across the inner mitochondrial membrane by the respiratory chain. The $\Delta\mu_H{}^+$ is composed by a proton concentration gradient (ΔpH) and by a difference in electric potential ($\Delta\psi$ or ''membrane potential'') across the membrane. One of the controversial aspects of the chemiosmotic theory is which $\Delta\mu_H{}^+$ component is responsible for driving the ATP synthesis. Although ΔpH and $\Delta\psi$ have been considered to be thermodynamically equivalent, Kaim and Dimroth [29] have shown that the membrane potential is essential for ATP synthesis. On that grounds, Dimroth et al [30] have suggested that $\Delta\psi$ controls the kinetics of the unidirectional ion translocation through the F_o sector of the ATP synthase. It is also matter of discussion whether the ΔpH relevant for the energy transduction is the pH difference between the bulk of the aqueous phases separated by the membrane (ΔpH^B) or between both membrane–water interfaces (ΔpH^S). Cherepanov et al. [31] have postulated that the low permittivity of water at a charged surface [32] generates a potential barrier for the diffusion of ions. Such a barrier, located 0.5–1 nm away from the membrane surface, could generate a significant difference between ΔpH^S and ΔpH^B. The height of that barrier and hence the difference between ΔpH^S and ΔpH^B would depend – among other factors – on the surface charge density [31–33]. A protonophore collapses the proton electrochemical gradient increasing the proton permeability of the membrane as a result of its structural characteristics: protonophores are weak acids that permeate bilayers either in their protonated neutral form or in their unprotonated anionic form. Despite its ionic character, the latter is still soluble in the membrane because the negative charge is delocalized by an extensive π-orbital system [34]. By cycling across the membrane they catalyze the net electrical uniport of protons, collapsing both $\Delta\psi$ and ΔpH and uncoupling $\Delta\mu_H{}^+$ generation from ATP synthesis.

It has been claimed that the ANS fluorescence enhancement induced by succinate oxidation can be used for estimating the ''membrane potential'' ($\Delta\psi$). Although the ANS fluorescence can be influenced by other factors [35], its enhancement induced by succinate oxidation or by ATP hydrolysis is a useful tool for monitoring the ''energized state'' of the inner mitochondrial membrane. A typical protonophore such as FCCP completely collapses

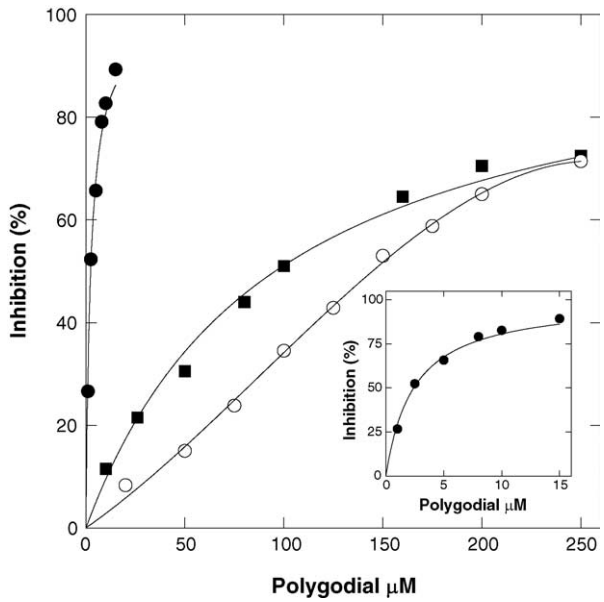

Fig. 3. Inhibition by polygodial of energy-linked reactions in submitochondrial particles. ATP synthesis (●), succinate oxidation (○) and ATPase activity (■) were measured as indicated under Section 2 in the absence and in the presence of different polygodial concentrations. IC_{50} values for the inhibition of ATP synthesis and of ATPase activity were estimated by non-linear regression to the following equation: Inhibition(%) = (100 × [Polygodial])/(IC_{50} + [Polygodial]), whereas the IC_{50} value for succinate oxidation was estimated by interpolation. The data obtained when the inhibition of the ATP synthesis was studied are also shown in an expanded scale in the insert.

