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THE STIMULATION OF COUPLING FACTOR 1 ATPase BY TENTOXIN AND ITS ANALOGS[1]

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SUMMARY:

Tentoxin and two of its analogs, Sar[1]-tentoxin and D-MeAla[1]-tentoxin, all of which inhibit coupling factor 1 ATPase at low concentrations, are capable of significantly enhancing enzymatic activity at high concentrations.

Tentoxin [*cyclo* (-N-methyl-L-alanyl-L-leucyl-N-methyl-(*Z*)-dehydrophenylalanyl-glycyl-)][2] is a species-selective inhibitor of chloroplast coupling factor 1 ATPase (1). This inhibition is uncompetitive with respect to ATP (2) and involves the binding of tentoxin to a single site located on the α and β subunit complex (3).

The inhibition of CF_1 ATPase by tentoxin and tentoxin analogs has been investigated, and the relationship between conformation and inhibitory activity examined (4). During these investigations we found that tentoxin and some of its analogs also are capable of significantly enhancing CF_1 ATPase activity, provided that their concentration is sufficiently great. Some details of this phenomenon are reported herein.

MATERIALS AND METHODS

Coupling factor 1 was prepared from lettuce chloroplasts following the method of Lien and Racker (5). It was purified by 5% polyacrylamide gel electrophoresis as previously described (2), then activated by mild trypsin treatment (5). Tentoxin was purified from culture filtrates of *Alternaria alternata* (6). The tentoxin analogs, Sar[1]-tentoxin [*cyclo* (-N-methyl-L-glycyl-L-leucyl-N-methyl-(*Z*)-dehydrophenylalanyl-glycyl-)] and D-MeAla[1]-tentoxin [*cyclo* (-N-methyl-D-

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[2]Nomenclature according to IUPAC Nomenclatural Committee, J. Org. Chem. 35, 2849 (1970).

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alanyl-L-leucyl-N-methyl-(Z)-dehydrophenylalanyl-glycyl-)], were prepared synthetically (7, 8). The biologically active conformers of D-MeAla^1-tentoxin (conformer 2 L) were isolated at 4°C by thin-layer chromatography of D-MeAla^1-tentoxin on silica gel G F254 plates developed with ethyl acetate:methanol (95:5), followed by recovery of the slower-moving conformers (R_f 0.13) using methanol. This separation was performed immediately before use because of the rapidity of conformer interconversion into inactive forms (R_f 0.28, $t_{1/2} \approx 200$ min in water at 27°C) (4). The concentrations of tentoxin and its analogs were determined by UV absorption at 282 nm.

Assays for CF_1 ATPase were done by incubating varying amounts of tentoxin or an analog with 5-10 µg of protein in 0.8 ml of 40 mM Tricine-NaOH buffer (pH 8.0) for 18 minutes. Following this treatment, the assay tubes were brought to 27°C over a 2-min period and then ATP and $CaCl_2$ were added to give concentrations of 2 mM ATP and 10 mM Ca^{+2} in a total volume of 1 ml. After an additional 15 minutes, the reaction was stopped with 1 ml of 0.5 N trichloroacetic acid and phosphate release determined by the method of Taussky and Shorr (9).

RESULTS AND DISCUSSION

The response of CF_1 ATPase to tentoxin over a millionfold concentration range is quite unusual (Fig. 1). At a concentration of about 3×10^{-7} M, tentoxin almost completely inhibits CF_1 ATPase (Ki $\approx 5 \times 10^{-9}$ M) (4), but as the tentoxin concentration is increased to 5×10^{-5} M, enzymatic activity is restored; 10^{-4} M, catalytic activity is stimulated almost 3-fold with respect to the control.

The phenomenon does not appear to be caused by a stimulation of a "latent" ATPase that might have been present in the enzyme preparation. Activated and nonactivated forms, produced by either trypsin or heat treatments, were separated from each other on 5% polyacrylamide gels, eluted and assayed in the presence of 10^{-9} to 10^{-4} M tentoxin. All forms behaved similarly, although the nonactivated ones had comparatively little activity. Thus, it appears that the same enzyme species that is inhibited, is also stimulated depending upon the tentoxin concentration.

The inhibition-stimulation appears to show structural specificity. Sar^1-tentoxin, an analog which can adopt a conformation identical to tentoxin except for the deletion of a methyl group (10), strongly inhibits CF_1 ATPase (Ki = 14×10^{-9} M) and also restores catalytic activity at concentrations comparable to those required by tentoxin (Fig. 1). In contrast, D-MeAla^1-tentoxin, an analog that is a much weaker inhibitor (Ki = 6-10 $\times 10^{-6}$ M) (4), restores enzymatic activity only at very high concentrations (Fig. 1). Conformational studies have

