TETRADIFON: AN OLIGOMYCIN-LIKE INHIBITOR OF ENERGY-LINKED ACTIVITIES OF RAT LIVER MITOCHONDRIA

Ernesto Bustamante and Peter L. Pedersen

Department of Physiological Chemistry
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

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SUMMARY

Tetradifon (p-chlorophenyl-2,4,5-trichlorophenyl sulfone) at concentrations between 4.5 and 27.0 nmoles/mg mitochondrial protein provides half-maximal inhibition of the following energy-linked activities of rat liver mitochondria: ADP-stimulated respiration, DNP-stimulated ATPase activity, Mg^{++}-stimulated ATPase activity, and P_i-ATP exchange activity. Tetradifon has no effect on the activity of soluble ATPase purified from rat liver mitochondria. Respiration inhibited by tetradifon is restored upon addition of 2,4-dinitrophenol. It is concluded that tetradifon acts at or near the oligomycin sensitivity conferring complex located in the mitochondrial inner membrane.

The use of specific inhibitors of oxidative phosphorylation is an important tool for the elucidation of the molecular mechanisms involved in this complex mitochondrial process. Unfortunately, the chemical structures of most of these compounds are not completely known, a fact that restricts the understanding of the mechanisms of action of these inhibitors. Tetradifon is an acaricide (1) of known chemical structure (p-chlorophenyl-2,4,5-trichlorophenyl sulfone) that has been shown recently by Desaiah et al. (2) to be a rather potent inhibitor of Mg^{++}-stimulated ATPase activity in fish brain homogenates. In this communication we report the effects of tetradifon on energy-linked functions of rat liver mitochondria and on purified mitochondrial ATPase and conclude that the mode of action of tetradifon is very similar to that of oligomycin.

MATERIALS AND METHODS

Mitochondria were prepared according to the procedure of Schnaitman and Greenawalt (3) and resuspended in a medium containing 220 mM D-mannitol, 70 mM sucrose, 2.0 mM HEPES buffer, pH 7.4, and 0.5 mg/ml crystalline bovine serum albumin.

Respiration rates and acceptor control ratios were determined polarographically in a 2.87 ml system containing 0.33 M mannitol, 0.11 M sucrose, 3.7 mM HEPES buffer, 3.7 mM potassium phosphate buffer, 2.7 mM $MgCl_2$, and 8.0 mM succinate, at pH 7.4 and 20°. Mitochondria, ADP, tetradifon, and 2,4-dinitrophenol (DNP) were added at concentrations noted in Fig. 1.

DNP-stimulated ATPase activity was determined by measuring the release of inorganic phosphate (P_i). A 1.1 ml system was used containing 9.1 mM imidazole buffer, 0.045 M sucrose, 4.5 mM $MgCl_2$, 0.18 mM DNP, 7.3 mM ATP, and 2.0 mg of intact mitochondria, at pH 6.9 and 30°. Tetradifon was added at concentrations indicated in Fig. 2B.

Mg^{++}-stimulated ATPase activity was determined by following the release of P_i in a 1.1 ml system. The reaction medium contained 9.1 mM imidazole buffer, 0.045 M sucrose, 4.5 mM $MgCl_2$, 7.3 mM ATP, and 1.9 mg of sonicated mitochondria, at pH 6.9 and 30°. Tetradifon was added at concentrations noted in Fig. 2C.

P_i-ATP exchange activity was measured by following the incorporation of $^{32}P_i$ into ATP in a 0.6 ml system. The assay medium contained 8.3 mM imidazole buffer, 0.042 M sucrose, 4.2 mM $MgCl_2$, 4.0 mM sodium phosphate buffer, 4.0 mM ADP, 10.0 mM ATP, 0.75 μC $^{32}P_i$, and 1.0 mg of intact mitochondria, at pH 6.9 and 30°. Tetradifon was added at concentrations noted in Fig. 2D.

