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Inhibition of electron transport of rat liver mitochondria by unnatural (−)-antimycin A_3

Hideto Miyoshi[1], Hitoshi Kondo[2], Takayuki Oritani[2], Isao Saitoh[1] and Hajime Iwamura[1]

[1]*Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan* and [2]*Department of Agricultural Chemistry, Tohoku University, Sendai 981, Japan*

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The inhibition of electron transport by unnatural (−)-antimycin A_3 was examined with rat liver mitochondria and compared with that of natural (+)-antimycin A_3. (−)-Antimycin A_3 inhibited respiration about 1/100th as strongly as natural (+)-antimycin A_3. (−)-Antimycin A_3 binding to the cytochrome *bc*1 complex did not seem to induce a conformational change in this proteinous complex. The binding site of (−)-antimycin A_3 was probably the same as that of (+)-antimycin A_3 (at the Q_i center). However, the mode of interaction with the Q_i center by (−)-antimycin A_3 and (+)-antimycin A_3 was somewhat different.

Antimycin A (unnatural); Cytochrome *bc*1 complex; Mitochondria; Rat liver

1. INTRODUCTION

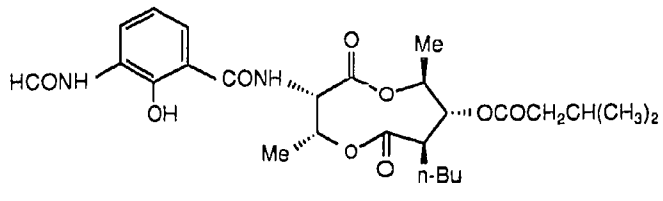
The study of antimycin A, a potent specific inhibitor of the cytochrome *bc*1 complex (ubihydroquinone: cytochrome *c* oxidoreductase, EC 1.10.2.2), has given results useful in the understanding of the mechanism and evolution of this complex [1,2]. The structural aspects of antimycin A needed for inhibitory activity are fairly well understood [3–6], the phenolic hydroxy group of the salicylic acid moiety of the antimycin molecule is necessary for its inhibitory activity, but the dilactone ring moiety may be replaced by other hydrophobic groups such as long alkyl chain. The stereochemical aspects of antimycin A that govern its inhibitory activity are not known. In this study, the inhibition by unnatural (−)-antimycin A_3 and natural (+)-antimycin A_3 of electron transport in rat liver mitochondria was compared.

2. MATERIALS AND METHODS

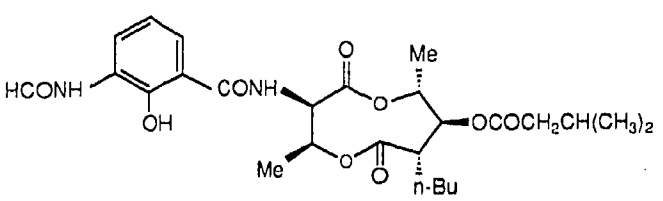
The unnatural (−)-antimycin A_3 studied was synthesized by Kondo and Oritani [7], mp 184–185°C, $[\alpha]_D^{22}$ −74.4° (*c* = 0.47, $CHCl_3$), IR_{max} (KBr) cm^{-1}: 3400, 1750, 1690, 1640, ^{13}C-NMR($CDCl_3$) δ: 13.8, 15.0, 17.9, 22.4, 25.5, 28.2, 29.2, 43.2, 50.1, 53.7, 70.9, 74.9, 75.4, 112.6, 119.0, 120.1, 124.8, 127.5, 150.6, 159.1, 169.4, 170.1, 171.7, 173.0, MS m/z: 521(M^++1, 8%), 520(M^+), 264(20), 40(100). Natural (+)-antimycin A_3 and myxothiazol were purchased from Sigma.

Mitochondria were isolated from the livers of adult male Wistar rats in a medium containing 250 mM sucrose and 2 mM Tris-HCl (pH 7.4) as described by Myers and Slater [8]. Mitochondrial respiration with 10 mM succinate as the respiration substrate was measured with a Clark-type oxygen electrode at 25°C. The final mitochondrial protein concentration in the medium was 0.7 mg/ml. The incubation medium consisted of a mixture of 200 mM sucrose, 2 mM $MgCl_2$, 1 mM EDTA, and 2.5 μM rotenone in 2.5 mM potassium phosphate buffer (pH 7.4), and the total volume was 2.5 ml. The respiration inhibitory activity of (+)-antimycin A_3 and (−)-antimycin A_3 was calculated from their effects on fully stimulated respiration by 40 nM SF6847, because this uncoupler-stimulated respiration is readily reduced by the presence of a respiration inhibitor [9].

The redox status of cytochrome *b* of the intact mitochondria was identified before and after each treatment with the wavelength pair of 563 and 577 nm [10]. The absorbance spectra were measured with a Shimadzu UV3000 spectrophotometer with a 1-nm bandwidth. The reaction medium was the same as that used for the respiration experiment except that 1 mM KCN was included. The final mitochondrial protein concentration was 1.4 mg/ml.



