SHORT COMMUNICATION

Conformational Changes of Acetylcholinesterase

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SUMMARY

The conformation of highly purified acetylcholinesterase was measured with a spectropolarimeter. It was demonstrated that heat, strong base, a substrate, and anticholinesterase agents induce alterations in the conformation of this enzyme.

Acetylcholinesterase is an important enzyme in human physiology by virtue of its essential action in ganglionic and cholinergic neural-effector organ transmission. Recent developments in the study of protein structure indicate that specific enzyme inhibitors and substrates may produce their effects by binding to the active site and inducing appropriate conformational changes essential to further reaction (1, 2). Indirect evidence indicates that conformational alterations in structure may provide an explanation for some of the reactions of acetylcholinesterase (3, 4). Similarly, by binding a molecule at a site other than the active surface, the enzyme's tertiary structure may be altered with a resultant change in catalytic activity (5, 6). This type of allosteric mechanism has recently been proposed for acetylcholinesterase (7). In this preliminary study we report direct spectropolarographic measurement of conformational changes induced in highly purified acetylcholinesterase by heat, strong base, a substrate, and anticholinesterase agents.

Acetylcholinesterase was purified from the Electrophorus electricus (8). The specific activity was between 660 and 700 mmoles acetylcholine hydrolyzed/mg protein/hr at 25°, pH 7, and substrate concentration of 3×10^{-3} M, measured as previously described (8); the protein concentration was 0.10 mg/ml.

Optical rotatory dispersion (ORD) measurements were made with a Cary model 60 recording spectropolarimeter. The data were recorded at least twice on 0.1-ml samples in a 10-mm pathlength silica cell thermostated at 25° and other temperatures as indicated. The reproducibility was excellent. Some observations were repeated two months after the original study. A new preparation of purified enzyme was used; the original traces could be superimposed on the later observations.

The ORD curve for highly purified acetylcholinesterase is recorded in Fig. 1 and has the following characteristics: Negative rotations over the ranges 500–230 mμ; a negative Cotton effect with maximum negative rotation at about 235 mμ ($[\alpha] = -.0050°$); zero rotation (λ_0) at 222.5 mμ; positive rotations from 222 mμ to a sharp but forked peak maximum at 205 mμ ($[\alpha] = +.020°$) and 197 mμ ($[\alpha] = +.0198°$). When data from similar curves are plotted in accordance with the Moffitt equation, the helical parameter, b_0, may be evaluated. A value of approximately −600 was obtained for b_0. If this type of data analysis is relatable to helix content, then purified acetylcholinesterase derived from

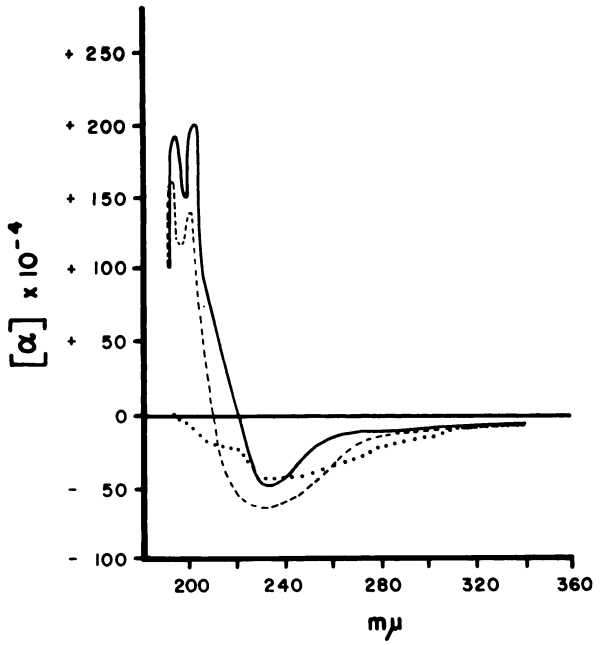

FIG. 1. Optical rotatory dispersion of acetylcholinesterase. The sample had a specific activity of 660 mmoles acetylcholine hydrolyzed/mg protein/hr. Measurements were made at an enzyme concentration of 0.10 mg protein/ml in 0.25 M NaCl, 0.03 M phosphate buffer, pH 7, with a Cary model 60 recording spectropolarimeter. The smooth curves are drawn from the experimental trace. (——) Active acetylcholinesterase, 25°, pH 7; (- - - -) acetylcholinesterase inactivated by heating to 60° for 30 min, pH 7; (●) acetylcholinesterase inactivated by 0.1 N KOH, 25°.

the electric eel is apparently in a greater than 90% right-handed helical conformation.

The effect of temperature variation and strong alkali on the enzyme as indicated by the ORD curve is illustrated in Fig. 1. When the enzyme is totally inactivated by raising the temperature to 60°, the trough of the negative Cotton effect is deeper and wider, the wavelength of zero rotation (λ_0) is 210 mμ, and the positive rotation component is sharply peaked with maxima at 205 mμ ([α] = +.0135°) and 197 mμ ([α] = .0160°). This curve contrasts sharply with that measured for another sample of enzyme inactivated with 0.1 N KOH. Only negative rotation is recorded with a wide shallow trough.

