BIOORGANIC CHEMISTRY 17, 79–85 (1989)

Spatial Orientation of *n*-Alkanesulfonyl Chlorides in the Active Center of Cholinesterases

A. SEPP, P. PALUMAA, U. LANGEL, J. JÄRV, AND M. ZORKO*

*Laboratory of Bioorganic Chemistry, Tartu State University, 202400 Tartu, Estonian SSR, USSR, and *Institute of Biochemistry, Medical Faculty, University of Ljubljana, Ljubljana, Yugoslavia*

Received April 20, 1988

Acetylcholinesterase and butyrylcholinesterase inhibition by *n*-alkanesulfonyl chlorides $C_nH_{2n+1}SO_2Cl$ at $n = 1–4$ has been investigated at the level of second-order rate constants. It has been found that an increase in chain length of the alkyl group causes a decrease in their reactivity in the case of acetylcholinesterase and an increase in the case of butyrylcholinesterase. On the grounds of similar structure–activity relationships for *n*-alkanesulfonyl chlorides and ester substrates with variable acyl parts, it has been shown that the alkyl substituents of inhibitors interact with the areas responsible for the accommodation of the acyl part of the ester substrates in the active center of cholinesterases. It is proposed that this special orientation of the alkanesulfonyl chlorides in the active center of these enzymes is stabilized by two hydrogen bonds to the oxygen atoms of the inhibitor molecule.

INTRODUCTION

Proceeding from the structure–activity relationships of the substrates and organophosphorus inhibitors of acetyl- and butyrylcholinesterases, a spatial model of the active center of these enzymes has been proposed by Järv (*1*). According to this model the esteratic center of cholinesterases is surrounded by three areas, revealing different topography and hydrophobic properties. Because alkanesulfonyl chlorides are supposed to sulfonylate the active center of cholinesterases, the above-mentioned model was used to analyze the orientation of the alkanesulfonyl chloride molecules in the active center of these enzymes. It has been found that the alkyl substituents of alkanesulfonyl chlorides interact with the area responsible for the accommodation of the acyl part of the ester substrates. The structural background of this spatial requirement is discussed.

EXPERIMENTAL

Acetylcholinesterase (EC 3.1.1.7) from cobra (*Naja naja oxiana*) venom was purified by affinity chromatography at the Institute of Chemical and Biological Physics of the Academy of Sciences of the Estonian SSR (Tallinn) and the enzyme preparation used was a generous gift from Dr. Raivo Raba. Butyrylcholinesterase

0045-2068/89 $3.00

(EC 3.1.1.8) from horse serum was purchased from the Mechnikov Institute of Sera and Vaccines (Moscow) and was used without further purification. The enzyme stock solutions were prepared in 0.15 M KCl and stored at 4°C. All alkanesulfonyl chlorides $C_nH_{2n+1}SO_2Cl$ (n = 1–4, Kodak, USA) were used after microdistillation and their solutions in acetonitrile were prepared immediately before kinetic experiments. All other chemicals of analytical grade were obtained from Reakhim (USSR).

The enzyme sulfonylation reactions were carried out in 0.15 M phosphate buffer (pH 7.5) containing of 2.5% (v/v) acetonitrile. The enzyme inhibition reaction was stopped by diluting the samples (15 μl) into the enzyme assay solution (3 ml).

The activity of the cholinesterases was followed spectrophotometrically as described by Ellmann *et al.* (*2*) by measuring the initial rate of the enzymatic hydrolysis of 1 mM acetylthiocholine. All kinetic measurements were performed on a Perkin–Elmer 402 spectrophotometer in temperature-controlled cells at 25°C.

The bimolecular rate constants for the sulfonylation reaction k_i were determined according to the reaction scheme

$$E + I \xrightarrow{k_i} EI', \qquad [1]$$

where E is enzyme, I is alkanesulfonyl chloride, and EI′ is sulfonylated enzyme, by means of

$$k_i = -\frac{1}{t[I]_0} \ln \frac{v_0}{v}, \qquad [2]$$

where v_0 is the initial rate of the substrate hydrolysis, v is the rate at time t (in seconds) and $[I]_0$ the initial concentration of the inhibitor.

The pseudo-first-order rate constants of the hydrolysis of the alkanesulfonyl chlorides were measured titrimetrically on a Radiometer pH-stat (pHM-82, TTT-80, ABU-80, REC-80) in 0.15 M KCl from pH 7.5 to 9 at 25°C. The rate constants of alkaline and neutral hydrolysis were calculated from these data according to

$$k_s = k_{H_2O} + k_{OH^-}[OH^-]. \qquad [3]$$

Data processing was performed on a IBM PC/XT computer using linear and nonlinear regression programs.