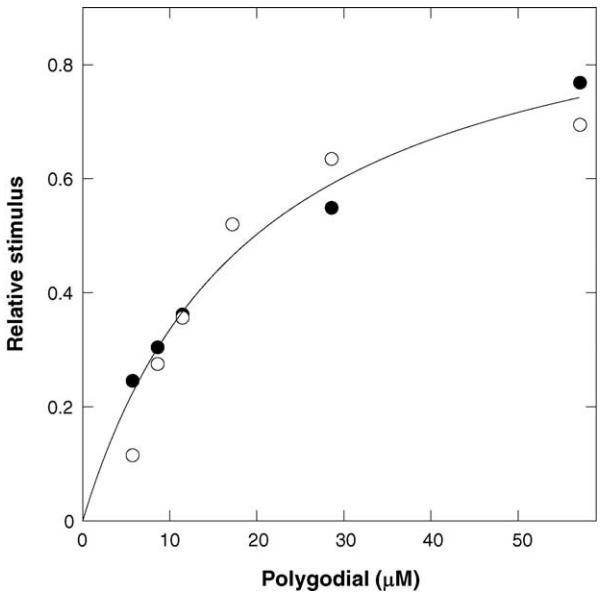

Fig. 4. Stimulus by polygodial of oxygen consumption in state 4 in rat liver mitochondria. Oxygen consumption by RLM in state 4 was measured as described in Section 2 in the absence (v_0) and in the presence (v) of polygodial, using either malate + glutamate or succinate as oxidizable substrates. The experimentally determined values were fitted by a non-linear regression procedure to the following equation: $((v/v_0) - 1) = \frac{((v/v_0)-1)_{max}[\text{polygodial}]}{A_{0.5}+[\text{polygodial}]}$. Maximal stimuli ($((v/v_0) - 1)_{max}$) estimated for malate + glutamate and for succinate oxidation were 8.9 ± 1.8 and 5.1 ± 0.4, respectively. Symbols in the Figure are the relative stimuli for malate + glutamate (○) and succinate (●) oxidation, calculated as the ratio between the experimentally determined ($((v/v_0) - 1)_{max}$) values and the maximal stimulus estimated, accordingly to the procedure described above. The line is the fit of the relative stimuli to a rectangular hyperbola on polygodial concentration.

such fluorescence enhancement (Fig. 5A). Polygodial partially abolished the ANS fluorescence enhancement at concentrations that did not inhibit succinate oxidation (compare Figs 5B and 3). This result indicates that polygodial dissipates most of the "energized state" of the mitochondrial membrane (19% of the FCCP-sensitive ANS fluorescence enhancement is not affected by polygodial, see Fig. 6). Half of the maximal amplitude and half of the maximal rate constant of the polygodial-induced decay of the ANS fluorescence enhancement are attained at polygodial concentrations (2.2 and 4.5 μM, see Fig. 6) similar to that responsible for the inhibition of ATP synthesis (2.5 μM, Fig. 3). Thus, it is reasonable to postulate that the inhibition of ATP synthesis and the dissipation of the "energized state" of the membrane are closely related phenomena.

FCCP increases the proton permeability of the inner mitochondrial membrane and hence it completely collapses the energy-driven ΔpH (see Fig. 7A). Since polygodial does not collapse the ΔpH (Fig. 7B), it can be concluded that it does not increase the proton permeability of the inner mitochondrial membrane. Therefore it neither disrupts the integrity of the membrane nor does it behave as a protonophore. This last conclusion is not surprising since polygodial is not a weak acid and it lacks the extensive π-system, properties that are characteristic of protonophores [34].

