Nature of Anionic or α-Site of Cholinesterase

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Abstract ☐ The nature of the so-called anionic binding site of acetylcholinesterase was investigated using a technique called receptor mapping using model interaction calculations. The results support the suggestion that coulombic forces play only a minor role in the binding event at this enzyme site.

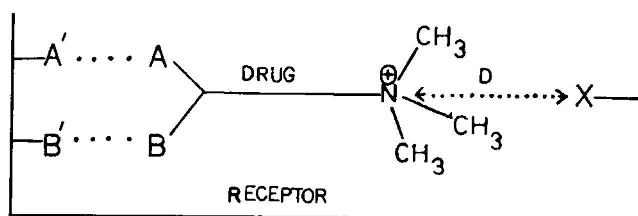
Keyphrases ☐ Acetylcholinesterase—anionic binding studied, receptor mapping using model interaction calculations, role of coulombic forces ☐ Binding sites, acetylcholinesterase—anionic binding site, receptor mapping using model interaction calculations, coulombic forces ☐ Enzymes, acetylcholinesterase—theoretical studies on nature of anionic binding site ☐ Receptor mapping using model interaction calculations—theoretical studies on nature of anionic binding site of acetylcholinesterase ☐ Coulombic forces—role in binding of acetylcholinesterase, anionic site

The widely held view (1) concerning the active features of cholinesterase is that two principal regions exist, a catalytic or esteratic site and a binding or anionic site. The esteratic site is thought to be a serine residue, engaging in acylation with acetylcholine or a *similar ester.* This site can be phosphorylated or carbamylated, leading to a slowly dissociable ester and, hence, to effective inhibition of the enzyme. The binding site is that region of the enzyme near the esteratic site capable of interacting or binding to the onium groups of acetylcholine or similar compounds. It has been postulated that this feature is anionic, since the complimentary onium group of the substrate bears a net positive charge. This interaction was presumed to involve an ion-pair. In addition to an ionic component, Wilson (1) postulated a significant role for dispersion forces.

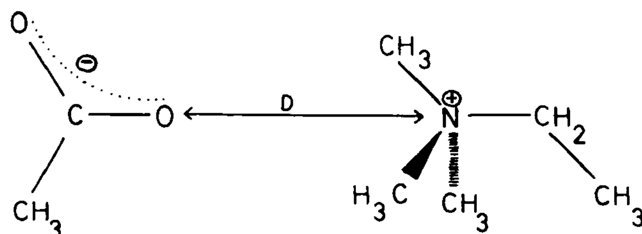
DISCUSSION

In a recent treatise, O'Brien (2) discussed work leading to some new views on the nature of the binding site and proposed that binding sites exist on cholinesterase, each responsive to different types of substrates or inhibitors. O'Brien renamed the classical anionic site the α-site. This enzyme feature must bind to acetylcholine for hydrolysis to occur. Other sites, β and γ, are engaged by different classes of inhibitors.

From accumulated evidence, O'Brien made a case for the view that the α-site is not ionic but is a region of the enzyme (side chain) essentially nonpolar in character and engaging in a noncoulombic interaction with the onium group of acetylcholine. Specifically, O'Brien proposed two alternatives to the general view of an anionic α-site: *(a)* an α-site composed of an inducible dipole which



Scheme I—Drug–receptor model showing binding features A and B locked at A' and B', respectively, and distance D between receptor feature X and onium group



Scheme II—Mode of interaction between acetic acid-ion and acetylcholine model

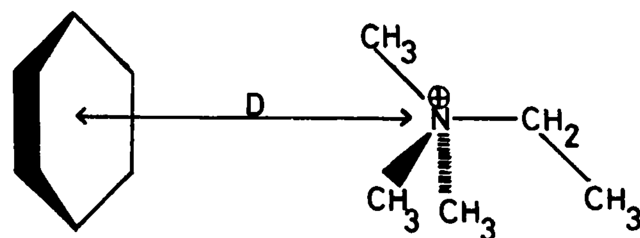
forms an ion-induced dipole with the acetylcholine onium group, or *(b)* a completely nonpolar α-site engaging in van der Waals interaction with the acetylcholine onium residue.