RESULTS

It was found that the reaction of cholinesterases with alkanesulfonyl chlorides did not proceed as a pseudo-first-order reaction when the reaction time exceeded 2 min. These deviations turned out to be caused by the spontaneous hydrolysis of the alkanesulfonyl chlorides. Therefore shorter reaction times from 0.5 to 2 min were used. Under these conditions the influence of the spontaneous hydrolysis of alkanesulfonyl chlorides on enzyme inhibition can be eliminated and the reaction follows first-order kinetics (Fig. 1).

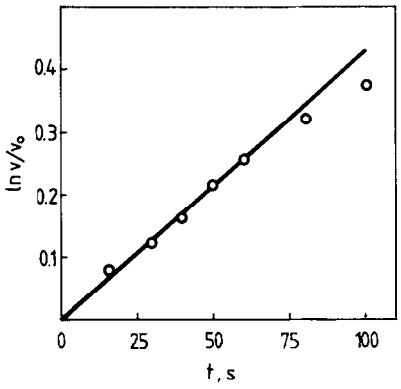


FIG. 1. Acetylcholinesterase inhibition by 6 mM propanesulfonyl chloride.

The pseudo-first-order rate constants, calculated from the slope of the straight line in the coordinates $\ln(v/v_0)$ versus t, were found to depend linearly upon the initial concentration of the alkanesulfonyl chlorides up to the solubility limits of these reagents (Fig. 2). This allowed us to calculate the second-order rate constants for both cholinesterases from the slopes of these linear dependences (Table 1).

An increase in the length of the *n*-alkyl group of the alkanesulfonyl chlorides caused a decrease in the reactivity of these reagents in the case of acetylcholinesterase, and an increase in the case of butyrylcholinesterase (Fig. 3).

The intrinsic reactivity of alkanesulfonyl chlorides, which depends upon the structure of the alkyl substituent, is one of the factors influencing the rate of enzyme sulfonylation. The latter effect can be characterized by the rate of the alkaline hydrolysis of these compounds. It was found that the reactivity of ethane-, propane-, and butanesulfonyl chlorides was quite similar to, but lower than, that for methanesulfonyl chloride (Table 1). The same trend could be found

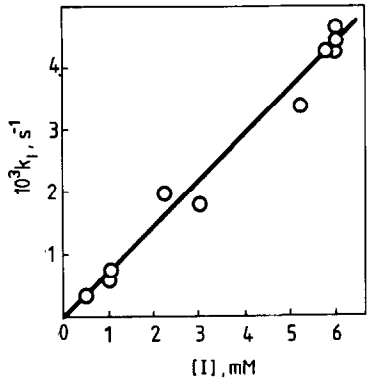


FIG. 2. Plot of the pseudo-first-order rate constant of the acetylcholinesterase inhibition reaction vs propanesulfonyl chloride concentration.

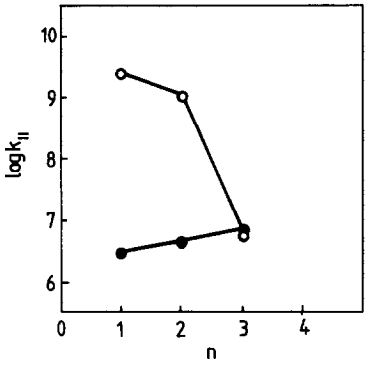


FIG. 3. Structure–activity relationships for acetylcholinesterase (○) and butyrylcholinesterase (●) inhibition with a series of alkanesulfonyl chlorides $C_nH_{2n+1}SO_2Cl$; n = 1–4.

in the case of the substituent constants σ^* and E_s for the alkyl substituents, which do not depend upon the length of the n-alkyl groups beginning from the ethyl group (*3*).

DISCUSSION

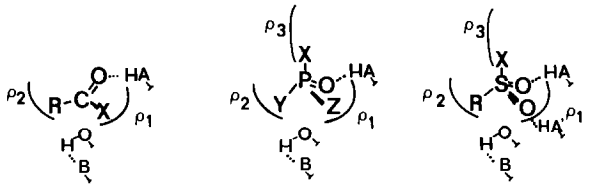
The structure–activity relationships obtained for the sulfonylation reaction of acetyl- and butyrylcholinesterases are different. Thus, the anticholinesterase activity of alkanesulfonyl chlorides cannot be explained using only the conventional parameters of the structural effects quantified by the inductivity, steric, and hydrophobicity constants, but is closely related to some properties of the enzyme active center and seems to be governed by some specific interactions of the alkyl groups with the protein molecules.

