EFFECTS OF TRIPHENYLTIN COMPOUNDS ON THE ADENOSINE TRIPHOPHATASE ACTIVITY OF BEEF HEART SUBMITOCHONDRIAL PARTICLES* **

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SUMMARY

Low concentrations of several TPT compounds inhibit the ATPase activity of ETPH. Higher concentrations of these compounds cause a partial release of the inhibition and a concomitant loss of rutamycin sensitivity. Inhibition produced by low levels of TPT chloride is noncompetitive and the inhibitor constant is 2.8×10^{-8} M. Several sulfur compounds are capable of partially protecting the ATPase from inhibition by low levels of TPT chloride. Heat treatment destroys the sensitivity of the ATPase to inhibition by TPT chloride and rutamycin.

INTRODUCTION

Several trialkyltin and triphenyltin compounds have been shown to influence mitochondrial and submitochondrial preparations in a manner similar to oligomycin or rutamycin (1-5). Relative to oligomycin or rutamycin, these organotin compounds are of special interest as inhibitors because their chemistry is well understood.

This communication is a report of studies completed to explore the potential of TPT compounds as tools for investigation of the chemistry of the rutamycin sensitive ATPase activity associated with mitochondrial preparations.

MATERIALS AND METHODS

TPT derivatives were purchased from Ventron Alfa Inorganics, Eastman Kodak, and K & K Laboratories. Rutamycin was a gift from the Eli Lilly Co. All other materials were purchased from the usual sources.

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** Unusual abbreviations: Submitochondrial particles from beef heart mitochondria, ETPH; Triphenyltin, TPT.

The 8-hydroxyquinoline complex of TPT was prepared by the method of Roncucci, Faraglia, and Barbier (6). The dimethyl sulfoxide complex of TPT chloride was prepared by the method of Langer and Blut (7).

TPT compounds were added to the ATPase assay in aqueous ethanol solutions. In all cases, the final level of ethanol in the ATPase assay was 1% (v/v) or less.

ETPH were prepared from beef heart mitochondria by the method of Hansen and Smith (8) as modified by MacLennen, Smoly, and Tzagoloff (9). Protein was determined by the method of Gornal, Bardawill, and David (10). Inorganic phosphate was determined by the method of Martin and Doty (11).

The standard ATPase assay was conducted at 30°C and contained the following in a final volume of one ml: 50 μmoles of Tris-HCl, pH 8.5; 2 μmoles of $MgCl_2$; 50 μg of ETPH protein; and 10 μmoles of sodium ATP, pH 8.5. The reaction mixture, complete except for ATP, was preincubated for five min in the presence of the experimental compound or a control volume of ethanol. The reaction was then started by the addition of ATP and stopped after 10 min by adding the reagents for phosphate extraction to the assay.

RESULTS AND DISCUSSION

As illustrated in Figure 1, in the absence of rutamycin low concentrations of TPT chloride inhibit the ATPase activity of ETPH and relatively high concentrations produce a partial release of inhibition. The I_{50} for inhibition by low concentrations of TPT chloride ranged from 10^{-8} M to 5 X 10^{-8} M with an average of 2.7 X 10^{-8} M for three titrations. The maximum degree of inhibition obtainable with TPT chloride alone was never less than 85% and, for a given preparation of ETPH, was equal to the degree of inhibition observed with rutamycin alone.

The titration conducted in the presence of rutamycin demonstrates that the ATPase of ETPH exposed to high concentrations of TPT chloride is insensitive to rutamycin inhibition and that, in the presence of low levels of TPT chloride, the remaining ATPase activity is sensitive to rutamycin inhibition. These

