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Influence of ubiquinone on the rate of antimycin binding to submitochondrial particles

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SUMMARY

A spectrophotometric method is described for measuring the rate of antimycin binding to submitochondrial particles from beef heart. It is shown that the rate of antimycin binding to the particles is increased when these are made ubiquinone deficient by extraction with pentane, and decreased again when ubiquinone is reincorporated into the particles. On increasing the H^+ concentration of the suspending medium, antimycin binding to both ubiquinone-containing and ubiquinone-deficient particles is accelerated, although with different kinetics. The significance of these results is discussed relative to a role for ubiquinone as a regulator of the state of protonation of the cytochrome b–c_1 complex.

Previous studies with ubiquinone-deficient submitochondrial particles have led to the conclusion that ubiquinone (Q), in addition to acting as an oxidation-reduction catalyst, exerts a regulatory effect on the interaction of succinate dehydrogenase and the cytochrome b–c_1 complex of the respiratory chain[1-4]. This conclusion was based primarily on the observations that in Q-deficient particles, antimycin induces a reduction of cytochrome b by succinate[1], with antimycin-titration kinetics different from those found in Q-containing particles[2]. Extraction of Q was also shown to modify the kinetics of succinate dehydrogenase[3] and its interaction with cytochrome b in the presence of thenoyltrifluoroacetone[4].

Data reported in the present paper provide further support for the concept of a regulatory function of Q, based on measurements of the initial rates of antimycin binding

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to Q-containing and Q-deficient submitochondrial particles. It is shown that extraction of Q accelerates in a reversible manner the binding of antimycin to the particles, and that this effect probably reflects a Q-dependent regulation of the state of protonation of the cytochrome b–c_1 complex. Some of these data were briefly presented orally at the 7th FEBS Meeting[5].

The experiments were performed with submitochondrial particles derived from beef-heart mitochondria by sonication in the presence of EDTA[6]. The particles were lyophilized, and extraction and reincorporation of Q were carried out as described previously[1]. Cytochrome b reduction was measured with an Aminco-Chance dual-wavelength spectrophotometer at 565 *minus* 575 nm.

The rates of antimycin binding were estimated from the rates of cytochrome b reduction by succinate in the presence of bovine serum albumin. In the absence of bovine serum albumin (Fig. 1, A and B) addition of small amounts of antimycin to a buffered (pH 7.9) suspension of submitochondrial particles caused a rapid increase in the low level of cytochrome b reduction by succinate both in Q-containing and Q-deficient particles, in accordance with previous findings[1]. When bovine serum albumin (5 mg/ml) was included in the suspension medium (Fig. 1, C and D), addition of antimycin resulted in a slow reduction of cytochrome b. Moreover, the initial rate of cytochrome b reduction following the addition of antimycin was markedly lower in the Q-containing than the Q-deficient particles. When bovine serum albumin was added to the particle suspension together with antimycin, 3 min prior to the addition of succinate (Fig. 1, E and F), the latter caused a rapid and extensive reduction of cytochrome b, similar to that found in the absence of bovine serum albumin (*cf.* Fig. 1, A and B).

On the basis of earlier observations of Reporter[7], Berden and Slater[8] and Berden[9], these findings may be interpreted to indicate that antimycin, when added to a particle suspension containing bovine serum albumin, is first rapidly bound to bovine serum albumin, but is subsequently transferred to the particles, which bind antimycin with a higher association constant than does bovine serum albumin[9]. Once bound to the particles, it inhibits electron transfer between cytochromes b and c_1, thereby causing an increase in the level of reduced cytochrome b. Thus, the rate of cytochrome b reduction under these conditions is a measure of the rate of binding of antimycin to its inhibitory site in the cytochrome b–c_1 complex. The slow rates of cytochrome b reduction in the presence of bovine serum albumin were not due to an inhibitory effect of bovine serum albumin *per se* on electron transfer from succinate to cytochrome b, as shown by the rapid reduction of cytochrome b observed when antimycin was allowed to equilibrate between bovine serum albumin and the particles before the addition of succinate (Fig. 1, E and F). These latter data also show that the cytochrome b–c_1 complex binds antimycin when cytochrome b is oxidized, in agreement with earlier reports[8–10]. It may thus be concluded from the data in Fig. 1 (C and D) that extraction of Q from the particles enhances the rate of antimycin binding to the cytochrome b–c_1 complex.