Figure 2 shows the influence of the reaction between enzyme and substrate on the shape of the ORD curve. The acetate of γ-trimethylammonium-*n*-propanol chloride (acetylhomocholine chloride) was used as the substrate rather than acetylcholine because the latter compound would be very rapidly hydrolyzed by the relatively high concentration of enzyme required for ORD measurement. In the presence of this substrate, acetylhomocholine at 1×10^{-2} M, the enzyme had an activity of 30 mmoles hydrolyzed/hr and a measured K_m value of 2.7×10^{-4} mole/liter. The ORD trace appears unaltered (Fig. 2) except for slightly higher values for the twin peaks of positive rotation ([α] = +.0250° and +.0240°).

3-Hydroxyphenyl dimethylethylammonium chloride (Tensilon) is a potent reversible anticholinesterase agent used in the diagnosis and treatment of some diseases. The value of the K_i for its reaction with this enzyme was measured at 9.7×10^{-8} M. Because of the high concentration of enzyme used in these studies a concentration of 3×10^{-4} M reversible inhibitor was required for 100% inhibition. The ORD curve of acetylcholinesterase in the presence of this concentration of inhibitor is similar to that recorded for the enzyme denatured at

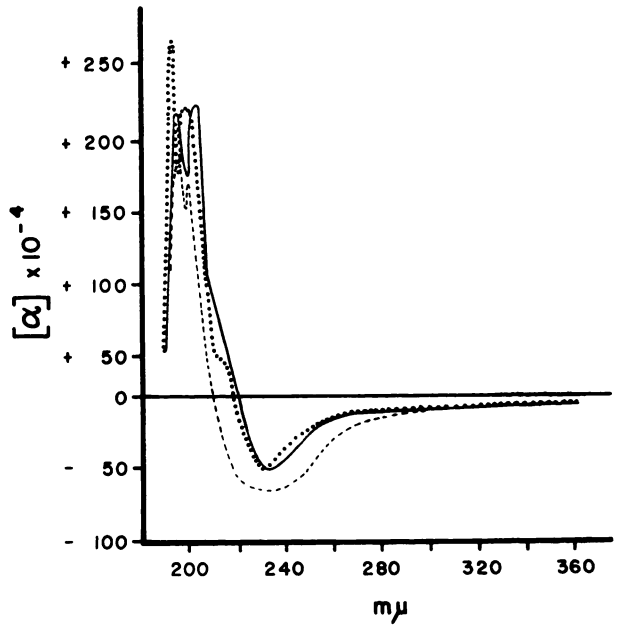

FIG. 2. Optical rotatory dispersion of acetylcholinesterase in the presence of inhibitors and a substrate. (——) Active acetylcholinesterase in the presence of 0.01 M acetylhomocholine, pH 7, 25°; (- - - -) acetylcholinesterase completely inhibited by 3×10^{-4} M 3-hydroxyphenyl dimethylethylammonium chloride (Tensilon), pH 7; 25°; (●) acetylcholinesterase completely inhibited by incubation with 5×10^{-4} M tetraethylpyrophosphate for 30 min, pH 7, 25°. The control curve obtained with native enzyme alone is the trace listed as "(——)" in Fig. 1.

60° and very much different from the curve measured when the enzyme is incubated with 0.1 N KOH (Fig. 2).

Tetraethylpyrophosphate (TEPP) is a potent, irreversible organophosphate anticholinesterase agent frequently used in the treatment of glaucoma. The second-order rate constant value for its reaction with this enzyme is 2.1×10^6 liter·mole^{-1} min^{-1}. After reaction with 5×10^{-4} M TEPP for 30 min at pH 7, 25°, the enzyme was completely inhibited. The ORD curve for this form of inhibited enzyme is recorded in Fig. 2. The negative Cotton effect is shifted to lower wavelength values, the point of zero rotation (λ_0) is at 218 mμ, and a prominent shoulder appears at about 210 mμ ([α] = +.0040°) on the positive rotation portion of the curve. The area of maximum positive rotation is forked with peaks at 200 mμ ([α] = +.0225°) and at 195 ([α] = +.0270).

This specimen of TEPP-inhibited enzyme was later reactivated to 80% of its original activity by dilution in a solution of 1×10^{-3} M pyridine-2-aldoxime methiodide.

These spectropolarographic data indicate that highly purified acetylcholinesterase derived from the electric eel is principally in a right-handed helical form and that the conformation of this enzyme is altered by heat, strong base, a substrate, an irreversible inhibitor, and a potent, reversible, quaternary ammonium anticholinesterase agent. Further studies designed to correlate structure, ORD, and reaction rates are planned.

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