INTERACTION OF TELLURITE WITH THE RESPIRATORY CHAIN IN RAT LIVER MITOCHONDRIA

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1. Introduction

It has been previously reported that the sulfhydryl reagent, tellurite, at concentrations in the range 0.2 to 1 mM, inhibits the oxidation of the NAD-linked substrates L-glutamate, L-malate, α-ketoglutarate, pyruvate, DL-isocitrate, and DL-β-hydroxybutyrate in rat liver mitochondria without affecting the oxidation of succinate, α-glycerolphosphate, or ascorbate plus TMPD* [1, 2]. There are evidently a number of sites at which tellurite can act. One is the mitochondrial β-hydroxybutyrate dehydrogenase after its detachment from the membrane by sonication, for which evidence has been presented [2]. Another is quite probably the lipoate dehydrogenase component of the α-keto acid dehydrogenases, since the inhibition of tellurite is readily reversed by dithiothreitol but not by mercaptoethanol [1]. Further, there is also evidence that, in this concentration range, tellurite can cause the oxidation of endogenous pyridine nucleotide in liver mitochondria [3]. This implies that tellurite may bind directly to, or react with, one of the respiratory chain carriers associated with the mitochondrial NADH dehydrogenase. The results reported here strongly imply that tellurite binds or reacts in such a way as to make this region of the respiratory chain accessible to the electron acceptor ferricyanide, which is impermeant to the inner mitochondrial membrane [4].

* *Abbreviations*:
TMPD: *N,N'*-Tetramethyl-*p*-phenylenediamine; ROT: Rotenone; BOH: β-Hydroxybutyrate; TELL: Tellurite; ANT: Antimycin A.

2. Results

The reduction of ferricyanide by β-hydroxybutyrate plus NAD^+ in azide-inhibited rat liver mitochondria is shown in fig. 1A. The concentration of ferricyanide in these experiments is 0.5 mM, which is sufficient for maximal rates of reduction via the cytochrome *c* pathway [4, 5], but still low enough to minimise reduction directly via the mitochondrial dehydrogenases. Accordingly, the rate of ferricyanide reduction by β-hydroxybutyrate plus NAD^+ (fig. 1A) is decreased 6-fold by the inhibitor rotenone (fig. 1B). If 0.25 mM tellurite is added with the rotenone (fig. 1C), however, the decrease in the rate of ferricyanide reduction is only 3.5-fold just after NAD^+ addition; the rate of ferricyanide reduction then increases with time until it attains 60% of the rate observed in the absence of rotenone. If the concentration of tellurite is increased to 2 mM, the lag in attaining the final rate of ferricyanide reduction seen in fig. 1C is abolished and the final rate is significantly increased. The same patterns of reduction are also observed in the presence of antimycin A.

