Vol. 82, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1978 Pages 289-295

DIMINISHED INHIBITION OF SUCCINATE-CYTOCHROME c REDUCTASE ACTIVITY OF RESOLVED REDUCTASE COMPLEX BY THENOYLTRIFLUOROACETONE IN THE PRESENCE OF ANTIMYCIN

Bernard L. Trumpower and Zachary Simmons

Department of Biochemistry
Dartmouth Medical School
Hanover, N.H. 03755

Received March 30,1978

Summary Antimycin, when added to resolved succinate-cytochrome c reductase complex in amounts sufficient to partially inhibit succinate-cytochrome c reductase activity, causes a decrease in inhibition of the residual succinate-cytochrome c reductase activity by 2-thenoyltrifluoroacetone. Antimycin has no effect on the inhibition of succinate-ubiquinone reductase activity by 2-thenoyltrifluoroacetone. We propose that antimycin increases the steady state concentration of ubisemiquinone in the reductase complex, and that 2-thenoyltrifluoroacetone is competitive with ubisemiquinone.

Introduction

Antimycin, a fungicide, and 2-thenoyltrifluoroacetone (TTFA), a metal chelator, are both potent inhibitors of succinate-cytochrome c reductase activity in intact mitochondria and resolved succinate-cytochrome c reductase complex. However, these inhibitors differ in their sites of action. Antimycin binds tightly ($K_d=10^{-11}$ M) to the b-c_1 segment (1) and inhibits ubiquinol-cytochrome c reductase activity (2,3). TTFA inhibits succinate-ubiquinone reductase activity (4), in reversible fashion (5), and does not inhibit ubiquinol-cytochrome c reductase activity (6).

Understanding how TTFA inhibits electron transfer is of interest because this inhibitor appears to block electron transfer between membranous succinate dehydrogenase and ubiquinone, and the mechanism of electron transfer in this region of the respiratory chain is not understood. Nelson and coworkers (7) reported that TTFA is competitive with ubiquinone and proposed that the inhibitor disrupts an interaction between ubiquinone and an iron-sulfur center of succinate dehydrogenase. More recent experiments

0006-291X/78/0821-0289$01.00/0

by Ackrell and coworkers (8) on the oxidation kinetics of succinate dehydrogenase indicate that TTFA acts on the oxygen side of and vicinal to the high potential iron-sulfur center (S-3) of the dehydrogenase. These findings have been supplemented by EPR measurements which suggest that TTFA disrupts a spin-spin interaction between a ubisemiquinone pair closely associated with center S-3 (9,10).

In this communication we report that the efficacy of TTFA is lowered by antimycin, an inhibitor which acts on the respiratory chain at a site different than that of TTFA. We propose that this effect is due to an increase in the concentration of ubisemiquinone caused by antimycin.

Materials and Methods

Succinate-cytochrome c reductase complex was prepared from phosphate-washed bovine heart mitochondria, and succinate-cytochrome c reductase activity was measured as described previously (11). Succinate-ubiquinone reductase was measured with 48 μM DBH, a decyl substituted analog of ubiquinone-2 (12), using 52 μM dichloroindophenol as terminal acceptor (13). Rates of electron transfer, measured at 30°C, are expressed in units of one electron equivalent, a unit being defined as one microgram equivalent per minute.

Results and Discussion

TTFA inhibits both succinate-cytochrome c reductase and succinate-ubiquinone reductase in resolved succinate-cytochrome c reductase complex. This inhibition is like that observed with submitochondrial particles or resolved complex II (14), in that a residual amount of activity (5-10 percent of the total) is resistant to TTFA as shown below (see Figure 2). As shown in Figure 1, a Dixon plot of [succinate-cytochrome c reductase activity]$^{-1}$ versus concentration of inhibitor is linear from 1-500 μM TTFA, over which concentration range 95 percent of the activity is inhibited. Likewise a linear Dixon plot is obtained for inhibition of succinate-ubiquinone reductase activity in the resolved complex (results not shown).