Purified ATPase activity was measured spectrophotometrically in a 0.97 ml system by following the oxidation of NADH at 340 nm essentially as described by Pullman et al. (4). The medium contained 0.26 M sucrose, 0.058 M Tris, 4.7 mM KCN, 4.2 mM $MgCl_2$, 4.1 mM ATP, 0.62 mM phosphoenolpyruvate, 0.39 mM NADH, 85 units lactate dehydrogenase, 0.15 mg crystalline bovine serum albumin, 4.5 units pyruvate kinase, and 2.0 μg purified mitochondrial ATPase, at pH 7.4 and 20°. Tetradifon was added at concentrations noted in Fig. 2E.

The solvent used for DNP was ethanol. The solvent for tetradifon

was chosen to be either ethanol or methanol, so that the amounts of solvent used caused no detectable effect on the systems studied. Ethanol was used in the polarographic assays and in the assay systems for DNP- and Mg^{++}-stimulated ATPase activities. Methanol was used in the spectrophotometric assays of purified ATPase, and in the measurements of P_i-ATP exchange activities.

Mitochondrial ATPase was purified according to the method of Catterall and Pedersen (5). Inorganic phosphate determinations were carried out according to the method of Gomori (6). Protein concentrations were determined by the biuret method (7). Lactate dehydrogenase and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Pyruvate kinase was obtained from Calbiochem (San Diego, Calif.). $^{32}P_i$ was purchased from New England Nuclear Corp. (Boston, Mass.). Tetradifon was obtained from Niagara Chemical Division (Middleport, N.Y.). All other reagents used were analytical grade or of the highest purity available.

RESULTS AND DISCUSSION

The polarographic traces presented in Fig. 1 show that tetradifon

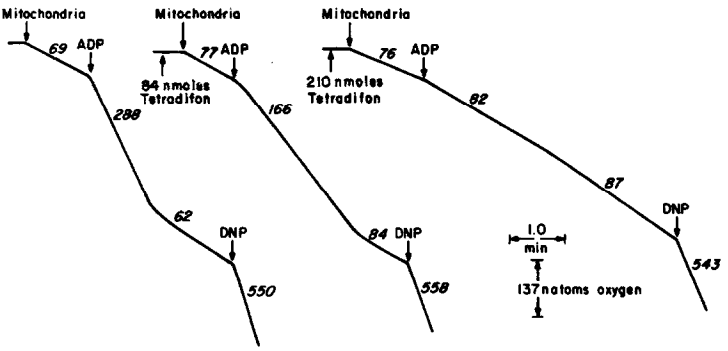


Fig. 1. Typical polarographic traces showing the effect of tetradifon on mitochondrial respiration stimulated by ADP. Experimental conditions are described in Materials and Methods. Where indicated, 3.0 mg mitochondria, 400 nmoles ADP and 500 nmoles DNP were added. Rates of respiration shown on the traces are expressed as natoms oxygen consumed/min.

markedly inhibits ADP-stimulated respiration (State 3). Similar to the inhibition of respiration by oligomycin (6), inhibition by tetradifon can be restored by addition of low concentrations of DNP (174 μM). Results presented in Fig. 2A show that half-maximal inhibition of State 3 respiration is obtained with about 21 nmoles tetradifon/mg mitochondrial protein.