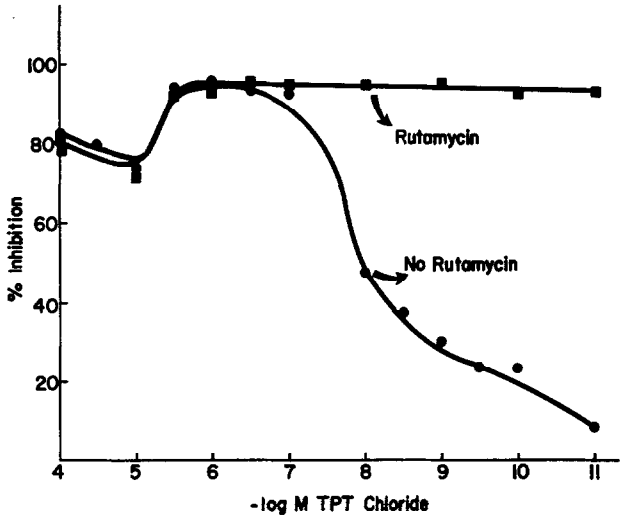

Figure 1. Titration of the ATPase activity of ETPH with TPT chloride in the absence and presence of rutamycin (1 µg/mg protein). The control ATPase rate was 1.25 µmole of Pi/min and mg of protein. The ATPase assay was as described in Materials and Methods.

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results suggest that low levels of TPT chloride and rutamycin interact with the same site(s) to inhibit the ATPase activity of ETPH.

In addition to TPT chloride, high concentrations of tri-n-butyltin chloride (4) and diethylstilbesterol (12) have been reported to destroy the rutamycin sensitivity of mitochondrial ATPase. The high concentrations of the organotins required for destruction of rutamycin sensitivity, relative to the levels producing inhibition of the ATPase, and the observation that diethylstilbesterol is not an inhibitor of the ATPase suggest that the interactions which result in inhibition are more specific than are the interactions resulting in a loss of rutamycin sensitivity. It is probable that high concentrations of many lipid soluble compounds are capable of destroying the sensitivity of the ATPase to rutamycin.

Contained in Table 1 are the I_{50} values obtained for the titrations of the ATPase of a single ETPH preparation with several TPT compounds. In all cases the complete titration curves were similar to those seen in Figure 1. The small range of I_{50} values seen in Table 1 suggests that the compounds

TABLE 1

CONCENTRATIONS OF TPT COMPOUNDS REQUIRED FOR

50% INHIBITION OF ETPH ATPase ACTIVITY

TPT Compound	I_{50} M X 10^8
Chloride	2.7*
Acetate	4.5
Hydroxide	3.0
8-Hydroxyquinoline complex	2.0
Chloride-DMSO complex	3.5

The ATPase assay was as described in the Materials and Methods section. * The I_{50} reported for TPT chloride is the average value for three ETPH preparations.

tested were all equally active or that the derivatives listed were all converted to a common, active material possibly TPT hydroxide or TPT oxide.

Figure 2 is a Dixon plot (13) for the hydrolysis of ATP by ETPH exposed to low levels of TPT chloride. The intersection of the extrapolated lines at the abscissa is indicative of noncompetitive inhibition, and the inhibitor constant, K_i, the negative of which is given by this intersection is 2.8 X 10^{-8}M. Large positive deviations from linearity occurred in the Dixon plots when concentrations of TPT chloride producing more than a 50% reduction of the ATPase rate were present. This suggests that there may be more than one inhibitory mode of interaction between TPT chloride and the ATPase.

Several compounds which would be expected to interact with TPT chloride under conditions of the standard assay were checked for their ability to alter the I_{50} of TPT chloride. Sodium fluoride (10^{-2} M), sodium EDTA (10^{-4} M), and 8-hydroxyquinoline (10^{-5} M) had no effect on the control ATPase rate or the I_{50} of TPT chloride. Imidazole, in the mM concentration range, produced a partial inhibition of control ATPase rate; this inhibition was approximately additive with that produced by I_{50} levels of TPT chloride or rutamycin. Two