In summary, the strong inhibition of the mitochondrial ATP synthesis cannot be explained either by the inhibition of the respiratory chain or the ATP synthase or by an increase in the proton permeability of the inner mitochondrial membrane. Nonetheless, 81% of the $\Delta\mu_H$+ generated by the respiratory chain is abolished by polygodial. Since the ΔpH^B – which is the component measured by the 9AA + ACMA fluorescence quenching – is not dissipated by polygodial, the "membrane potential" component ($\Delta\psi$) of the $\Delta\mu_H{}^+$ is a good candidate as the primary target for polygodial action.

Polygodial is an amphipathic molecule. Therefore, it can be postulated as a working hypothesis that polygodial penetrates the lipid bilayer and modifies the membrane dipole potential. The membrane dipole potential (ψ_d) is due to the alignment of the dipolar residues of the lipids and/or water dipoles at the membrane–solution interface [36] and could be modified by the insertion in the bilayer of dipolar molecules such as polygodial. As a matter of fact, preliminary experiments have shown that polygodial can penetrate a lipid monolayer and modify the dipole potential estimated as the electrical potential difference across the monolayer.

The membrane dipole potential contributes – together with the surface (Guoy-Chapman, ψ_s) and the transmem-

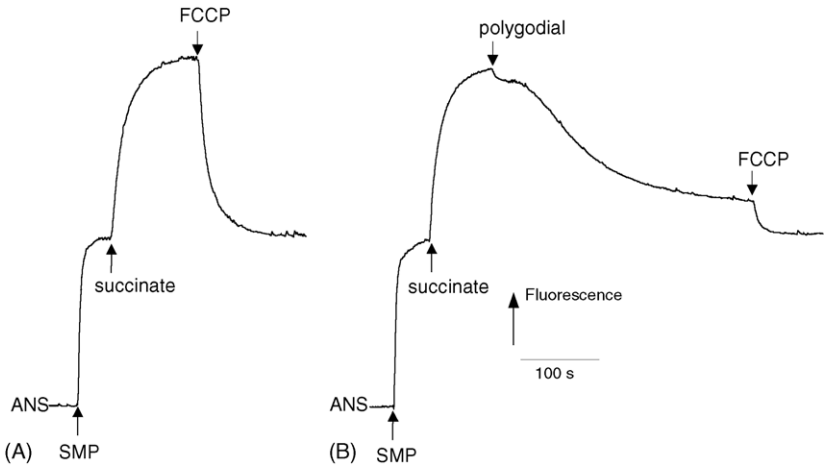

Fig. 5. Effect of polygodial on the succinate-induced ANS fluorescence enhancement. The succinate-induced ANS fluorescence enhancement was measured as described in Section 2. Additions were 0.5 mg SMP, 10 mM succinate, 20 μM polygodial and 1 μM FCCP.

brane potentials ($\Delta\psi$) – to the potential difference between the membrane surfaces ($\Delta\phi$) and determines the electrical potential profile in the membrane interior [37]. Therefore, the postulated modification of the dipole potential by polygodial would affect the potential profile in the membrane and the interfacial membrane potential difference ($\Delta\phi$). It can be argued that the modification of the latter requires a differential effect of polygodial on the dipole potential in opposite membrane surfaces. There is an intrinsic asymmetric distribution of phospholipids between both sides of the inner mitocondrial membrane: cardiolipin and phosphatidylinositol are located mainly on the matrix side whereas phosphatidylcholine and phosphatidylethanolamine on the cytoplasmic side [38]. Either this intrinsic asymmetry and/or a functional asymmetry induced by the $\Delta\mu_H{}^+$ can justify a differential effect of polygodial on the matrix and cytoplasmic faces of the inner mitochondrial membrane. Based on the above-presented working hypothesis, several possible mechanisms that could explain the inhibition of the mitochondrial ATP synthesis by polygodial can be considered:

