INTERACTION OF QUATERNARY AROMATIC ISOQUINOLINE ALKALOIDS WITH ACETYLCHOLINESTERASE FROM *Electrophorus Electricus*

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Fluorescence data for the interaction of acetylcholinesterase (EC 3.1.1.7, ACHE) from *Ele phorus electricus* with berberine, 13-ethylberberine, coralyne, and chelerythrine have been sented. By a comparison with known inhibitors of acetylcholinesterase, tetramethylammo and tacrine, the electrostatic interaction of quaternary alkaloids with the γ-anionic site o enzyme could be confirmed. During the interaction, two molecules of the ligand are bound t molecule of the enzyme.

Quaternary protoberberine and benzo[*c*]phenanthridine alkaloids are inhib of various enzymes[1]. Our earlier studies have shown that quaternary isoquinoli alkaloids are inhibitors of cholinesterases[2,3]. We have now studied the intera of chlorides of berberine (*I*), 13-ethylberberine (*II*), coralyne (*III*), and chelerythr (*IV*) with acetylcholinesterase (EC 3.1.1.7, ACHE) from the electric eel (*Ele phorus electricus*) by fluorescence measurements. The main aim of this paper been to check our hypothesis that these compounds bind into the γ-anionic site of the enzyme[2].

EXPERIMENTAL

Material. Acetylcholinesterase (specific activity 22·5 μkat/mg protein) from the electric organ of the electric eel was obtained from Sigma Chemical. The enzyme was dissolved in 0·1 mol l^{-1} phosphate buffer of pH 7·4 prior to use. The enzyme concentration was determined spectrophotometrically at 280 nm (M_r 260 000)[3]. Berberinium sulphate was a Merck product, 13-ethylberberinium iodide was prepared from berberinium chloride[4], coralynium sulfoacetate (m.p. 278–280°C, methanol) from papaverine[5], and chelerythrine was isolated from plant material[6]. All the substances were used in form of chlorides. Tacrine (9-amino-1,2,3,4-tetrahydroacridine hydrochloride, THA) was donated by Dr J. Patočka.

Methods. The fluorescence and fluorescence-polarizing measurements were performed in a Aminco–Bowman spectrofluorimeter equipped with Glan-prism polarizers. All the measurements were performed in 0·1 mol l^{-1} phosphate buffer of pH 7·4 at 25°C. The polarization (p) was

calculated as described[7]. The values of the dissociation constants (K_D^{fluor}) were obtained by linearization of the quenching values of the fluorescence of the enzyme. The values of the distance between the donor and acceptor at which the rate constant for transfer is equal to the rate constant for spontaneous deactivation of the donor (R_0) and the distance between the chromophores (alkaloid–tryptophan, R) have been calculated according to the relations[8]:

$$R_0 = (1{\cdot}69 \,.\, 10^{-33}\tau_s J_{\bar{\nu}})^{1/2}/n\bar{\nu}_0 \,,$$

$$R^6 = (R_0^6/T) - R_0^6 \,,$$

where τ is the lifetime of tryptophan in the absence of transfer, n refractive index of the solvent, $\bar{\nu}_0$ the 0—0 wave number for the emission of tryptophan, $J_{\bar{\nu}}$ the overlap integral, T the efficiency of energy transfer is defined by using the maximum quenching values. The parameters for tryptophan were taken from the literature[8], the absorption spectra of alkaloids were measured on a Perkin–Elmer 552 spectrophotometer.

RESULTS AND DISCUSSION

Alkaloids *I*–*IV* absorb the energy in the emission region of tryptophan (*c.* 340 nm) and decrease the intensity of the fluorescence of ACHE (Fig. 1). The quenching of the enzyme can be explained by the resonance energy transfer from tryptophan to the bound alkaloid. The quenching of acetyltryptophan by the alkaloids *I*–*IV* was negligible. The dissociation constants (calculated from the quenching curves $-K_D^{fluor}$) were compared with the inhibition constants from kinetics measurements (K_I^{kin}) (Table I). The results from the two measurements are in good agreement and the differences between the K_D^{fluor} and K_I^{kin} values are due to the different quality of the enzyme preparations.

For the compounds *I*–*IV* there was characteristic the decrease in their fluorescence and the increase in polarization on addition of the enzyme. This dependence for coralyne (*III*) is shown in Fig. 2. On saturation with the enzyme, the polarization reached maximum values $p_{max} = 0{\cdot}1-0{\cdot}2$. The low P_{max} values suggest that the mobility

TABLE I
Inhibition of acetylcholinesterase

Alkaloid	K_I^{kin} [a] μmol l^{-1}	K_D^{fluor} [b] μmol l^{-1}
Berberine (*I*)	0·98	0·64
13-Ethylberberine (*II*)	0·91	0·58
Coralyne (*III*)	1·30	0·43
Chelerythrine (*IV*)	9·40	1·50

[a] Values from ref.[2]; [b] For the values see Experimental.

of the alkaloids bound to ACHE is rather high; this might be the result of a superficial binding of these compounds to the enzyme.