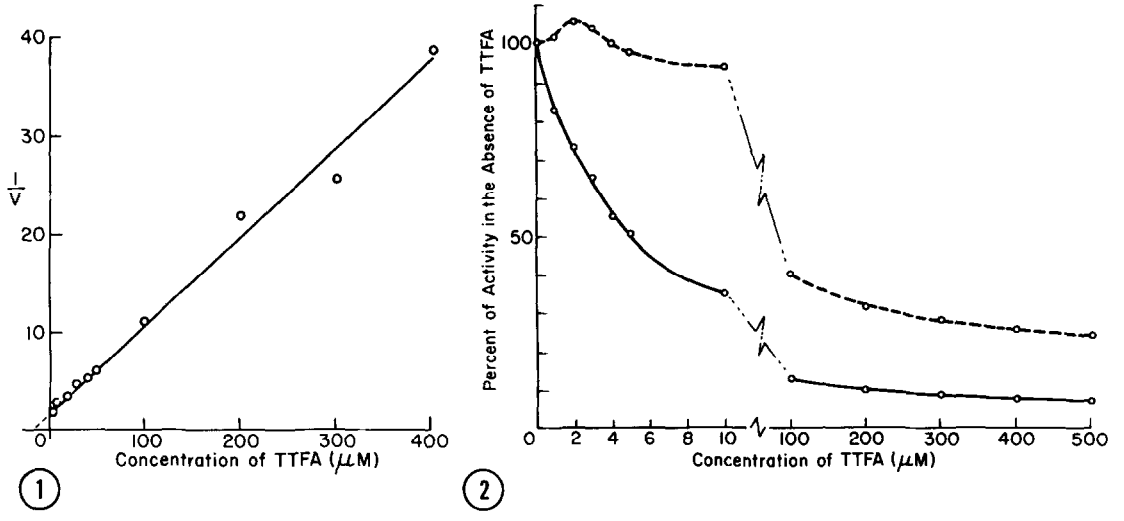

Figure 1 Dixon plot of the inhibition of succinate-cytochrome c reductase activity of resolved succinate-cytochrome c reductase complex by TTFA. For calculating the reciprocal, velocities were expressed as the fraction of the velocity in the absence of TTFA, which was 4.2 units per mg.

Figure 2 Inhibition of succinate-cytochrome c reductase activity of resolved succinate-cytochrome c reductase complex by TTFA in the absence (solid line) and presence (dashed line) of antimycin. In the absence of TTFA the succinate-cytochrome c reductase activity of the sample not treated with antimycin was 3.77 units per mg and that of the antimycin-treated sample was 0.68 units per mg.

As pointed out by Mowery and coworkers (14), the linear Dixon plots reflect simple saturation kinetics of inhibition. Either there is only one site of TTFA inhibition, or alternatively, if there are multiple sites, their affinities are indistinguishable. In view of findings reported here, the possibility of multiple TTFA sites is relevant. Our results (Figure 1) and those of Mowery and coworkers (14) suggest there is only a single site of TTFA inhibition.

If a small amount of antimycin is added to the reductase complex so that succinate-cytochrome c reductase activity is partially inhibited by antimycin, the remaining activity is resistant to inhibition by TTFA. This effect of antimycin is manifested as a lag in titration curves of activity versus TTFA concentration as shown in Figure 2. In the absence of antimycin 10 μM TTFA inhibits more than 60 percent of the succinate-cytochrome c reductase activity.

TABLE I

Effect of Thenoyltrifluoroacetone and Antimycin on Succinate-Cytochrome c Reductase Activity of Resolved Reductase Complex

Additions to Assay	Cytochrome c Reductase Activity (units/mg)
None	4.00
Antimycin	0.14
5 μM TTFA	2.01
5 μM TTFA + Antimycin	0.12
30 μM TTFA	0.83
30 μM TTFA + Antimycin	0.11
500 μM TTFA	0.23
500 μM TTFA + Antimycin	0.14

Where indicated, the inhibitors were added to the ongoing succinate-cytochrome c reductase reaction. The amount of antimycin added was 2 μg per mg of reductase complex. When both inhibitors were added, antimycin was added after TTFA.