Some insight into these three possibilities may be gained using a theoretical approach recently employed by Kier and Aldrich (3) and Höltje and Kier (4, 5). This approach, referred to as receptor mapping using model interactions calculations, involves the calculation of interaction energies (electrostatic, polarization, dispersion, and repulsion) between a series of reactive or biologically active molecules and a group of model compounds simulating a variety of likely receptor or enzyme active site moieties. These moieties are models derived from amino acid side chains.

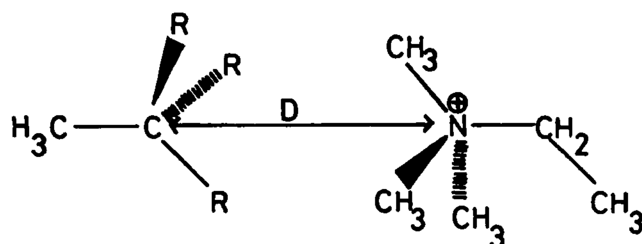
The assumption has been made that the receptor or enzyme active site is composed of a constellation of amino acid side chains. It is also assumed that one amino acid side chain is the dominant structural feature engaging a particular part of the substrate molecule or drug pharmacophore.

The problem is then to calculate the interaction energy between a structural feature under study in the drug or substrate molecule and a model compound simulating a possible receptor or enzyme active site. By performing these calculations on a series of drugs or substrates in which a variation in structure has been introduced at the moiety under study, a pattern of interaction energies can be obtained for each of several candidates for the receptor or active site. An attempt is made to correlate the trend in calculated interaction energies with a catalytic reactivity index or a biological property. The better the correlation, the more reasonable is this candidate for the receptor or active site.

Two concepts of enzymatic rate enhancement lead to the belief that the binding of a structural feature, remote from the catalyzed structure of the substrate, can have a direct bearing on the rate of reaction. The concept of catalysis by induction of strain, developed by Jencks (6), views the substrate as going through significant structural alteration as it is transformed through a transition state while bound at the enzyme active site. The binding forces probably vary as a function of these structural changes. At some point in the conversion of substrate to product, these binding forces are maximal. If the binding is maximal when the substrate reaches the transition state for the rate-determining step, a powerful driving force for the reaction could be provided. In effect, the enzyme is using a potential binding energy to help force the substrate toward the critical transition state structure. It is felt that these strain



Scheme III—Mode of interaction between benzene and acetylcholine model



Scheme IV—Mode of interaction between ethane (R = H) and tert-*butane (R = CH_3) and acetylcholine model*

forces involve noncovalent forces between enzyme and substrate. Such could be the role of structural features on a substrate, remote from the region of catalytic change.

A second concept of enzymatic rate enhancement is called substrate anchoring (7). Based upon nuclear magnetic evidence, substrates have a relatively long residence time at the active sites of enzymes. This binding as a complex is believed to be capable of accelerating the reaction rate by factors between 10^6 and 10^9. The concept presumes that the anchoring of a substrate to the enzyme greatly increases the probability of the formation of an activated complex, thereby increasing the reaction rate by large factors. The role of substituent groups on a substrate, remote from the region of catalytic change, could provide this anchoring.

Further significance can be attached to a correlation if the assumption is made that, in a series of drugs or substrates, the ratio, ρ, of any two activities in a series is related to the difference in strength of binding of the feature under study by the expression $\Delta E = -RT \ln \rho$. The better the calculated ΔE values in a series fit to the experimental ρ values, the more realistic is the model of the receptor or active site.

It is assumed that the mode of approach of the structural feature under study is constrained to a single mode of approach to the receptor model because of other points of binding between drug or substrate and receptor or enzyme (Scheme I). The calculations of interaction energies are thus made for a single mode of approach, varying the distance between the two molecules.