Analogously to the situation for organophosphorus compounds, the stereochemical configuration of the reaction center of alkanesulfonyl chlorides is tetrahedral, but the latter compounds cannot possess chirality. Because the enzyme inhibition by alkanesulfonyl chlorides proceeds as a nucleophilic substitution re-

TABLE 1

Reaction of n-Alkanesulfonyl Chlorides $C_nH_{2n+1}SO_2Cl$ with Cholinesterases and Their Alkaline and Spontaneous Hydrolysis

	k_i (M^{-1} s^{-1})			
n	Acetylcholinesterase	Butyrylcholinesterase	k_{OH^-} (M^{-1} s^{-1})	$10^3 * k_{H_2O}$ (s^{-1})
1	13.1 ± 1	0.29 ± 0.03	14400 ± 900	1.83 ± 0.2
2	6.0 ± 0.2	1.45 ± 0.02	234 ± 15	1.83 ± 0.2
3	0.71 ± 0.03	7.76 ± 0.2	234 ± 15	1.83 ± 0.2
4	0.4 ± 0.03	17.4 ± 0.4	234 ± 15	1.83 ± 0.2



1 2 3

action, the leaving group of these reagents must also be positioned on the side opposite to the attacking group, i.e., in the locus ρ_3 of the enzyme active center (Schemes **1, 2** and **3**). In this configuration the *n*-alkyl group of *n*-alkanesulfonyl chlorides must be located in either the subsite ρ_1 or the subsite ρ_2. Because these sites have been characterized in the case of cholinesterases by different structure–activity relationships (Fig. 4), it is possible to specify the location of the alkyl substituent of the alkanesulfonyl chloride molecule on the active surface of these enzymes (*1*).

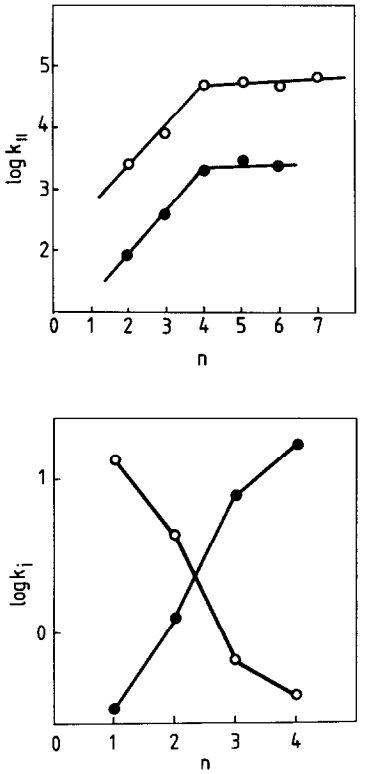


FIG. 4. Comparison of acetylcholinesterase (○) and butyrylcholinesterase (●) specificities in their reactions with ester substrates: (A) $CH_3C(O)OC_nH_{2n+1}$. Data are taken from Refs. (*4, 5, 12*). (B) $C_nH_{2n+1}C(O)SC_2H_4N^+(CH_3)_3$. Data are taken from Refs. (*6, 7*).

Comparison of the structure–activity relationships of ester substrates with variable acyl parts and alkanesulfonyl chlorides (Figs. 3 and 4B) clearly points to the similarity of these dependences. Consequently, the set of structural factors determining the specificity of the enzymes in these reactions is common for both types of compounds and the variable alkyl groups R of the choline esters $RCOSC_2H_4N^+(CH_3)_3$ and the *n*-alkanesulfonyl chlorides RSO_2Cl interact with the same area ρ_2 near the esteratic center.

Thus, by comparing the structure–activity relationships of the cholinesterase-catalysed hydrolysis of ester substrates and the inhibition reaction of these enzymes by means of a series of *n*-alkanesulfonyl chlorides, it has been possible to draw conclusions about the spatial location of the latter compounds in the active center of cholinesterases.