This same concentration of TTFA inhibits less than 5 percent of the activity remaining in the presence of antimycin. This effect of antimycin extends to concentrations of TTFA as high as 500 μM (Figure 2).

In Table I we have summarized results of a control experiment which shows that in the presence of low (5 μM), intermediate (30 μM), and high (500 μM) concentrations of TTFA the reduction of cytochrome c which is not inhibited by TTFA remains fully sensitive to antimycin. This illustrates that the population of reductase complexes with which TTFA interacts is homogeneous in its sensitivity to antimycin.

In contrast to the effect which antimycin has on inhibition of cytochrome c reductase activity by TTFA, inhibition of succinate-

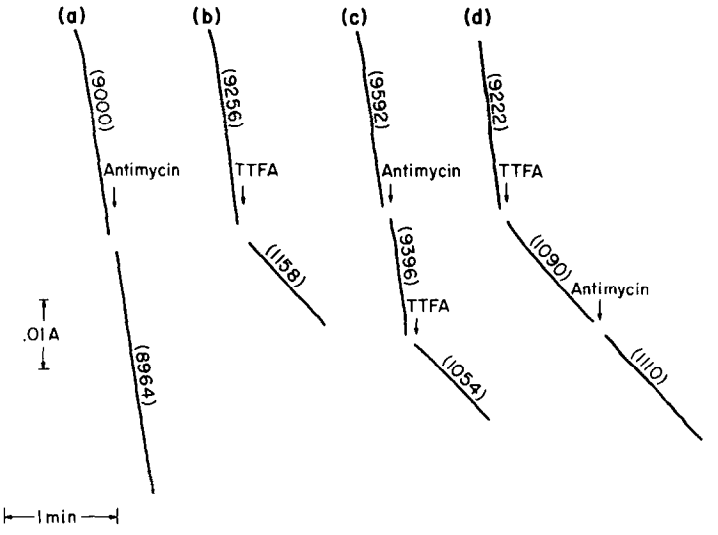

Figure 3 Effect of antimycin and TTFA on the succinate-ubiquinone reductase activity of resolved succinate-cytochrome c reductase complex. The tracings show the reduction of dichloroindophenol. Numbers in parentheses are rates of reduction of dichloroindophenol, in units per mg. Where indicated, 2 μg of antimycin per mg of reductase complex or 500 μM TTFA were added to the reaction mixture.

ubiquinone reductase activity by TTFA is unchanged by antimycin. Traces showing the rate of reduction of dichloroindophenol in the succinate-ubiquinone reductase reaction are shown in Figure 3. As mentioned above, antimycin itself has no effect on succinate-ubiquinone reductase activity (Figure 3a), while this activity is inhibited by TTFA (Figure 3b). If antimycin is added before (Figure 3c) or after (Figure 3d) TTFA, the TTFA-inhibited rate is not significantly different than in the absence of antimycin. As suggested by these results, titration curves of succinate-ubiquinone reductase activity versus TTFA concentration showed no difference in efficacy of TTFA in the presence of antimycin (results not shown), in contrast to the effect seen in the cytochrome c reductase reaction (Figure 2).

There are several possible explanations for how antimycin might lower the efficacy of TTFA. One possibility is that maximal inhibition of succinate-cytochrome c reductase by TTFA requires interaction of TTFA with two sites

in the reductase complex and that antimycin interferes with one of these. However, as noted above, there is no evidence for multiple sites of TTFA inhibition.