(+)-Antimycin A_3



(-)-Antimycin A_3

Abbreviation: SF6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidene malononitrile

Correspondence address: H. Miyoshi, Department of Agricultural Chemistry, Kyoto University, Kyoto 606, Japan. Fax: (81) (75) 753 6128.

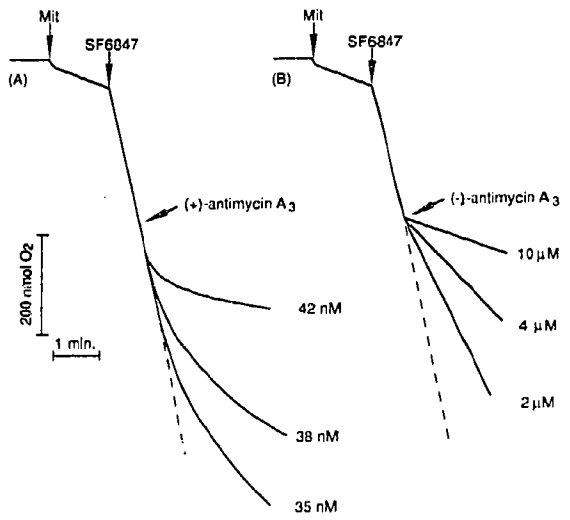


Fig. 1. Effects of natural (+)-antimycin A_3 (A) and unnatural (−)-antimycin A_3 (B) on uncoupler-stimulated respiration. The concentration of SF6847 was 40 nM.

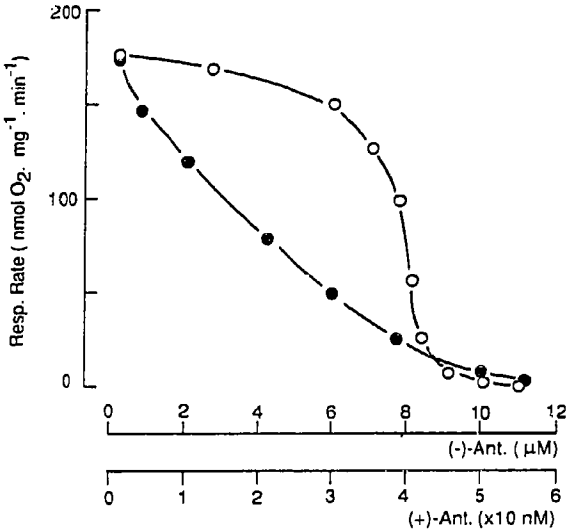


Fig. 2. Titration curves for the inhibition of respiration stimulated by SF6847 (40 nM). (○) (+)-antimycin A_3; (●) (−)-antimycin A_3.

3. RESULTS

Fig. 1 shows the inhibition by (+)-antimycin A_3 and (−)-antimycin A_3 of fully stimulated respiration by SF6847. With (+)-antimycin A_3, there was always a lag phase, which had a length dependent on the concentration of (+)-antimycin A_3. The extent of inhibition increased with time after the lag phase. A lag phase was not observed with (−)-antimycin A_3 at any of the three concentrations tested.

The titration curve for the inhibition of respiration by (+)-antimycin A_3 and (−)-antimycin A_3 is shown in Fig. 2. The respiration rate by (+)-antimycin A_3 was read

