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THE REDUCTION OF COENZYME Q_{10} BY AN ANTICOAGULANT SENSITIVE ENZYME FROM DOG LIVER*

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Abstract—An enzyme obtained from dog liver, was found to catalyse the reduction of coenzyme Q_{10} by DPNH and TPNH. This activity was inhibited by various anticoagulants, such as, Dicumarol, Cumachlor, Tromexan, Warfarin, Phenindione, and Sintrom.

INTRODUCTION

AN ENZYME which catalyzes the reduction of menadione (2-methyl-1:4-naphthoquinone), and vitamin K_1 by the reduced pyridine nucleotides, is inhibited by various anticoagulants.[1, 2] The partially purified enzyme obtained from dog liver also catalyzes the reduction of benzoquinone; thus, it was of interest to test coenzyme Q_{10}, a naturally occuring benzoquinone derivative, as a possible electron acceptor. The purpose of the present report is to describe some of the properties of this enzyme, with coenzyme Q_{10} as an electron acceptor, and the effect of anticoagulants on this system.

METHODS

Since coenzyme Q_{10} is not readily soluble in water, it is solubilized as follows: 4 mg of coenzyme Q_{10}† was suspended in 8·0 ml of 3·6 per cent BRIJ-35 (polyoxyethylene lauryl alcohol) and heated in a tightly stoppered 25-ml volumetric flask for about 16 hr at from 105 to 115 °C, providing a clear yellow solution which was filtered if necessary. Estimation of the amount of coenzyme Q_{10} solubilized made use of the extinction coefficient reported by Wolf *et al.*[3] In the oxidized and reduced form these preparations exhibited essentially the same spectrum as coenzyme Q_{10} in ethanol.[4] When chromatographed, the solubilized coenzyme migrated in the same manner as reference samples dissolved in ethanol.[5] These findings indicate that the coenzyme was not destroyed by the solubilization procedure and such preparations appeared to be suitable for the present studies. Samples containing BRIJ-35 without coenzyme Q_{10}, for use as controls and in check cells, were treated in the same manner.

The preparation of the purified enzyme and the general procedures used are those previously described.[2] The activity of the heat-labile enzyme was determined by observing the rate of oxidation of reduced diphosphopyridine nucleotide (DPNH) at 340 mμ. The standard reaction mixture used in the present studies contained 50 μmoles of

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imidazole buffer of pH 7·4, 0·1 μmole of DPNH, 0·002 μmole of flavin adenine dinucleotide (FAD), 0·1 μmole of coenzyme Q_{10}, water and enzyme to a final volume of 1·0 ml. The enzyme preparations used in the present studies, which had been purified from 100- to 300-fold, had the absorbance of a typical protein in the ultraviolet region of the spectrum but no measurable absorbance in the visible region. The activity of the purified enzyme without added flavin was from 10 to 20 per cent of that observed with saturating amounts of FAD or flavin mononucleotide. During purification of the enzyme, the ratio of activity with vitamin K_1 to coenzyme Q_{10} did not change appreciably with the different enzyme fractions tested.

RESULTS AND DISCUSSION

The activity of the purified enzyme was inhibited by various anticoagulants when coenzyme Q_{10} served as an electron acceptor; of the compounds tested, dicumarol was the most potent, inhibiting about 59 per cent at 10^{-5} M. Other anticoagulants tested, at a concentration of 10^{-4} M, caused the following percentage inhibitions: Cumachlor, 50; Tromexan, 45; Warfarin, 45; Phenindione, 25; Sintrom, 15. At higher concentrations of anticoagulant the inhibition was greater. Cations such as Mg^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Zn^{2+} and Ni^{2+}, at final concentrations of 10^{-4} M, did not appear to affect the rate of the enzymatic reaction when added to the standard assay system, while Cu^{2+}, at the same concentration, increased the rate several-fold.