In the case of butyrylcholinesterase, the spatial orientation of the alkyl group of the inhibitor molecule in the ρ_2 locus is supported by the favorable hydrophobic binding effect because this site reveals hydrophobic properties resulting from the increase in the reactivity of the compounds with alkyl substituents. On the other hand, the location of the *n*-alkyl groups of the alkanesulfonyl chlorides in the ρ_2 site of acetylcholinesterase are probably not supported by the hydrophobic effect as an increase in the length of the acyl part of the ester substrates causes a decrease in their activity (*1*). For this reason the results described above point to the possibility of the existence of some additional stabilizing factors, which support the spatial orientation of the alkanesulfonyl chloride molecule in the active center, as shown in **3.** The latter conclusion seems to be important, because the *n*-alkyl group of the inhibitor might be located at the ρ_1 site, which also provides favorable mutual positioning of the reaction center of the inhibitor molecule and the nucleophilic group of the enzyme active center.

The factors determining the orientation of the inhibitor molecule in the active center of acetylcholinesterase must meet with the stereochemical requirements of the reaction mechanism of the enzyme sulfonylation step following the formation of the "productive" enzyme–reagent complex. In the case of *n*-alkanesulfonyl chlorides, we propose that both oxygen atoms of the sulfonyl group are fixed in the active center by two hydrogen bonds as shown in **3.** It is noteworthy that in this model these oxygen atoms are located in a position similar to that of the C=O and C—O—C oxygen atoms of ester substrates (Scheme **1**). The latter conclusion is compatible with the idea of the important role of the carbonyl oxygen atom in the enzyme acylation mechanism, as thiono esters C=S groups are not hydrolyzed by the enzyme (*8*). On the other hand, however, interaction of the second oxygen atom of the ester group with the enzyme is not considered to be important for the acylation mechanism, because thiol esters are good substrates of cholinesterases (*8*). Therefore, there are some contradictions between the latter data and the results of the present studies, although the participation of two acidic groups in the enzyme acylation reaction has been discussed by Krupka (*9*) on the basis of experimental data about pH dependences of the acetylcholinesterase-catalyzed substrate hydrolysis.

In summary, it is important to note that independently of the structural reasons for the above-mentioned orientation of the molecules of the *n*-alkanesulfonyl chlo-

rides in the active center of cholinesterases, it can be concluded that the binding site ρ_1 for the leaving group of specific substrates is not occupied in the enzyme sulfonylation reaction. Consequently, the anionic site of cholinesterases responsible for the specific recognition of choline esters, and putatively located in the vicinity of this locus on the enzyme active surface, can bind the anionic site-directed cationic ligands independently of the enzyme interaction with alkanesulfonyl chlorides. The latter fact gives us a chance to study the functional interrelationship between the esteratic and anionic sites of cholinesterases through their simultaneous interaction with *n*-alkanesulfonyl chlorides and tetraalkylammonium ions, respectively. Indeed, the reactions of cholinesterases with methanesulfonyl fluoride, if carried out under the second-order conditions, can be accelerated by alkylammonium ions, as shown by Pavlic *et al.* (*10, 11*).

REFERENCES

1. JÄRV, J. (1984) *Bioorg. Chem.* **12,** 259–278.
2. ELLMANN, G. L., COURTNEY, K. D., ANDUS, V., JR., AND FEATHERSTONE, R. M. (1961) *Biochem. Pharmacol.* **7,** 88–95.
3. PALM, V. A. (Ed.) (1975) Tables of Rate and Equilibrium Constants of Heterolytic Organic Reactions 5, p. 163, VINITI Press, Moscow.
4. JÄRV, J., KESVATERA, T., AND AAVIKSAAR, A. (1976) *Eur. J. Biochem.* **67,** 315.
5. JÄRV, J., AND LANGEL, U. (1979) *Bioorg. Khim.* **5,** 764.
6. BRESTKIN, A. P., ZHUKOVSKI, YU. G., AND SIPENKOVA, T. M. (1974) *Biokhimiya* **39,** 13–19.
7. KESVATERA, T., USTAV, M., AND AAVIKSAAR, A. (1978) *Comp. Biochem. Physiol.* **62C,** 193–197.
8. BRESTKIN, A. P., AND ROZENGART, E. V. (1965) *Nature* (*London*) **205,** 388–389.
9. KRUPKA, R. M. (1964) *Canad. J. Biochem.* **42,** 677–693.
10. PAVLIC, M. R. (1973) *Biochim. Biophys. Acta* **327,** 393–397.
11. PAVLIC, M. R. (1984) *Per. Biologorum* **86,** 343–348.
12. PEDAK, A. E. (1987) *in* Abstracts of the VII Conference of Young Chemists 1, p. 14, Bit Press, Tallinn.