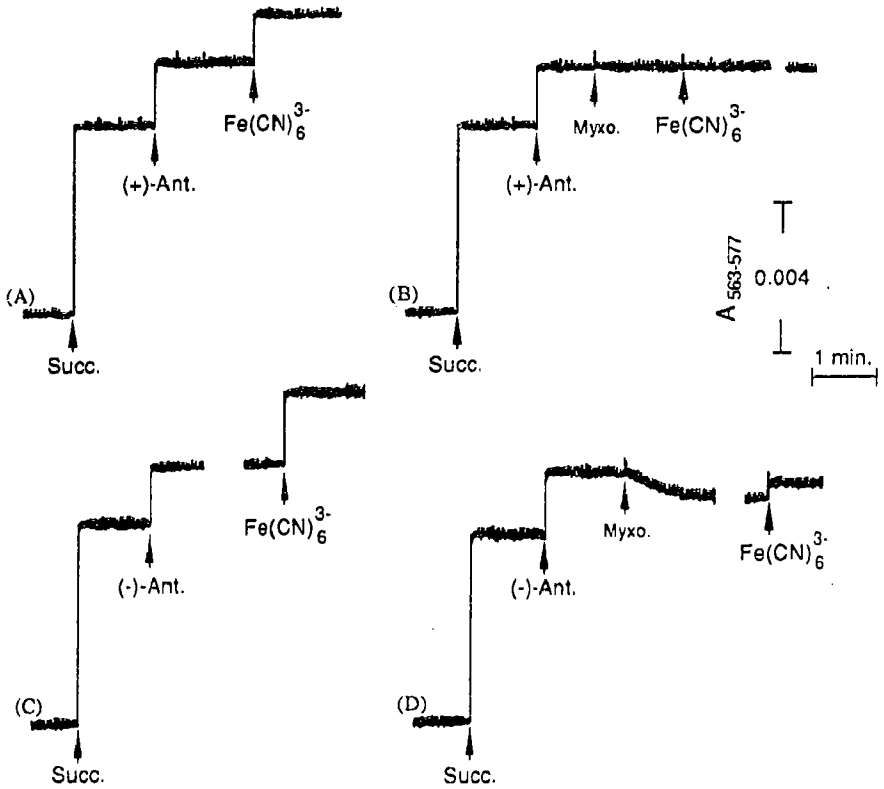


Fig. 3. Effects of (+)-antimycin A_3 and (−)-antimycin A_3 on the reduction of cytochrome *b* by succinate. The reduction of cytochrome *b* was monitored at the wavelength pair of 563 and 577 nm. Mitochondria were incubated in the reaction medium for 10 min before the addition of succinate. Where indicated, 10 mM succinate, 95 nM (+)-antimycin A_3, 35 μM (−)-antimycin A_3, 95 nM myxothiazol, and an excess of potassium ferricyanide were added. Discontinuities in the absorbance trace indicate discontinuities in the time scale.

when it had become stable. The inhibition by (−)-antimycin A_3 was weaker than that of (+)-antimycin A_3. The averaged I_{50} value from three runs (I_{50} being the molar concentration needed to reduce the respiration rate fully stimulated by SF6847 to half) of (+)-antimycin A_3 and (−)-antimycin A_3 was 3.8×10^{-8} M (54.3 pmol/mg protein) and 4.2×10^{-6} M (6.0 nmol/mg protein), respectively. A sigmoidal relationship was obtained for (+)-antimycin A_3, but not for (−)-antimycin A_3. (+)-Antimycin A_3 binding to the cytochrome *bc*1 complex did induce a conformational change in this proteinous complex, as suggested by Rieske [11] and Ohnishi and Trumpower [12]. These results suggested that the mode of interaction with the binding cavity of the cytochrome *bc*1 complex may be different for (+)-antimycin A_3 and (−)-antimycin A_3. However, it is also possible that the interaction site may be different.

The binding site of natural (+)-antimycin A_3 is the Q_i reaction center of the cytochrome *bc*1 complex [2]. We set out to identify the binding site of (−)-antimycin A_3 from its effects on the redox status of cytochrome *b* (Fig. 3). The control experiments on the effects peculiar to (+)-antimycin A_3, i.e. reduction of cytochrome *b* after succinate and oxidant-induced reduction of cytochrome *b* [2], are shown in Fig. 3A. The oxidant-induced reduction was completely prevented by the presence of myxothiazol, a Q_o center inhibitor (Fig. 3B) [2]. (−)-Antimycin A_3 amplified the reduction of succinate-reduced cytochrome *b* to a level close to that observed with (+)-antimycin A_3 (Fig. 3C). The oxidant-induced reduction of cytochrome *b* was also seen in the presence of (−)-antimycin A_3 (Fig. 3C). Unlike with (+)-antimycin A_3, the addition of myxothiazol after (−)-antimycin A_3 caused further oxidation of the cytochrome *b* (Fig. 3D). The combined addition of (−)-antimycin A_3 and myxothiazol did not completely prevent the oxidant-induced reduction of cytochrome *b* (Fig. 3D), although the reduction level was lower than that without myxothiazol.

4. DISCUSSION

The inhibitory activity by (−)-antimycin A_3 was about 1/100th that of (+)-antimycin A_3 . In contrast to (+)-antimycin A_3 , a lag phase before the respiratory inhibition began was not observed for (−)-antimycin A_3. (−)-Antimycin A_3 binding did not seem to cause a conformational change in the binding cavity in the cytochrome *bc*1 complex. Perhaps the (−)-antimycin A_3 molecule, esspecially its salicylic acid moiety, does not adequately fit into the binding cavity of natural (+)-antimycin A_3 .

The binding site of (−)-antimycin A_3 seemed to be the same as that of (+)-antimycin A_3 from the results shown in Fig. 3. However, the effects on the redox status of cytochrome *b* were different between (+)-antimycin A_3 and (−)-antimycin A_3 ; cytochrome *b* reduced with (−)-antimycin A_3 was then oxidized by the addition of myxothiazol, and the oxidant-induced reduction of cytochrome *b* in the presence of (−)-antimycin A_3 was not completely abolished by myxothiazol. The interaction of (−)-antimycin A_3 with the Q_i center might be somewhat weakened by the conformational change in the cytochrome *bc*1 complex caused by the binding of myxothiazol [10].

The dilactone moiety of antimycin A probably reinforces the interaction of the antimycin A molecule with the binding cavity by increasing the hydrophobicity of antimycin A [11]. Our findings suggested that the configuration of the antimycin A molecule is a very important factor to its inhibitory activity (to its binding to the Q_i center). The configuration of the antimycin molecule, when appropriate, may allow the tight fitting of the salicylic acid moiety into the binding cavity. The amino acid residues of cytochrome *b* needed for interaction with antimycin A have been identified by a molcular genetic approach [1,13–16]. The binding model of antimycin A to the Q_i center of the cytochrome *bc*1 complex should take into account the stereochemical factors governing such interactions.

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