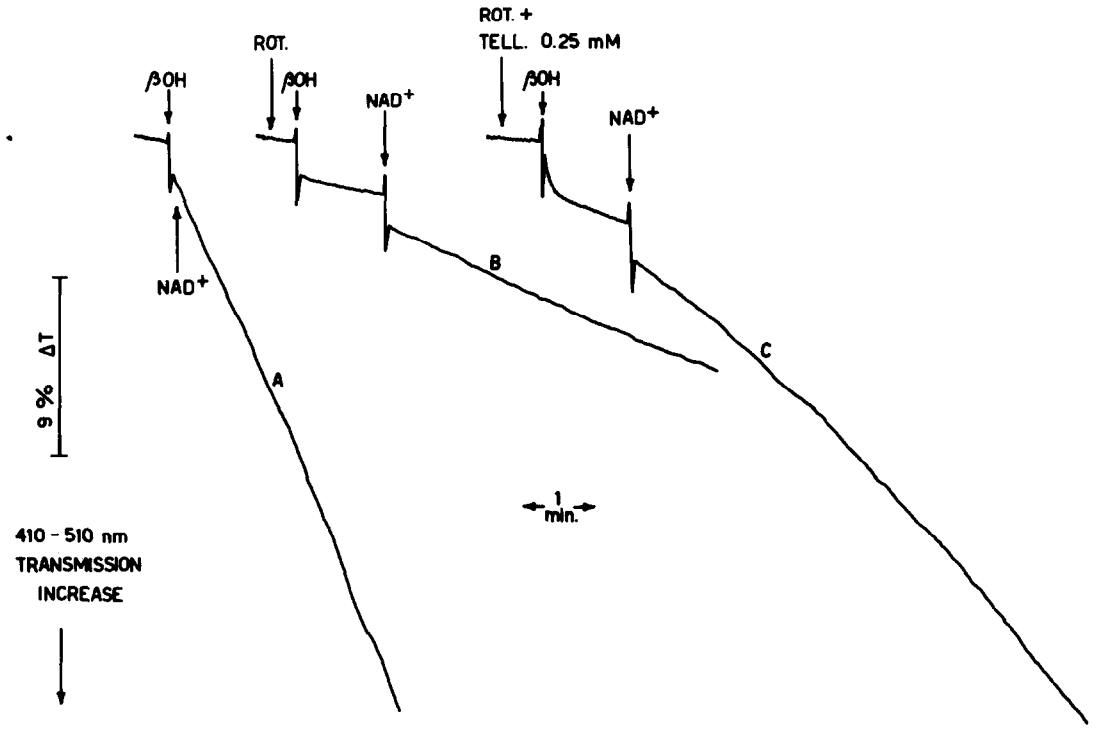

Fig. 1. Ferricyanide reduction induced by tellurite in the presence of β-hydroxybutyrate. 1.5 mg rat liver mitochondrial protein was added in 2 ml medium containing 9.75 mM K_2HPO_4, 2.25 mM KH_2PO_4, 9 mM NaF, 19.5 mM NaCl, 43.4 mM KCl, 4.5 mM $MgCl_2$, 0.25 mM azide, 0.5 mM ferricyanide, pH 7.4. *Trace B*: 10 μM Rotenone (ROT) present; *Trace C*: 10 μM rotenone, 0.25 mM tellurite (TELL) present. Additions: 5 mM β-hydroxybutyrate (BOH), 1 mM NAD^+. The absorbance was recorded on a dual wavelength spectrophotometer built in the workshop of the Department of Biochemistry, Bristol Medical School.

The analagous experiments carried out with succinate as substrate are shown in fig. 2. The rate of ferricyanide reduction by succinate in azide-treated mitochondria is shown in fig. 2A; this rate is reduced 13-fold by addition of antimycin A (fig. 2B) as expected [4, 5]. In the presence of both 0.25 mM tellurite and antimycin A (fig. 2C) the inital rate of reduction is nearly twice that observed in the absence of tellurite and increases with time until it attains a rate about half that observed with the control in the absence of antimycin A. If rotenone is also present with antimycin A and 0.25 mM tellurite, as in the experiment of fig. 2D, the rate of ferricyanide reduction is the same as the initial rate seen in fig. 2C, but the time-dependent increase of this rate is inhibited. If the experiment of fig. 2D is now modified by increasing the tellurite concentration to 2 mM with rotenone and antimycin A still present (fig. 2E), the rate of ferricyanide reduction by succinate rapidly attains a value equal to half that of the control (fig. 2A) and equal to that attained in the experiment of fig. 2C some 5 min after addition of succinate.

3. Discussion

These results strongly suggest that tellurite can interact directly with the respiratory chain very close to the rotenone binding site at a carrier which can be reduced both by endogenous NADH and by succinate in the absence of reversed electron transport. This interaction has the effect of modifying the carrier so that it can donate electrons to the membrane-impermeant acceptor, ferricyanide, by means of a reduction which is insensitive to inhibition by antimycin A and which proceeds at a rate about half of that observed with cytochrome *c* as electron donor. The most likely candidate for this carrier is the iron–sulfur protein com-

