

The Probable Site of Action of Thenoyltrifluoroacetone on the Respiratory Chain

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1. It is shown that the electron-transfer inhibitor thenoyltrifluoroacetone abolishes a respiratory-chain electron-paramagnetic-resonance absorbance due to spin-spin interactions of ubisemiquinones at concentrations similar to those required for inhibition of succinate oxidation. 2. A specific site of interaction of thenoyltrifluoroacetone with the respiratory chain is proposed to be on the ubisemiquinone with which succinate dehydrogenase reacts. 3. Our results further demonstrate the close association of the HiPIP (high-potential iron-sulphur) centre of succinate dehydrogenase with ubisemiquinone.

2-Thenoyltrifluoroacetone is a potential metal-chelating agent which was shown to inhibit succinate oxidation at a concentration one to two orders of magnitude lower than that required for similar inhibition of NADH oxidation (Tappell, 1960). It was subsequently reported that at micromolar concentrations it does not block electron transfer from succinate to artificial redox dyes, but inhibits almost completely succinate oxidase activity (King, 1966), and succinate-ubiquinone reductase or succinate-cytochrome *b* reductase activities (Baginsky & Hatefi, 1969). The precise site of action of this compound is as yet unknown, although kinetic studies indicate inhibition between the iron-sulphur centres of succinate dehydrogenase and the ubiquinone pool (Singer *et al.*, 1975). Nelson *et al.* (1971) have suggested that 2-thenoyltrifluoroacetone competes with ubiquinone, altering a component (which they suggest is non-haem iron) in the succinate dehydrogenase-cytochrome *b* region, thereby causing an inhibitory conformational change. This proposal was formulated after studies on the effect of 2-thenoyltrifluoroacetone on ubiquinone-depleted submitochondrial particles, where it did not inhibit the residual rates of succinate oxidation (Rossi *et al.*, 1970; Nelson *et al.*, 1971).

The mode of action of 2-thenoyltrifluoroacetone on succinate oxidation appears to be a perturbation of a complex interaction involving the HiPIP[†]-type iron-sulphur centre of succinate dehydrogenase

(centre S-3) and two or more ubisemiquinones. 2-Thenoyltrifluoroacetone abolishes the e.p.r. signal that arises from the interaction of the ubisemiquinones. In the native system at intermediate redox potentials, additional e.p.r. signals are seen on both sides of the HiPIP signal; these signals are attributed to the spin-spin interaction of ubisemiquinone (Ruzicka *et al.*, 1975; Ingledew & Ohnishi, 1975; Ingledew *et al.*, 1976). This series of e.p.r. signals ($g = 2.04, 2.01, 1.99$ and 1.96) and their properties are explained by the relaxation of one ubisemiquinone by centre S-3 and the splitting of this ubisemiquinone radical by a slower-relaxing radical of the same species (Ingledew *et al.*, 1976).

Methods

Pigeon heart mitochondria were prepared by the method of Chance & Hagihara (1963) and submitochondrial particles were obtained from them by use of a mechanical press (Lindsay *et al.*, 1972). Ox heart mitochondria were prepared by the method of Löw & Vallin (1963) and frozen before use. Redox potentials were clamped as described by Dutton (1971), by using phenazine ethosulphonate ($50 \mu\text{M}$), plus phenazine methosulphonate ($50 \mu\text{M}$) and ascorbate (2 mM) to maintain the potential during the course of the experiment. Ubiquinone was extracted from ox heart mitochondria by the method of Lester & Fleischer (1959). Protein concentrations were determined by the biuret method (Gornall *et al.*, 1949). Details of the e.p.r. facilities are given by Ingledew *et al.* (1976). Thenoyltrifluoroacetone was dissolved in dimethyl sulphoxide.

Results

The effect of thenoyltrifluoroacetone on the e.p.r. spectra caused by the interaction between adjacent

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† Abbreviations: HiPIP, high-potential iron-sulphur protein (in this paper this is the same as Centre S-3); e.p.r., electron paramagnetic resonance; ubisemiquinone denotes the free-radical form of ubiquinone (Q_{10}).