In order to follow the reduction of coenzyme Q_{10} during the oxidation of DPNH, the reaction was also followed at 275 mμ, at which wavelength the reduced form of coenzyme Q_{10} has a lower absorbancy than the oxidized form.[4] Under the standard experimental conditions the reduced form was oxidized in the presence of air, therefore, the reduction was followed under anaerobic conditions using a modified system. As shown in Fig. 1, the oxidation of DPNH was accompanied by a decrease in absorption at 275 mμ, indicating reduction of coenzyme Q_{10}. On the basis of the change in absorbancy at 340 mμ, when the reaction reached a steady level, 0·12 μmole of DPNH, of the 0·55 μmole present, was oxidized by 0·14 μmole of coenzyme Q_{10}. This corresponds to about 1:1 relationship between the amount of coenzyme Q_{10} present and the amount of DPNH oxidized. The fact that benzoquinone also served as electron acceptor,[2] and that the absorbancy at 275 mμ decreased in an essentially stoichiometric manner, suggests that coenzyme Q_{10} might be reduced to the corresponding hydroquinone. Upon introduction of air to the system, the reduced coenzyme Q_{10} was reoxidized, as indicated by an increase in absorption at 275 mμ. The remaining DPNH was also oxidized, presumably as a result of a regeneration of oxidized coenzyme Q_{10}, which again acted as an electron acceptor. The rate of reoxidation of reduced coenzyme Q_{10} varied with different enzyme preparations; but whether this oxidation was catalyzed by trace metals or by coenzyme Q_{10} oxidase[6] in the enzyme preparation has not been determined. Under standard conditions, the rate of DPNH oxidation was greater than that illustrated in Fig. 1, because a modification of the standard assay was required in order that the spectral changes, occuring at 275 mμ, could be followed. DPN was identified as a product of the reaction by enzymatic regeneration with alcohol dehydrogenase. Chemically or enzymically reduced triphosphopyridine nucleotide also served as an electron donor in this system and, in this case, the product was identified by enzymatic procedures as oxidized triphosphopyridine nucleotide.

Although a number of quinones can serve as electron acceptors in this system, the identity of the natural electron acceptor is not certain and studies are in progress in order to clarify this point. However, it is of interest that this reduction of coenzyme Q_{10} was inhibited by various anticoagulants. In other studies the same anticoagulants were found to depress the respiration of rat liver slices,[7] and the relative order of

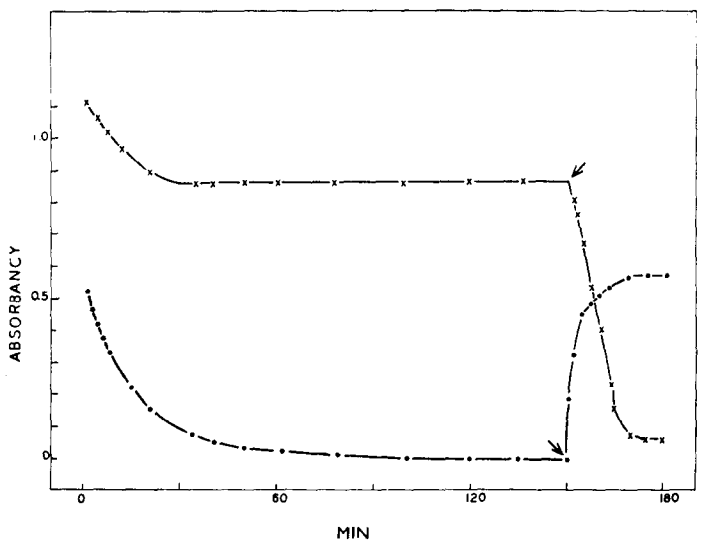

FIG. 1. Oxidation of DPNH and reduction of coenzyme Q_{10}. The reaction mixture was set up in anaerobic silica cells of the Thunberg type with 150 μmoles of imidazole buffer of pH 7·4, 0·005 μmole of FAD, 0·55 μmole of DPNH, and 0·14 μmole of coenzyme Q_{10} in a volume of 2·8 ml in the reaction chamber and 100 μg of enzyme in the side arm. After the cell was evacuated the reaction was started by tipping in the enzyme and the oxidation of DPNH was followed at 340 mμ while the reduction of coenzyme Q_{10} was followed at 275 mμ. In order to follow the reaction at 340 mμ and at 275 mμ, separate check cells were required for each wavelength. The check cell used at 340 mμ contained all components except DPNH and that used at 275 mμ contained all components except coenzyme Q_{10}. Air was introduced to the system at the time indicated by the arrows. ×——× 340 mμ; ●——● 275 mμ.

potency on the slices paralleled their inhibitory action on the purified enzyme. The present evidence does not permit any conclusion as to a causal relationship between the effects of these agents on the purified enzyme and their anticoagulant action.

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