For detailed study of the binding site of ACHE, use was made of corylane (*III*) and of the compounds whose binding sites at ACHE are known. Tetramethylammonium is bound in the α-anionic site which together with the esteratic site forms the catalytic center of the enzyme[9]. Fig. 3 shows the effect of tetramethylammonium on the inhibition of ACHE by coralyne (*III*). The decrease in the intensity of the fluorescence of the enzyme caused by tetramethylammonium and coralyne (*III*) is greater than that brought about by the individual compounds. This can be explained by the binding of coralyne (*III*) and tetramethylammonium to different binding sites of ACHE. The increase in the quenching of the fluorescence of ACHE in the ternary complex can be explained by some conformational changes in the enzyme molecule. A diminution of the distance or an increase in the "parallelism" between the ligand and tryptophan is caused by the mutual effect of the molecules of the ligands. The Ca^{2+} ions which are bound to the β-anionic site, do not affect the binding of coralyne (*III*) to the enzyme (Fig. 3).

Tacrine is specifically bound to the γ-anionic site at the periphery of ACHE (ref.[10]). The simultaneous interaction of tacrine and coralyne (*III*) with ACHE is shown in Fig. 4. The diagram indicates the competition between these two com-

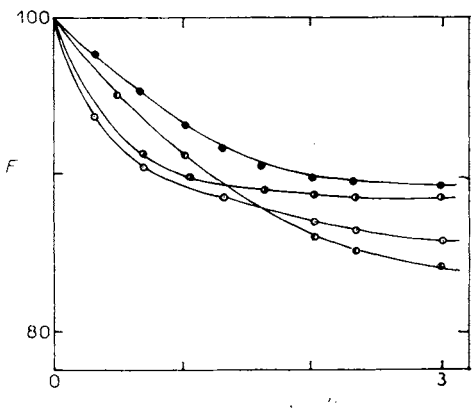


FIG. 1

Changes in the intensity of fluorescence during increasing alkaloid concentration. ● Berberine, ◑ coralyne, ⊙ 13-ethylberberine, ◐ chelerythrine $c_{ACHE} = 2{\cdot}5 \cdot 10^{-8}$ mol l^{-1}, $\lambda_{exc} = 280$ nm, $\lambda_{em} = 335$ nm; axis *x*: μmol l^{-1}

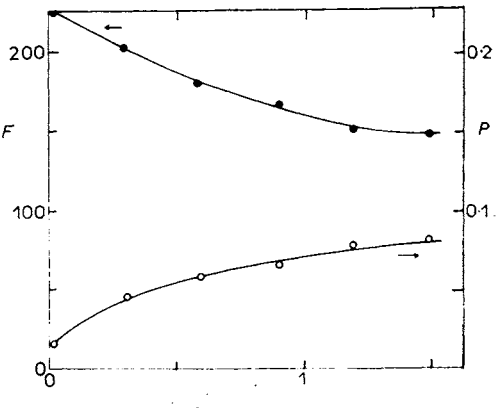
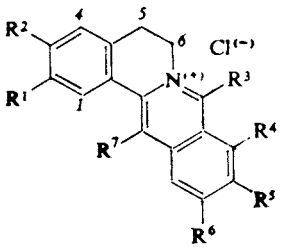


FIG. 2

Changes in the intensity of fluorescence (*F*, ●) and in the polarization of fluorescence (*p*, ○) during increasing ACHE concentration. $c_{coralyne} = 0{\cdot}3$ μmol l^{-1}, $\lambda_{exc} = 425$ nm, $\lambda_{em} = 480$ nm; axis *x*: μmol l^{-1}

pounds. A tacrine concentration higher than 2·5 μmol l^{-1} suppresses the effect of coralyne (*III*) on ACHE fluorescence.



I: $R^1 + R^2 = OCH_2O$, $R^3 = H$, $R^4 = R^5 = OCH_3$, $R^6 = R^7 = H$
II: $R^1 + R^2 = OCH_2O$, $R^3 = H$, $R^4 = R^5 = OCH_3$, $R^6 = H$, $R^7 = C_2H_5$
III: $R^1 = R^2 = R^5 = R^6 = OCH_3$, $R^3 = CH_3$, $R^4 = R^7 = H$, $\Delta^{5,6-\text{dehydro}}$

The kinetic measurements show that the protoberberine and the benzo[*c*]phenanthridine alkaloids are probably bound to the anionic binding site at the periphery