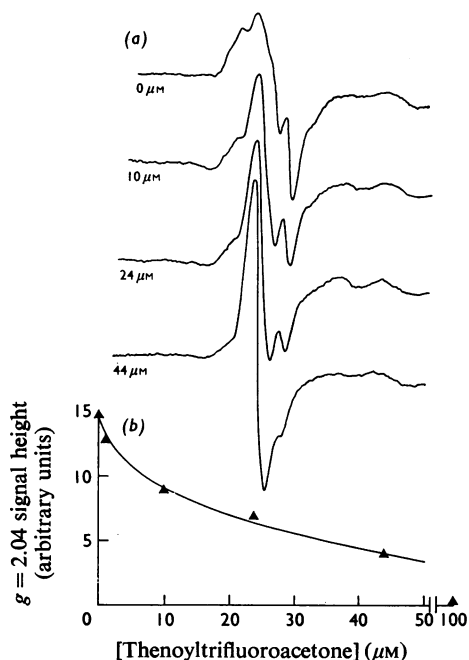


Fig. 1. Effect of thenoyltrifluoroacetone on the $g = 2.04$ and 1.99 signals and the HiPIP-centre absorption ($g = 2.02$). Samples of pigeon heart submitochondrial particles (10mg of protein/ml) were potentiometrically clamped at $+100\text{mV}$ ($\pm 5\text{mV}$) and titrated with thenoyltrifluoroacetone (see the Methods section). Samples were taken and freeze-clamped in e.p.r. tubes approx. 10s after addition of thenoyltrifluoroacetone. The e.p.r. spectra shown are those obtained in the presence of the indicated concentration of thenoyltrifluoroacetone (a). (b) shows the titration of the height of the $g = 2.04$ signal versus thenoyltrifluoroacetone concentration. The experiment was conducted in a medium of $50\text{mM-KCl}/50\text{mM-4-morpholine-ethanesulphonic acid}$ (pH 7.0). Thenoyltrifluoroacetone was dissolved in dimethyl sulphoxide. The e.p.r. conditions were: modulation amplitude 0.5mT , microwave frequency 9.12GHz , time constant 1s, scan rate 25mT/min .

ubisemiquinone radicals is shown in Fig. 1(a). In this experiment the system was redox-clamped at a potential of $+100 \pm 5\text{mV}$, the potential being monitored by a platinum electrode with a calomel reference electrode (corrected to hydrogen-electrode standard). This technique overcomes the objections to steady-state experiments, where loss of signal could mean either oxidation or reduction of the ubisemiquinones depending on whether they are on the substrate or oxygen side of a site of inhibition. The K_1 obtained for this titration of thenoyltrifluoro-

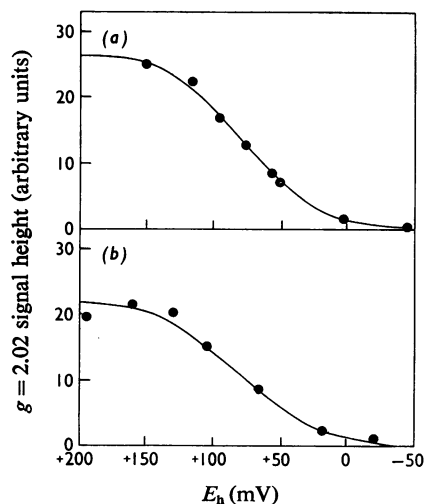


Fig. 2. Effect of (a) thenoyltrifluoroacetone addition and (b) ubiquinone extraction on the mid-point potential of centre S-3

(a) Pigeon heart mitochondria (10mg of protein/ml) were titrated potentiometrically in the presence of thenoyltrifluoroacetone ($100\mu\text{M}$). The results are plotted as E_h (electrode potential relative to a standard hydrogen electrode) versus centre S-3 signal height. (b) Ox heart mitochondria (10mg of protein/ml) depleted of ubiquinone were titrated potentiometrically. The HiPIP signal height is plotted against redox potential. The medium was $50\text{mM-KCl}/50\text{mM-morpholine-ethanesulphonic acid}$, pH 7.0; $50\mu\text{M-phenazine methosulphonate}$, $50\mu\text{M-phenazine ethosulphonate}$, $30\mu\text{M-pyocyanine}$, $40\mu\text{M-duroquinone}$ and $50\mu\text{M-diaminodurool}$, together, were used as redox mediators. The e.p.r. conditions were the same as for Fig. 1.

acetone against the $g = 2.04$ and 1.99 signals is approx. $18\mu\text{M}$ (Fig. 1b) and corresponds closely to that obtained for the succinate to oxygen reactions (Tappell, 1960). One point worthy of note in Fig. 1 is that as the $g = 2.04$ and 1.99 signals are abolished, the central HiPIP signal increases greatly. This implies that it was the HiPIP signal which was being split to give the absorptions at $g = 2.04$ and 1.99 and that addition of thenoyltrifluoroacetone abolishes its interaction with another species, allowing the absorption to be seen at its normal g value. However, it has been suggested that the electron spins seen at $g = 2.04$ and 1.99 come from ubisemiquinone radicals and not from the HiPIP centre (Ruzicka *et al.* 1975; Ingledew *et al.*, 1976).