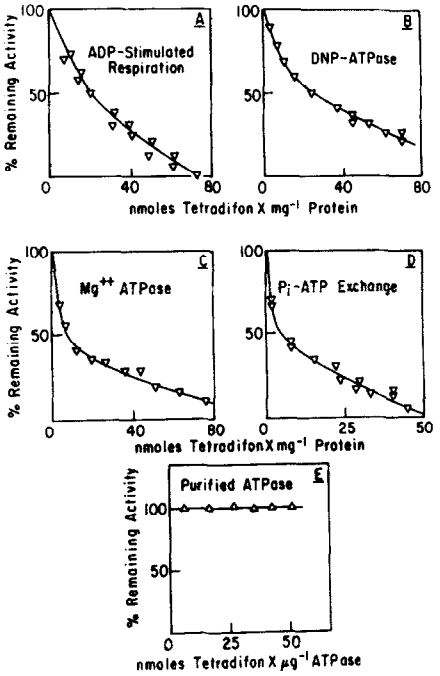


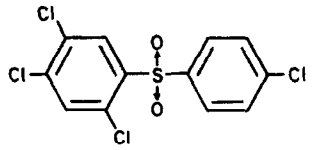
Fig. 2. Effect of tetradifon on energy-linked activities of rat liver mitochondria and on purified mitochondrial ATPase activity. A. ADP-stimulated respiration (100%= 95 natoms oxygen consumed/min/mg protein). B. DNP-stimulated ATPase activity of intact mitochondria (100%= 275 nmoles ATP hydrolyzed/min/mg protein). C. Mg^{++}-stimulated ATPase activity of sonicated mitochondria (100%= 392 nmoles ATP hydrolyzed/min/mg protein). D. P_i-ATP exchange activity of intact mitochondria (100%= 63 nmoles P_i exchanged/min/mg protein). E. Mg^{++}-stimulated ATPase activity of purified ATPase (100%= 19.2 μmoles ATP hydrolyzed/min/mg protein). Experimental conditions are described in Materials and Methods.

Since oligomycin is known to markedly inhibit other energy-linked activities of mitochondria including P_i-ATP exchange, Mg^{++}-stimulated ATPase activity, and DNP-stimulated ATPase activity (6, 7), it became of interest to

test the effectiveness of tetradifon as an inhibitor of these activities also. Results presented in Figs. 2B-2D clearly show that tetradifon is an effective inhibitor of all of these energy-linked activities. Half-maximal inhibition is obtained with 4.5, 7.5 and 27.0 nmoles tetradifon/mg mitochondrial protein in the case of P_i-ATP exchange activity, Mg^{++}-stimulated ATPase activity, and DNP-stimulated ATPase activity, respectively. The reason why half-maximal inhibition values obtained with tetradifon differ somewhat for various energy-linked activities is not fully understood at the present time but may well be related to differences in the permeability of mitochondria to the inhibitor under the variety of assay conditions employed.

As noted in Fig. 2E, tetradifon is without effect on soluble, homogeneous ATPase of rat liver mitochondria. Oligomycin is also without effect on this ATPase preparation (5) and on the soluble ATPase of bovine heart mitochondria (4).

It is of interest to compare oligomycin and tetradifon with respect to their relative effectiveness in inhibiting energy-linked activities of mitochondria, and with respect to their chemical structures and/or composition. On a nmole/mg mitochondria basis, oligomycin is a more effective inhibitor than tetradifon. Oligomycin provides 50 percent or more inhibition of most activities studied here at 1.3 nmole/mg mitochondrial protein, whereas tetradifon requires between 4.5 and 27 nmoles/mg mitochondrial protein to produce similar effects. Such differences may reflect differences in permeability of the two inhibitors or differences in their lipid solubility at or near their site of action. Oligomycin B, the complete structure of which has not been established, is known to have the chemical formula $C_{15}H_{72}O_{12}$ (MW 804), and to contain 3 double bonds in a conjugated diene, a separate unsaturated carbonyl structure, four secondary hydroxyl groups, and probably a lactone (8). Tetradifon (MW 356), on the other hand is a much simpler molecule of the following chemical structure:



Despite rather obvious differences in the chemical architecture of oligomycin and tetradifon, results reported here strongly suggest that tetradifon has a mode of action similar to that of oligomycin in support of the suggestion of Desaiah et al. (2). Sensitivity of soluble mitochondrial ATPase to oligomycin is known to be conferred by a lipoprotein complex consisting of lipid, a protein factor necessary to bind the ATPase, and a second protein factor of about 18,000 molecular weight called OSCP (oligomycin sensitivity conferring protein) (9, 10). It seems reasonable to conclude, therefore, that tetradifon may act at or near the level of this lipoprotein complex also, and in so doing, inhibit not only ATPase activities but associated energy-linked activities of mitochondria as well.

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