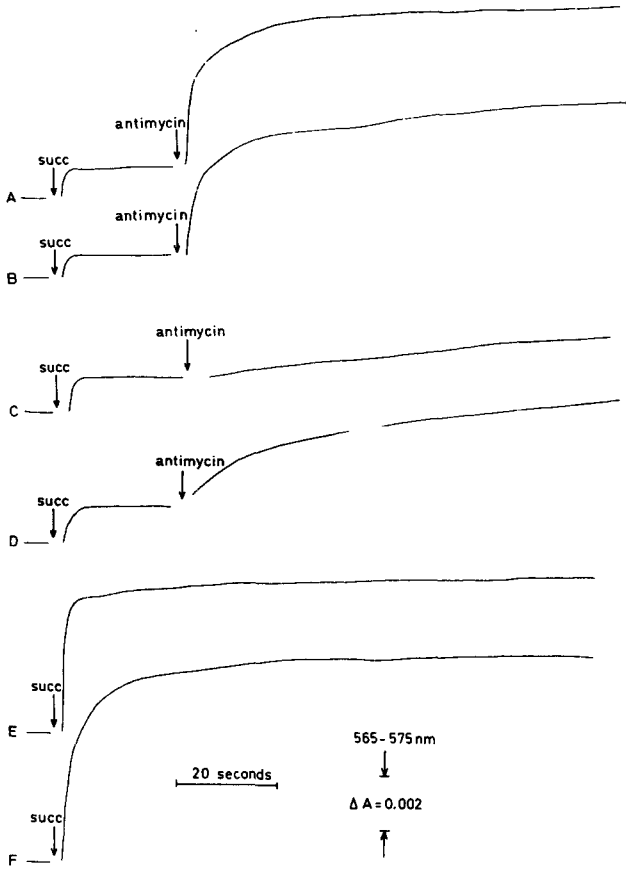

Fig. 1. Effect of antimycin on cytochrome *b* reduction in the absence and presence of bovine serum albumin in Q-containing and Q-deficient submitochondrial particles. The reaction mixture contained 1.8 mg particle protein, 0.66 M sucrose, 50 mM Tris-acetate, and 1 mM histidine (pH 7.9). When indicated, 5 mM succinate and 0.32 μg antimycin were added. In A and B, no bovine serum albumin was present. In C, D, E and F, the reaction mixture contained 5 mg/ml bovine serum albumin. In E and F, 0.32 μg antimycin was also added to the reaction mixture, 3 min prior to the additon of succinate. Final volume, 3 ml; temperature, 30 °C. Traces A, C, E: Q-containing particles; Traces B,D,F: Q-deficient particles.

This effect is further illustrated by the data in Fig. 2 which show the influence of extraction and reincorporation of Q on the rate of antimycin binding, measured as above in the presence of bovine serum albumin, at varying concentrations of antimycin and at two different pH values, 7.9 and 7.4. At all levels of antimycin and both pH values tested, the rates were higher in Q-deficient than in Q-containing particles, and reincorporation of Q into the particles restored the low rates of antimycin binding. In both the Q-containing and Q-deficient particles, the rate of antimycin binding increased with decreasing pH; his effect was not due to an altered binding of antimycin to bovine serum albumin, since the dissociation constant for the antimycin-bovine serum