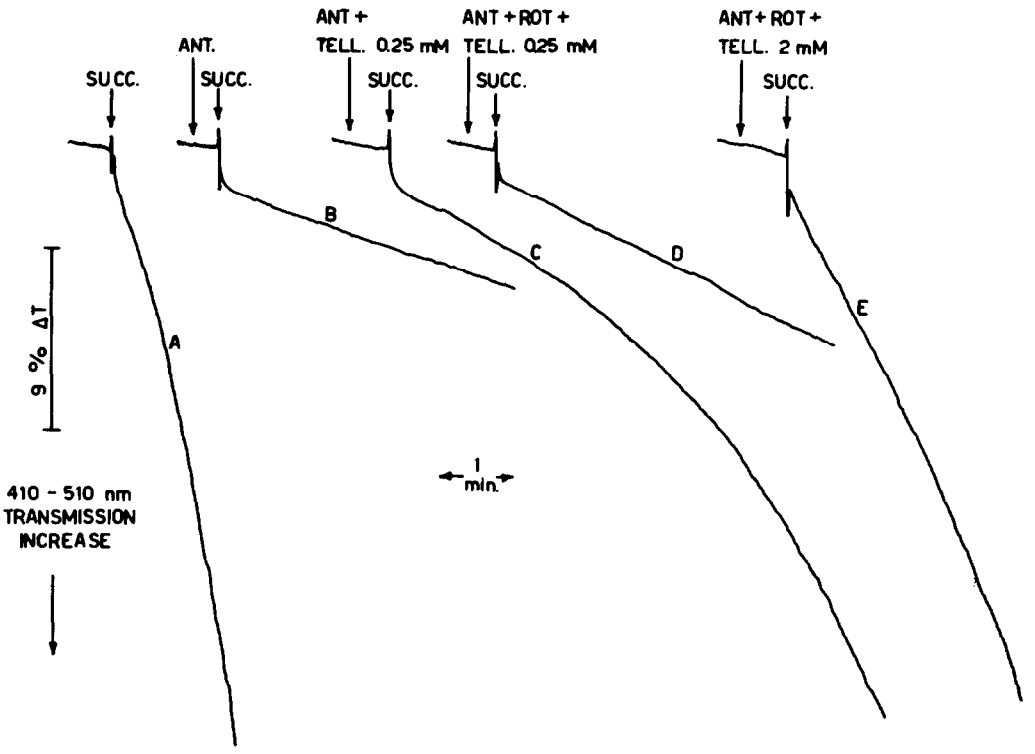

Fig. 2. Ferricyanide reduction induced by tellurite in the presence of succinate. Conditions as in fig. 1. *Trace B*: 3 μg Antimycin A (ANT) present; *Trace C*: 3 μg antimycin A, 10 μM rotenone, 0.25 mM tellurite present; *Trace D*: 3 μg antimycin A, 10 μM rotenone, 2 mM tellurite present. Additions: 5 mM succinate (SUCC).

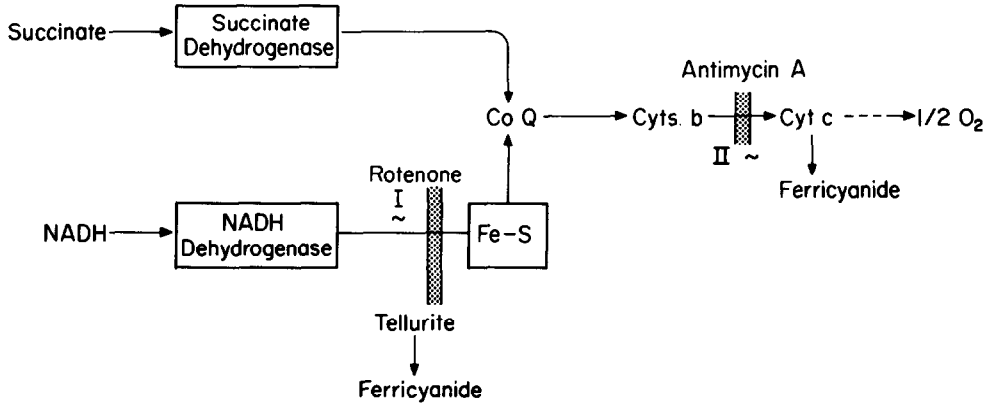

Fig. 3. Schematic representation of the site of tellurite interaction with the respiratory chain.

plex associated with the high potential side of the first energy conservation site [6]. This carrier is directly reducible by succinate via succinate dehydrogenase and ubiquinone, as well as being reducible by endogenous NADH. The various carriers comprising this site, including five iron–sulfur proteins distinguishable by ESR, are diagrammed and their role in energy conservation discussed by Chance [7] in a recent review.

In this discussion, we treat the carrier as a single iron–sulfur protein and visualize the respiratory chain at the energy conservation sites I and II in the simplified form shown in fig. 3. Both rotenone and tellurite are presumed to bind to the carrier at the point shown in the figure. In order to achieve full capacity for electron transport to ferricyanide, we postulate that the tellurite or, more probably, tellurite oligomer becomes reduced and incorporated into the (Fe–S) functional group of the iron–sulfur protein. The reaction is strongly dependent on tellurite concentration and is inhibited if rotenone is bound to the site. Thus, at low tellurite concentrations, the reduction of tellurite oligomer is slow; the full rate of ferricyanide reduction by succinate in the presence of antimycin A requires some minutes (fig. 2C) and is blocked by rotenone (fig. 2D). At the higher tellurite concentration (fig. 2E), the full rate of ferricyanide reduction is rapidly attained, and rotenone, being displaced by the higher tellurite concentration, has no effect. The incorporated tellurite will also be reduced if reducing equivalents are made available from NADH through the first coupling site and the same interaction of rotenone and tellurite should occur; this is precisely what is observed. Further, the reduction of the incorporated tellurite by this route should not be inhibited by antimycin A according to this mechanism, as is also observed experimentally.

This mechanism for tellurite interaction implies a competition between rotenone and tellurite for the same site, and suggests that tellurite may be a useful probe with which to characterize the rotenone binding site [8].

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