It is clearly recognized that simplifying assumptions and idealizing conditions are made. In reality, the receptor may be a region rather than a single moiety. The significance of these results may lie in the creation of a reasonable model which, although not proven to be reality, is isomorphic with reality. The value of such an isomorphic model lies in its abilities to explain and to predict.

EXPERIMENTAL

The calculations used were developed (8) and described previously (3–5). This approach was utilized in the first example of the prediction of a molecular conformation, specifically prostaglandin E_1 (9).

In this study, a series of acetylcholine derivatives in which the onium group was successively replaced by *tert*-butyl, isopropyl, ethyl, and methyl groups was selected. The reactivity against cholinesterase in terms of percentage of acetylcholine hydrolysis rates was measured in two separate studies (10).

The models simulating the α-site were chosen to reproduce the three proposed modes of interaction, *i.e.*, a carboxylate group (acetic acid) participating in an ionic interaction, a benzene ring participating in an ion-induced dipole interaction, and a *tert*-butyl (2,2-dimethylbutane) and methyl (ethane) group participating in a completely noncoulombic van der Waals interaction.

The modes of approach of the parent acetylcholine molecule and each model are shown in Schemes II–IV. The optimum distance separating the substrate model and the receptor model was chosen so that the entire series presented the best correlation with the hydrolysis rates. The distance separating the α-carbon atom of each member of the substrate series and the active site model was assumed to be constant for each test case of intermolecular distance. This is based on the assumption that the distal part of the molecule binds significantly to its complimentary feature (Scheme I), so that no matter what is appended on to the ethyl chain, the distance separating the active site from the α-methylene group will be constant.

RESULTS

The interaction energies of the model systems are given in Tables I–IV.

Using the thermodynamic formula $\Delta E = -RT \ln \rho$, the percentage of hydrolysis for the series of noncationic congeners compared

Table I—Interaction Energies (Kilocalories per Mole) Calculated between Acetic Acid-Ion and Acetylcholinesterase Substrate Models[a]

R	Distance, Å (Scheme II)			
	3.5[b]	3.75	4.0	4.25
$\overset{+}{N}(CH_3)_3$	12.50	11.49	9.99	8.51
$C(CH_3)_3$	3.48	4.26	4.04	3.49
$CH(CH_3)_2$	3.09	3.45	3.18	2.74
CH_2CH_3	2.68	2.64	2.32	1.94
CH_3	2.49	2.01	1.61	1.31

[a] Scheme II shows one typical mode of interaction listed in the table: R = $^+N(CH_3)_3$. [b] Set of values at this distance used to calculate the percentage of hydrolysis (Table V).

Table II—Interaction Energies (Kilocalories per Mole) Calculated between Benzene and Acetylcholinesterase Substrate Models[a]

R	Distance, Å (Scheme III)			
	5.0	5.25	5.5	5.75[b]
$\overset{+}{N}(CH_3)_3$	3.05	2.55	2.08	1.68
$C(CH_3)_3$	1.32	1.21	1.01	0.82
$CH(CH_3)_2$	1.05	0.94	0.78	0.63
CH_2CH_3	0.77	0.66	0.54	0.43
CH_3	0.48	0.39	0.30	0.24

[a] Scheme III shows one typical mode of interaction listed in the table: R = $^+N(CH_3)_3$. [b] Set of values at this distance used to calculate the percentage of hydrolysis (Table V).

Table III—Interaction Energies (Kilocalories per Mole) Calculated between *tert*-Butane and Acetylcholinesterase Substrate Models[a]

R	Distance, Å (Scheme IV)			
	5.75	6.0	6.25	6.5[b]
$\overset{+}{N}(CH_3)_3$	3.27	2.75	2.27	1.87
$C(CH_3)_3$	1.20	1.07	0.89	0.71
$CH(CH_3)_2$	0.95	0.83	0.69	0.55
CH_2CH_3	0.70	0.60	0.48	0.39
CH_3	0.44	0.35	0.27	0.22

[a] Scheme IV shows one typical mode of interaction listed in the table: R = $^+N(CH_3)_3$. [b] Set of values at this distance used to calculate the percentage of hydrolysis (Table V).