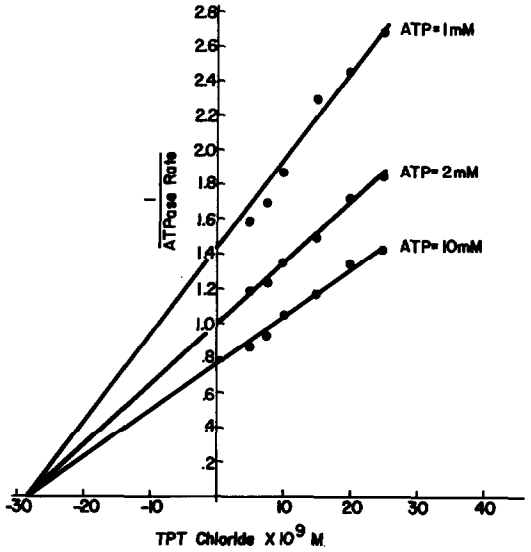

Figure 2. Dixon plot for the inhibition of the ATPase by TPT chloride. Except for the ATP concentration the ATPase assay was as described in Materials and Methods.

thiol compounds and sodium sulfide were found to be capable of partially protecting the ATPase from inhibitory concentrations of TPT chloride (see Table 2). Aldridge and Cremer (14, 15) concluded that trialkyltin compounds have little affinity for thiols; it is possible that trialkyltins and TPT compounds differ in this regard.

TABLE 2

EFFECT OF SULFUR COMPOUNDS ON THE INHIBITION OF ATPase BY TPT CHLORIDE

Additions	Percent Inhibition	
	Minus TPT	Plus TPT
None	0.00	48.0
Sodium sulfide	7.0	15.6
Dithiothreitol	6.2	23.0
2-Mercaptoethanol	5.6	38.0

Sulfur compounds were at 10^{-4} M. TPT chloride was at 5 X 10^{-8} M. In this experiment sulfur compound and TPT chloride were added before the ETPH. Similar results were obtained if the sulfur compound was added after the TPT and ETPH.

The sensitivity of the ATPase activity of mitochondrial preparations to inhibition by oligomycin or rutamycin can be destroyed by heat treatment (4). As seen in Table 3, heat treatment also destroys the sensitivity of the ATPase activity of ETPH to inhibition by TPT chloride.

The work of others and the data presented here strongly suggest that low levels of TPT compounds interact with the rutamycin site to inhibit mitochondrial ATPase activity. It is possible that high levels of these compounds interact with the oligomycin sensitivity conferring protein described by MacLennen and Tzagoloff (16); however, further experimentation would be required to confirm this.

TABLE 3

TPT CHLORIDE AND RUTAMYCIN SENSITIVITY OF THE ATPase OF CONTROL AND HEAT TREATED ETPH

	ATPase Rate μmoles Pi/min and mg protein	
Additions	Control ETPH	Heated ETPH
None	0.93	0.82
TPT Chloride	0.17	0.90
Rutamycin	0.11	0.74

ETPH at a protein concentration of 10 mg/ml were suspended in a medium which was 1 mM in sodium EDTA, 4 mM in sodium ATP and 10 mM in Tris-HCl, pH 7.5. Heat treated ETPH were prepared by heating 10 ml of this suspension in a tube for 4 min in a 65° water bath. The preparations were diluted to a protein concentration of 0.5 mg/ml with 10 mM Tris-HCl, pH 7.5 and assayed by the standard ATPase method. TPT chloride was 10^{-7} M and rutamycin was at 1 μg/mg of protein in the ATPase assay.

TPT tends to form complexes in which the tin is pentacoordinated; thus TPT could combine with the rutamycin site by associating with two ligands. This interaction could be further strengthened by hydrophobic interactions between the phenyl groups of TPT and the binding site. The formation of such a complex, strengthened by multiple interactions between TPT and the rutamycin site, would explain the small K_i observed.

The I_{50} values reported in Table 1 range from 0.54 to 0.90 nmol of TPT compound per mg of ETPH protein. These values are of the same order of magnitude as the level of cytochrome c_1 (0.27 nmol/mg protein) present in ETPH (17) and suggest a highly specific interaction between TPT and two or three sites per respiratory assembly.

In conclusion, the data presented here are consistent with the thesis that low levels of TPT interact in a highly specific manner to form a stable complex with the rutamycin site of ETPH. The interactions of TPT with intact mitochondria are currently being investigated.

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