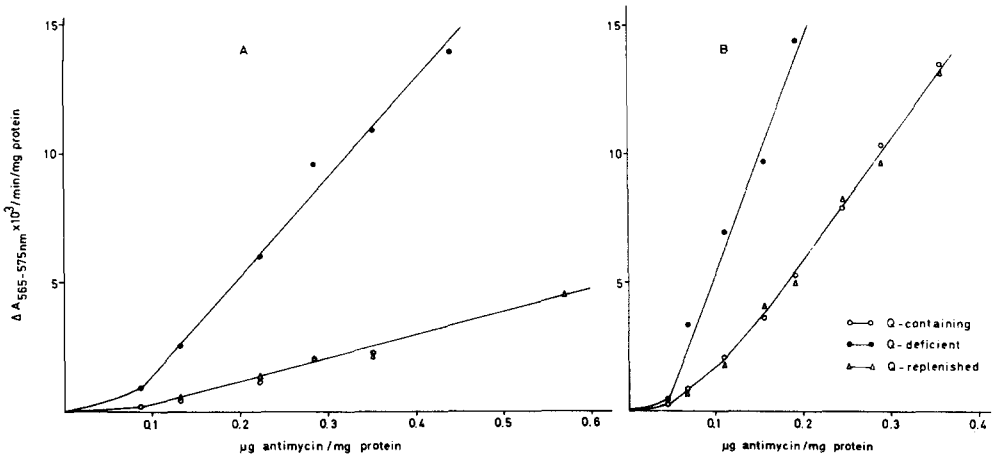

Fig. 2 Influence of varying antimycin concentrations on the rate of cytochrome *b* reduction in Q-containing, Q-deficient and Q-replenished submitochondrial particles in the presence of bovine serum albumin. The initial rates of cytochrome *b* reduction were measured following the addition of antimycin. The pH of the reaction mixture was 7.9 in A, and 7.4 in B. Other conditions were as in Fig. 1, C and D.

albumin complex, as determined fluorimetrically, was unchanged in the pH range 7.2–7.9, $K_D = 1.2 \cdot 10^{-7}$ M, a value identical with that reported by Berden[9]. Below a certain level of antimycin, approx. 0.05 μg/mg particle protein, there occurred little or no reduction of cytochrome *b*, possibly because of a very firm binding of this amount of antimycin to sites on either bovine serum albumin or the particles, unrelated to its site of inhibition of electron transport. Above this level, the rate of antimycin binding to its inhibitory site was linear with the antimycin concentration in the Q-deficient particles at both pH 7.9 and 7.4. In Q-containing particles, the rate was likewise linear at pH 7.9, but increased progressively with antimycin concentration at pH 7.4.

Fig. 3 shows the effect of varying pH on the rate of antimycin binding to Q-containing and Q-deficient particles. Other conditions were similar to those used in the

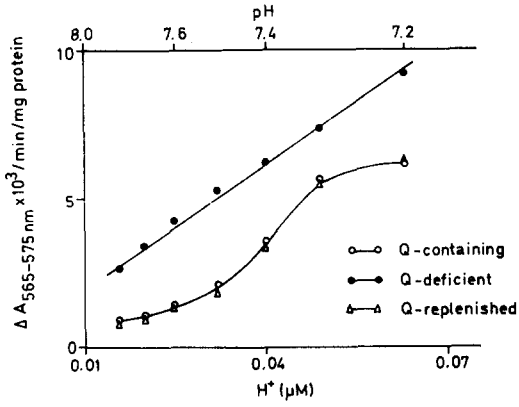

Fig. 3. Effect of pH on the rate of cytochrome *b* reduction in Q-containing, Q-deficient and Q-replenished submitochondrial particles in the presence of bovine serum albumin. Conditions were as in Fig. 2, except that the pH of the reaction mixture was varied as indicated. 0.2 μg antimycin was added.

experiment in Fig. 1 (C and D). It may be seen that, in the Q-deficient particles the rate of antimycin binding was linear with H^+ concentration, whereas in the Q-containing particles it was sigmoidal, with an inflection point at pH 7.4. Evidently, Q alters the kinetics of a reaction by which the pH of the medium influences the rate of antimycin binding to the particles.

There is now evidence[10] that antimycin binds to a protonated form of the cytochrome b-c_1 complex, in which cytochrome b is present in the oxidized form. Our data are consistent with this conclusion, and indicate that Q may regulate the state of protonation of the cytochrome b-c_1 complex. In the absence of Q, protonation (Fig. 3) and antimycin binding (Fig. 2) seem to proceed according to first-order kinetics. Q alters the protonation kinetics and lowers the rate of antimycin binding. Q may exert such an effect in several ways. Being a lipid-soluble hydrogen carrier, it may influence the proton activity in the surrounding of the cytochrome b-c_1 complex. It may also exert a direct, conformational effect on the cytochrome b-c_1 complex, resulting in an altered pK_a. Indeed, the two types of effect may occur simultaneously. In any case, it appears difficult to explain the present findings in terms of the simple model suggested by Kröger and Klingenberg[11] to account for the influence of Q on the antimycin-titration kinetics of cytochrome b reduction[2].

Recently several lines of evidence have been put forward[12-17] suggesting certain similarities between changes in the cytochrome b-c_1 complex taking place upon antimycin binding and during energization of the respiratory chain at Coupling Site II. There are also indications[18,19] that the energy-linked changes are related to changes in proton activity. It appears, therefore, that the present data concerning the influence of Q on the kinetics of antimycin binding to the cytochrome b-c_1 complex may be relevant to a regulatory function of Q in the mechanism of energy coupling at Site II.

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