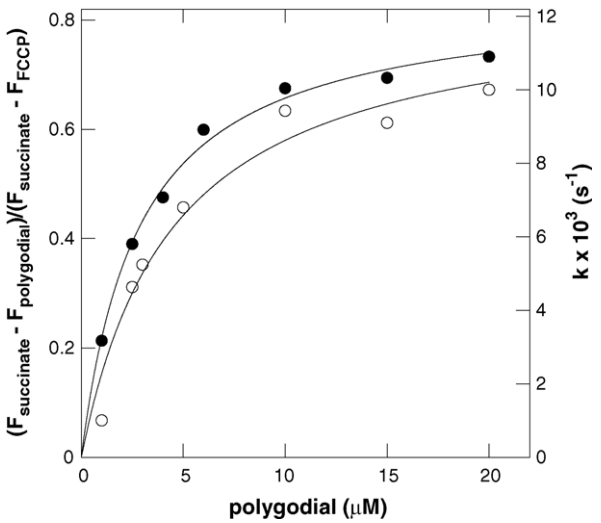

Fig. 6. Titration of the effect of polygodial on the succinate-induced ANS fluorescence enhancement. Assays similar to that shown in Fig. 5B were carried out at different polygodial concentrations. Fluorescence decays after the additions of polygodial were fitted using a non-linear regression procedure to the following equation: $F = F_{polygodial} + (F_0 - F_{polygodial})e^{-kt}$. F_0 and $F_{polygodial}$ were the fluorescence values estimated at zero and infinite time and k the decay rates constant. $F_{succinate}$ and F_{FCCP} (fluorescence values after the addition of succinate and FCCP, respectively) were determined from traces similar to that shown in Fig. 5B. Closed circles (●) represent the ratio $(F_{succinate} - F_{polygodial})/(F_{succinate} - F_{FCCP})$ and open ones (○) the estimated k values. The lines indicate the fit of each set of data to a rectangular hyperbola.

i. The modification of the interfacial membrane potential could lead to changes in ΔpH^S, which has been postulated to be the pH difference responsible for driving the ATP synthesis.
ii. The postulated modification of the dipole potential may affect the properties of intrinsic membrane proteins involved in the translocation of ions – other than protons – leading to an increased membrane conductance with the concomitant collapse of the transmembrane potential. It has been shown that the membrane dipole potential influences ion translocation among several membrane-related processes (see [37,39] and references quoted in there).
iii. Either the potential difference between the two membrane surfaces or the potential profile in the membrane interior would be kinetically relevant for driving the ATP synthesis instead of the transmembrane (bulk to bulk) potential difference.

Any of the above described mechanisms would explain the inhibition of ATP synthesis and also the polygodial-induced decay of the succinate-driven ANS fluorescence enhancement, provided that ANS fluorescence responds – under energized conditions – to a localized $\Delta\psi$, a delocalized $\Delta\psi$, or to the interfacial potential difference ($\Delta\phi$), respectively. Conversely, no differences are expected

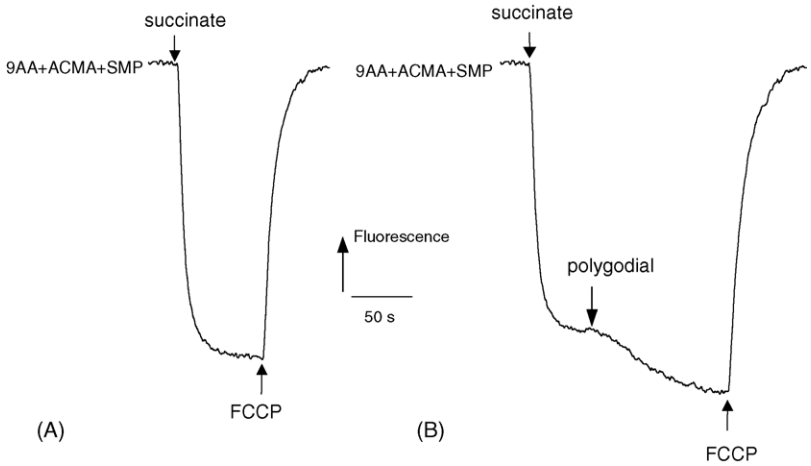

Fig. 7. Effect of polygodial on the succinate-driven H^+ translocation in SMP. Measurements were carried out as described under Section 2. Additions were 10 mM succinate, 20 μM polygodial and 1 μM FCCP.

between the ANS fluorescence values obtained with polygodial + FCCP and with FCCP alone (see Fig. 5) since FCCP collapses any potential difference across the membrane.