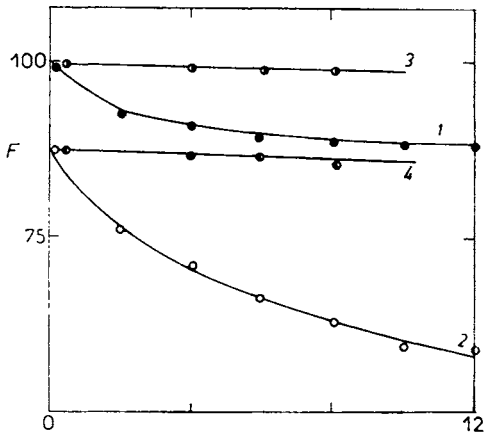


FIG. 3

Effect of coralyne (*III*) on ACHE interaction with tetramethylammonium ion and Ca^{2+} ions. $c_{ACHE} = 2{\cdot}4 \cdot 10^{-7}$ mol l^{-1}, $c_{coralyne} = 2{\cdot}3 \cdot 10^{-6}$ mol l^{-1}, $\lambda_{exc} = 280$ nm, $\lambda_{em} = 335$ nm; *1* tetramethylammonium; *2* tetramethylammonium + coralyne; *3* Ca^{2+}; *4* Ca^{2+} + coralyne; axis *x*: mmol l^{-1}

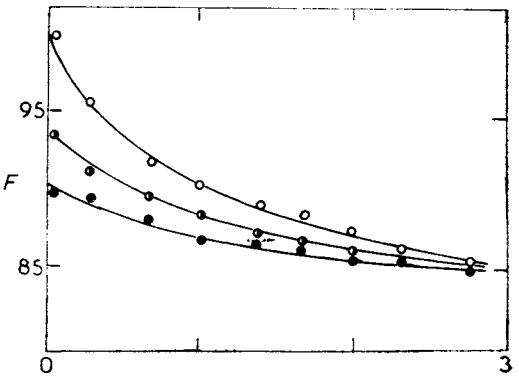


FIG. 4

Effect of tacrine (THA) on ACHE interaction with coralyne $c_{THA} = ○$ 0; ◑ 0·25; ● 2·5 μmol l^{-1}; axis *x*: μmol l^{-1}

of ACHE (ref.[2]). The fluorescence measurements provide evidence of the binding of quaternary alkaloids to the γ-anionic site of ACHE. The same binding site has been confirmed for compounds *I*–*IV* by the calculation of the distance R_0 and R between the chromophores. These values and the maximum quenching values are given in Table II. The high values of R and the fact that the polarization spectrum

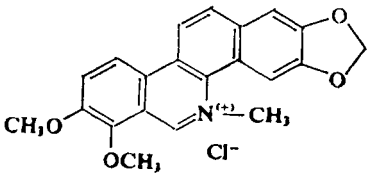


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TABLE II

Fluorescence parameters of the studied alkaloids

Alkaloids	Maximal quenching[a]	R_0 nm	R nm
Berberine (*I*)	18	2·8	3·5
13-Ethylberberine (*II*)	17	2·8	3·6
Coralyne (*III*)	13	2·5	3·4
Chelerythrine (*IV*)	22	2·8	3·5

[a] Determined from curves in Fig. 1. For the R_0, R values see Experimental.

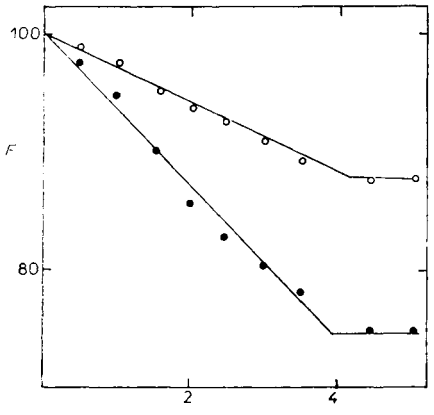

FIG. 5

Stechiometry of the interaction of ACHE with coralyne (○) and berberine (●). $c_{ACHE} = 2{\cdot}2 \cdot 10^{-6}$ mol l^{-1}, $\lambda_{exc} = 280$ nm, $\lambda_{em} = 335$ nm; axis *x*: μmol l^{-1}

of ACHE (λ_{exc} = 200–330 nm, λ_{em} = 350 nm) does not change in the presence of coralyne exclude the presence of tryptophan residues in the binding site of ACHE for alkaloids.

The subunit structure of ACHE and the number of active sites are not clear as yet[11]. It is assumed that in native state the ACHE molecule is a tetramer consisting of two α-chains and two β-chains[12]. The α-chain bears the catalytic center whilst the β- and γ-anionic sites are situated on the β-chain. This model is compatible with the stechiometry of interaction studied in this paper. The dependence shown in Fig. 5 as well as the number of binding sites for coralyne (*III*), found on the basis of the Scatchard dependence (n = 1·8), are consistent with the binding of two molecules of the ligand to one molecule of the enzyme.

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