of araC phosphorylation. The only other site of such reversal presently described is the competition for DNA polymerase noted above. The likelihood of competition between araC and CdR for entry into cells seems remote.[4]

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[4] A study of araC transport in a sub-line of the L1210 mouse leukemia lacking CdR kinase, to be published elsewhere, indicated that the drug transport process was not saturated, at 37°, until nucleoside levels were far in excess of those employed therapeutically.

The Nature of Inhibitor Binding Sites in Butyrylcholinesterase

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SUMMARY

Evidence suggesting the presence of a specific "hydrophobic" binding site in horse serum butyrylcholinesterase (BuChE) is presented. The data indicate that the site exhibits definite size limitations. Assuming van der Waals forces to be dominant in the binding of the alkyl side chain of some N^1-alkylnipecotamide homologs to BuChE, an enzyme-alkyl chain distance of the complex of 4.1 A can be calculated for a C_4–C_{10} chain.

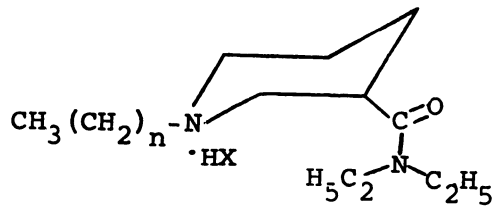
The controversy regarding the existence of an "anionic" site in butyrylcholinesterase (BuChE; acylcholine acylhydrolase, EC 3.1.1.8) has recently been discussed in a paper by Augustinsson (1) in which he proposed that this site is a "nonesteratic" site. The dominant factor in enzyme-substrate or enzyme-inhibitor complex formation is attributed to van der Waals forces (1).

Belleau (2, 3), in developing his macromolecular perturbation theory (MPT) of

drug-receptor interactions, has implicated van der Waals and hydrophobic interactions in specific conformational perturbations (SCP) and primarily hydrophobic forces in nonspecific conformational perturbations (NSCP).

Our interests in delineating the active sites of cholinesterases through structure activity correlation studies have yielded evidence[1] suggestive of a rather specific "hydrophobic" binding site in horse serum butyrylcholinesterase and supports the proposals of Augustinsson (1) and Belleau (3). This evidence, however, does not negate the existence of an "anionic" site.

Beasley and Christian,[1] in studying the effect on BuChE inhibition of increased alkyl chain length in a series of N^1-alkylnipecotamide homologs (I), showed



n = 3-11

(I)

that the value for the inhibitor dissociation constants (K_i) varied inversely with the alkyl chain length (Fig. 1). Changes in the free-energy of binding were calculated from Eq. 1 (ref. 4).

$$\Delta(\Delta F) = 2.3\,RT\,\Delta pK_i \qquad (1)$$

For the C_4–C_{10} ($n = 3$ to 9) homologs, the change in free-energy of binding per methylene group is 0.39 kcal/mole, and for the C_{10}–C_{12} ($n = 9$ to 11) compounds it is 0.13 kcal/mole. The value of 0.39 kcal/mole/CH_2 falls within the range of 0.36–0.95 kcal/mole/CH_2 associated with dispersion force interactions (5); therefore, it is interesting to consider the possibility that such increases in binding energy may be due to van der Waals forces alone.[2] Based on this assumption, Pauling and Pressman's equation (Eq. 2) derived for

$$\Delta W = (4 \times 10^5)(R_A - R_H)/r^6 \qquad (2)$$

the change in van der Waals energy, ΔW, for interactions between haptenes and antibodies (7) may be applied. The change in energy arises from the substitution of a group A for hydrogen; R_A and R_H are the molar refractions of the group A and hydrogen, and r is the distance between the molecule and the protein.

If one substitutes the change in the free-energy of binding per methylene group for ΔW, the molar refraction of a CH_3 group, 5.71 cm^3 (8), for R_A, and the molar refraction of hydrogen, 1.10 cm^3 (8), for R_H, a distance of 4.1 A is obtained for the C_4–C_{10} chain and a value of 4.9 A is found for the C_{10}–C_{12} tail. Therefore, one may interpret these data as indicative of van der Waals interactions since 4 A is of the right order of magnitude for van der Waals forces; the distance of intermolecular contact between two methylene groups, for example, is 3.96 A (9) or 4.2 A (5).

The fact that the free-energy of binding per methylene group is *less* for the C_{10}–C_{12} segment of the alkyl chain supports the model of a specific binding site.[3] It also

[1] Presented in part at the Nineteenth Southeastern Regional Meeting of the American Chemical Society, Atlanta, Georgia, November, 1967.

[2] Benjamin (6) in studies of the micellization of dimethyl-*n*-alkylamine oxides has shown that the entropy term is essentially independent of alkyl chain length. He suggests that the hydrocarbon chain curls up in order to reduce the degree of contact with water. A similar mechanism may be responsible for the experimental observations reported here. Further, since hydrophobic binding depends upon the entropy term, one may conclude that the hydrophobic interaction associated with the alkyl chain is constant with increasing chain length. Based on this comparison, one may assume that any observable changes in ΔF with increasing chain length may be associated with van der Waals forces.

[3] A constant or increasing $\Delta F/CH_2$ might indicate a nonpolar region of unlimited size or the influence of hydrophobic forces; a decrease in $\Delta F/CH_2$ is consistent with a partial saturation of the relatively specific "hydrophobic" site.

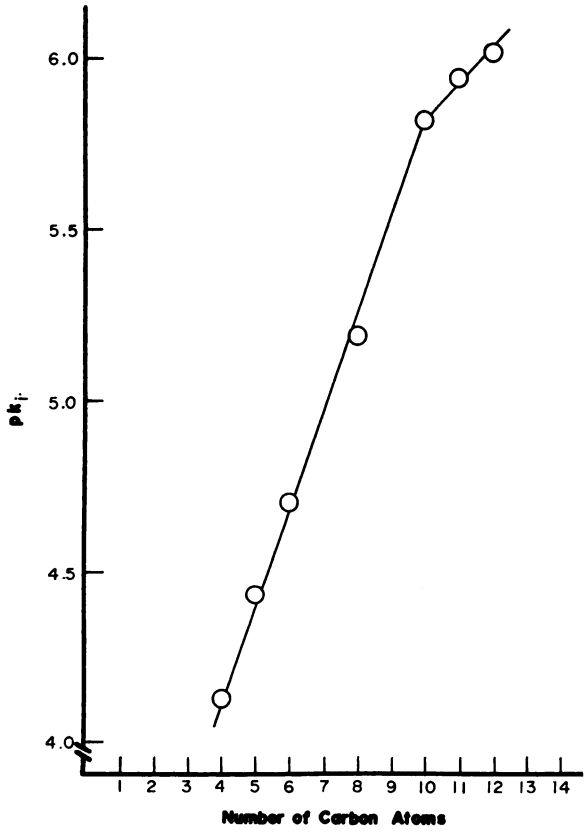


FIG. 1. *pK_i vs. the number of carbon atoms in the alkyl side chain of some N^1-alkylnipecotamides.* K_i values were determined titrimetrically at 26°C and pH 7.4 using the method of Beasley, *et al.* (J. G. Beasley, W. R. Smithfield, and L. L. Williford, *J. Med. Chem.* **10**, 1003, 1967), with acetylcholine as substrate.

suggests that the site has a limited spatial requirement and that the decrease in binding energy per methylene group may be attributed to folding or curling of the alkyl chain (6).

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