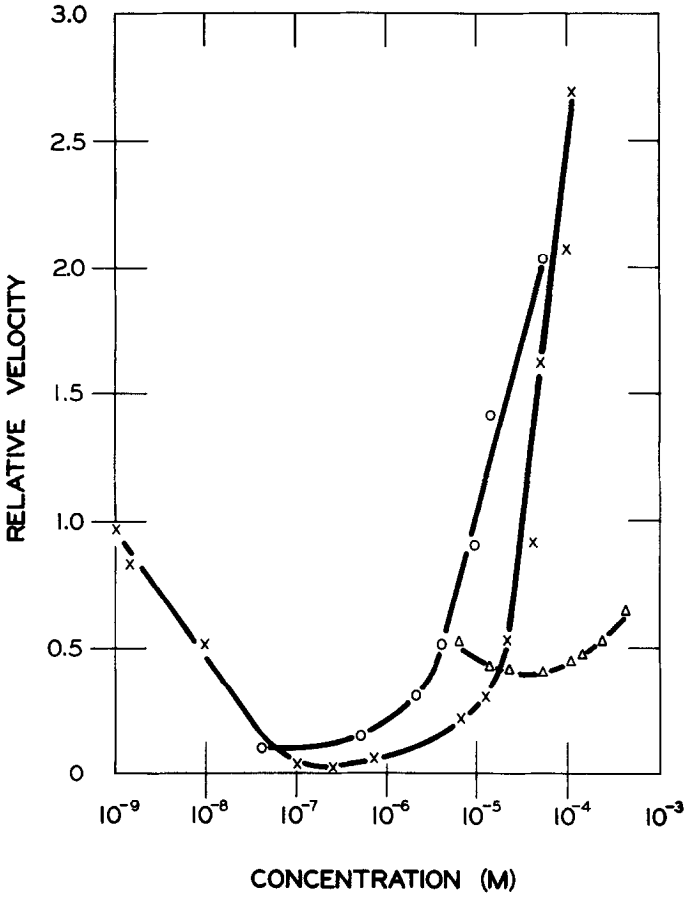

Figure 1. Effect on CF_1 ATPase activity of tentoxin (X), Sar^1-tentoxin (0) and D-$MeAla^1$-tentoxin (2 L) (Δ). The enzyme preparations had a specific activity of 8.2 - 11.1 μM Pi mg^{-1} min^{-1} when assayed as described in the text. The enzyme concentration varied from 15 - 30 nm. The tentoxin:CF_1 ratio at minimal activity was approximately 10:1.

shown that D-$MeAla^1$-tentoxin cannot adopt a conformation identical with that of tentoxin (11) although portions of both molecules are closely related (4). Thus, the stimulatory and inhibitory effects appear to be related. This implies that similar structural features are required for the interaction of tentoxin and its analogs with the site(s) responsible for these effects. This observation also suggests that a single site may be involved, but that two modes of binding are available.

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8. Boc-MeGly was coupled to methyl N-*tert*-butyloxycarbonyl-L-leucyl-N-methyl-(Z)-dehydrophenylalanylglycinate following the procedure reported for a homolog (7). Boc-MeGly-L-Leu-MePhe[(Z)Δ]-Gly-OMe was isolated: NMR ($CDCl_3$) δ 0.5-0.7 (6H, d, J = 9 Hz), 1.48 (9H, s), 0.9-1.2 (3H, m), 2.80 (3H, s), 3.17 (3H, s), 3.7 (3H, s), 4.17 (2H, d, J = 6.5 Hz), 4.23 (2H, s), 6.7 (1H, br. s), 7.39 (5H, s), 7.7 (1H, s), 7.8 (NH, m). Mass spectrum, m/e, M+ 533, 471, 444, 370, 332, 234, 118, 102, 90, 57 (100). Following deprotection and cyclization Sar^1-tentoxin was obtained in 30% yield as a mixture of conformers; *Rf* (ethyl acetate:ethanol. 95:5) 0.12 and 0.16; NMR ($CDCl_3$) δ 0.55, 0.65 (6H, d, d, J = 6 Hz), 1.3-1.7 (3H, m), 3.0 (0.5H, s, MeGly N-CH_3), 3.05 (0.5H, s, MeGly N-CH_3), 3.23 (0.5H, s, MePhe N-CH_3), 3.33 (0.5H, s, MePhe N-CH_3), 3.6 (1H, m, glycyl inner proton), 3.8 (1H, m, MeGly outer proton), 4.14 (1H, m), 4.4 (1H, m, MeGly inner proton), 5.13 (1H, m, Gly inner proton), 6.5 (0.5H, d, J = 7Hz, Leu NH), 7.22 (0.5H, d, 8 Hz, Leu NH), 7.04 (0.5H, s), 7.77 (0.5H, s), 7.46 (5H, s), and 8.15 (1H, m, Gly NH) ppm. Mass spectrum, m/e (% of base peak) M+ 402 (4), 287 (1), 202, 201, 200 (3), 118 (10), 117 (8), 102 (20), 100 (10), 58 (100).
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