An alternative explanation of the phenomenon observed in Fig. 1(a) is that on blocking with thenoyltrifluoroacetone the complex interaction between the

HiPIP centre and ubisemiquinones is perturbed, and the effect of this is to alter the midpoint potential of the HiPIP centre as well as to abolish the $g = 2.04$ and 1.99 signals. This suggestion was tested and the results are shown in Fig. 2(a). The effect of thenoyltrifluoroacetone on the midpoint potential at pH 7 of the HiPIP centre is to shift it to approx. +80 mV from a value of +120 to +140 mV obtained in the absence of thenoyltrifluoroacetone and in the presence of the interaction responsible for the $g = 2.04$ and 1.99 signals (Ingledeu & Ohnishi, 1975; Ingledeu *et al.*, 1976). Is this effect a specific one of thenoyltrifluoroacetone or is it an effect of the proximity of the ubisemiquinone interaction? Fig. 2(b) indicates that the presence of the ubisemiquinone interaction is a modulator of the midpoint potential of the HiPIP centre. In this latter case ubiquinone was removed from the system by organic-solvent extraction (Lester & Fleischer, 1959) and the midpoint potential of the HiPIP centre was found to be about +80 mV. These observations help to explain the diverse values reported for the midpoint potentials of the HiPIP centre (centre S-3) in various systems, because in some of these systems either ubiquinone was deficient or the interaction of ubisemiquinones responsible for the $g = 2.04$ and 1.99 signals was not present under the conditions of the redox titration.

Discussion

Thenoyltrifluoroacetone inhibits at the site involving mediation between the HiPIP centre and the ubiquinone pool (Singer *et al.*, 1975); however, the intermediate was unknown. We believe that we have demonstrated that this intermediate is bound ubisemiquinone. The situation pertaining to the involvement of centre S-3 and the mode of action of thenoyltrifluoroacetone is less clear. Thenoyltrifluoroacetone is a compound which readily forms metal co-ordination complexes with a variety of ions; it has therefore been concluded that the site of action of thenoyltrifluoroacetone must be an iron-sulphur protein (Nelson *et al.*, 1971). It is, however, an established observation that except under conditions of strong perturbation, iron chelators have no effect on iron-sulphur proteins (Malkin, 1973). Thenoyltrifluoroacetone could therefore act by associating with groups on the ubisemiquinone or by introducing into a hydrophobic milieu a co-ordinated proton. This latter possibility has been suggested for the action of the metal chelator *o*-phenanthroline on the primary acceptor in bacterial photosynthesis (Prince & Dutton, 1976), and its effect would be to raise the midpoint potential of the ubisemiquinone system.

The midpoint potential of centre S-3 has been reported to be approx. +65 mV in succinate-cytochrome *c* reductase (Ohnishi *et al.*, 1976), but approx.

+120 mV in ox heart submitochondrial particles and approx. +140 mV in those from pigeon heart (Ingledeu *et al.*, 1976). Generally, under conditions in which ubisemiquinone interaction is observable ($g = 2.04$ and 1.99) the midpoint potential of centre S-3 is greater than +100 mV, and in the absence of the interaction it is less than +100 mV (at pH 7.0). Thus the midpoint potential of centre S-3 is modified by an interaction in which it is involved with ubisemiquinone molecules. The increase in size of the HiPIP signal in Fig. 1 when thenoyltrifluoroacetone is added could then be explained by a change in midpoint potential resulting in further oxidation of the centre. An important notion derived from the observation of the spin-spin interaction of the ubisemiquinone radicals is that at least one of the ubisemiquinone radicals must arise from a protein-bound ubiquinone molecule, otherwise dismutation of the ubisemiquinone will readily occur, as discussed by Kröger & Klingenberg (1973). It is suggested that the primary site of action of thenoyltrifluoroacetone on succinate oxidation is on a bound ubisemiquinone which is involved in the transfer of reducing equivalents between the S-3 centre (HiPIP centre) of succinate dehydrogenase and the ubiquinone pool.

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