Further studies must be carried out to discriminate between the mechanisms mentioned above.

4.3. On the mechanism of action of polygodial as antifungal agent

It has been claimed that polygodial disrupts the cell membrane and induces leakage of intracellular components. On that grounds it was postulated that the plasma membrane is the target of polygodial [14]. Conversely, our results show that the inner mitochondrial membrane is not disrupted by polygodial since the latter did not increase its proton permeability (see Fig. 7). Moreover, Lunde and Kubo have previously reported that $CaCl_2$ suppresses the membrane leakage induced by polygodial but does not protect yeast cells against polygodial's antifungal activity, clearly indicating that there is not a good correlation between both phenomena [17].

It has also been postulated that polygodial exerts a major portion of its antifungal action through inhibition of ATP synthesis [17]. Our studies, using mitochondrial preparations from mammals, support this hypothesis, since ATP synthesis by SMP was inhibited at polygodial concentrations similar to the MIC values reported for several yeast and filamentous fungi [14]. However, our results show that such inhibition is better explained by uncoupling ATP synthesis from electron transport and not by a direct effect of polygodial on the mitochondrial ATPase as it has been suggested [17].

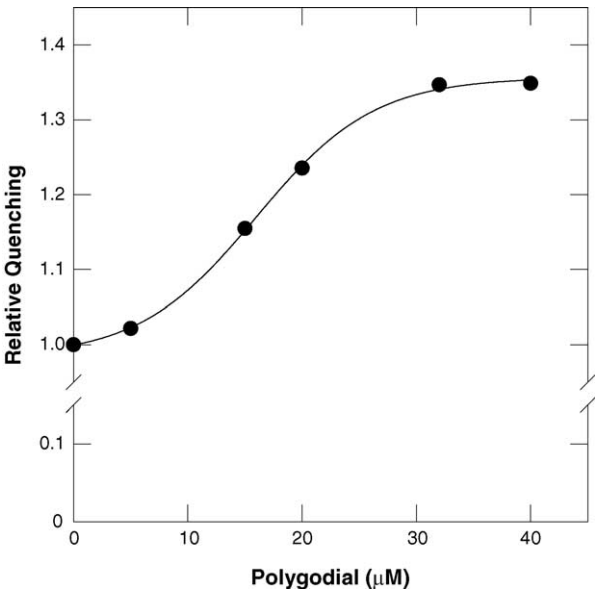

Fig. 8. Titration of the effect of polygodial on the quenching of ACMA + 9-AA fluorescence induced by succinate oxidation in SMP. Assays at different polygodial concentrations were performed as shown in Fig. 7. Symbols indicate the ratio between the quenching of fluorescence induced by succinate in the presence of polygodial and in its absence.

4.4. Concluding remarks

The results described here show that polygodial is a strong inhibitor of the mitochondrial ATP synthesis. In spite of the fact that it does not increase the proton permeability of the inner mitochondrial membrane, the more likely mechanism for such inhibition is the uncoupling of ATP synthesis from electron transport. The possibility that this uncoupling – as well as other membrane-related biological activities of polygodial – could be explained by modification of the electric properties of the membrane surface is currently being studied.

Regardless of the inhibitory mechanism of polygodial, the fact that the mitochondrial ATP synthesis – a central and essential metabolic process – is strongly inhibited by polygodial in mammals, must be taken into account when its potentiality as a therapeutic agent is evaluated.

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