Table IV—Interaction Energies (Kilocalories per Mole) Calculated between Ethane and Acetylcholinesterase Substrate Models[a]

R	Distance, Å (Scheme IV)			
	5.0[b]	4.75	4.5	4.25
$\overset{+}{N}(CH_3)_3$	2.02	2.51	2.98	3.16
$C(CH_3)_3$	0.86	1.01	0.98	0.32
$CH(CH_3)_2$	0.67	0.80	0.82	0.45
CH_2CH_3	0.48	0.59	0.67	0.56
CH_3	0.28	0.37	0.49	0.66

[a] Scheme IV shows one typical mode of interaction listed in the table: R = $^+N(CH_3)_3$. [b] Set of values at this distance used to calculate the percentage of hydrolysis (Table V).

Table V—Experimental and Calculated Hydrolysis Rates (Percent) Relative to Acetylcholine

R	Experimental		Calculated from Model			
			Acetate	Benzene	*tert*-Butane	Ethane
$\overset{+}{N}(CH_3)_3$	100[a]	100[b]	100	100	100	100
$C(CH_3)_3$	24	60	4.37×10^{-5}	25	15	15
$CH(CH_3)_2$	14	24	2.4×10^{-5}	18	12	11
CH_2CH_3	7	16	1.23×10^{-5}	13	9	8
CH_3	3	10	0.91×10^{-5}	10	7	6

[a] Horse plasma cholinesterase. [b] Human erythrocytes cholinesterase.

to a 100% hydrolysis rate for the acetylcholine model was calculated. For this purpose, the set of data that fits best in view of the biological data was chosen for each model approach. Table V shows the values obtained together with the experimental hydrolysis rates of human erythrocytes and horse plasma cholinesterase.

Table V shows clearly that the anionic α-site model cannot explain the rather moderate differences in activity between the cationic and the noncationic acetylcholinesterase substrates, whereas the three uncharged α-binding site models behave much more realistically in terms of the hydrolysis rates. These results support the suggestion of O'Brien (2) that an anionic binding site is not a good assumption. The benzene model provides the best fitting set of values in this series, but the superiority over the two nonaromatic α-site models is not significant enough to decide clearly between an ion-induced dipole or a van der Waals interaction binding mechanism. However, the results of this study make an ion-induced dipole interaction at the α-binding site of the acetylcholinesterase, typified by a benzene ring, most likely.

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Microbiological Determination of Drug Partitioning IV: Drug–Protein Interactions

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Abstract □ The protein binding characteristics of chloramphenicol, furazolium chloride, benzalkonium chloride, and phenylmercuric nitrate were described from their partitioning behavior in gelatin-acacia complex coacervate systems. Although the partitioning was determined by two different methods (microbiological and chemical), the microbiological method was more reliable for this type of investigation. Drug-protein parameters were calculated for the four antimicrobials. The advantages of the coacervate systems over other models for protein binding studies of drugs are discussed.

Keyphrases □ Protein binding characteristics—chloramphenicol, furazolium chloride, benzalkonium chloride, and phenylmercuric nitrate, partitioning behavior in gelatin–acacia complex coacervate systems, microbiological determination □ Drug–protein interactions—microbiological determination of drug partitioning, gelatin–acacia complex coacervate systems, four antimicrobial agents □ Drug partitioning—microbiological determination, drug–protein interactions, gelatin–acacia complex coacervate systems □ Microbiology—determination of drug partitioning, drug–protein interactions, four antimicrobials, gelatin–acacia complex coacervates

Binding of drugs to the protein constituents in biological systems has been demonstrated to have a tremendous effect on the ultimate action of drugs in these systems (1). Different model systems, including dialysis membranes (2–5), lipid-like organic solvents (6–10), more polar liquids (11–18), and other types of systems (19–33), have been used in the study of protein binding drugs. The use of organic solvent systems for the study of protein binding involves the tacit assumption that the organic solvents closely