A second possibility is that antimycin, which binds to the $\underline{b}$-$\underline{c}_1$ segment, causes a conformational change in the vicinity of succinate dehydrogenase where TTFA interacts. Although this possibility cannot be ruled out, it also may be difficult to test in any rigorous fashion.

A third possibility, and one which we propose is operative, is that antimycin alters the redox status of an electron transfer component at the site where TTFA acts. Addition of antimycin to submitochondrial particles (15) or resolved electron transfer complexes (16) causes production of superoxide anion, and there is evidence that this reflects increased levels of ubisemiquinone (15,16). Thus it seems likely that antimycin increases the concentration of semiquinone formed from ubiquinone in the succinate-cytochrome $\underline{c}$ reductase reaction.

We propose that there is an inverse relationship between ubisemiquinone concentration and efficacy of TTFA. This agress with and extends previous work indicating a competition between TTFA and ubiquinone (7). Likewise, our explanation of our results agrees with evidence that TTFA disrupts a semiquinone interaction on the oxygen side of center S-3 of the dehydrogenase (8-10). The finding that the antimycin-dependent change in efficacy of TTFA is manifested in the succinate-cytochrome $\underline{c}$ reductase reaction, but not in the succinate-ubiquinone reductase reaction, is explainable if in the ubiquinone reductase reaction the use of external acceptor (DBH or dichloroindophenol) prevents the accumulation of ubisemiquinone.

Acknowledgements This investigation was supported by a National Institutes of Health Research Grant, 1-RO1-GM20379. BLT is an Established Investigator of the American Heart Association.

References

1. Berden, J.A. and Slater, E.C. (1972) Biochim. Biophys. Acta 256, 199-215

2. Rieske, J.S. and Zaugg, W.S. (1962) Biochem. Biophys. Res. Comm. 8, 421-431
3. Teeter, M.E., Baginsky, M.L. and Hatefi, Y. (1969) Biochim. Biophys. Acta 172, 331-333
4. Tappel, A.L. (1960) Biochem. Pharmacol. 3, 289-296
5. Piper, P.A. and Thorn, M.B. (1965) Biochem. J. 94, 35P
6. Hatefi, Y., Haavik, A.G. and Griffiths, D.E. (1962) J. Biol. Chem. 237, 1681-1685
7. Nelson, B.D., Norling, B., Persson, B. and Ernster, L. (1971) Biochem. Biophys. Res. Comm. 44, 1312-1320
8. Ackrell, B.A.C., Kearney, E.B., Coles, C.J., Singer, T.P., Beinert, H., Wan, Y. and Folkers, K. (1977) Arch. Biochem. Biophys. 182, 107-117
9. Ingledew, W.J. and Ohnishi, T. (1977) Biochem. J. 164, 617-620
10. Ohnishi, T., Salerno, J.C., Blum, H., Leigh, J.S. and Ingledew, W.J. (1977) in Bioenergetics of Membranes (Packer, L., Papageorgiou, G.C. and Trebst, A., eds) pp 209-216, Elsevier-North Holland
11. Trumpower, B.L. and Katki, A. (1975) Biochem. Biophys. Res. Comm. 62, 282-288
12. Wan, Y.P., Williams, R.H., Folkers, K., Leung, K.H. and Racker, E. (1975) Biochem. Biophys. Res. Comm. 63, 11-15
13. Ziegler, D. and Rieske, J.S. (1967) in Methods in Enzymology, vol 10 (Estabrook, R.W. and Pullman, M.E., eds) pp 231-235, Academic Press
14. Mowery, P.C., Steenkamp, D.J., Ackrell, B.A.C., Singer, T.P. and White, G.A. (1977) Arch. Biochem. Biophys. 178, 495-506
15. Boveris, A., Cadenas, E. and Stoppani, A.O.M. (1976) Biochem. J. 156, 435-444
16. Cadenas, E., Boveris, A., Ragan, C.I. and Stoppani, A.O.M. (1977) Arch. Biochem. Biophys. 180, 248-257