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THE ACCELERATION OF METHANESULFONYLATION OF ACETYLCHOLINESTERASE WITH CATIONIC ACCELERATORS AS AN ELECTROSTATIC EFFECT

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Summary

1. In order to check our hypothesis of the electrostatic nature of the acceleration of methanesulfonylation of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) with cationic accelerators, equations were solved for methanesulfonylation with two accelerators and the reaction was studied in the presence of some single accelerators, including the sodium cation, and in the presence of two accelerators simultaneously.

2. The second-order rate constants for methanesulfonylation of the complexes between the enzyme and accelerators decamethonium, tetraethylammonium and tetramethylammonium are 90, 88 and 17 $1 \cdot mol^{-1} \cdot s^{-1}$, respectively, which corresponds to a maximal acceleration of 29, 28 and 5.5 times, respectively. The dissociation constants for the binding of these accelerators to the enzyme, obtained from our acceleration experiments, are $3.7 \cdot 10^{-6}$, $3.2 \cdot 10^{-4}$ and $1.4 \cdot 10^{-3}$ M, respectively. These values are in good agreement with the dissociation constants of these ligands as inhibitors of acetylcholinesterase. It is interesting to note that the sodium cation also accelerates the methanesulfonylation up to around three times, the corresponding second-order rate constant and the dissociation constant being 10 $1 \cdot mol^{-1} \cdot s^{-1}$ and 1.3 M, respectively.

3. All tested cations compete in the acceleration with each other; they seem to accelerate the reaction in the same way and from the same site, the catalytic anionic site.

4. These findings confirm the hypothesis of the electrostatic nature of acceleration.

Introduction

In 1963 Kitz and Wilson [1] found that some reversible cationic competitive inhibitors of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7), such as tetraethylammonium, accelerate irreversible sulfonylation of this enzyme by methanesulfonylfluoride. An elucidation of the mechanism of this acceleration would be important because the same acceleration mechanism may be involved in the hydrolysis of acetylcholine with acetylcholinesterase (see ref. 2), as well as in some other acceleration phenomena.

Our findings [3—5] suggested that the acceleration effect of an accelerator on methanesulfonylation of acetylcholinesterase is an electrostatic effect: the cationic accelerator, bound to the anionic site of the enzyme, electrostatically effects the methanesulfonylation at the esteratic site. If the acceleration effect is electrostatic indeed, one would expect that, in the first place, the acceleration site would be near the esteratic site. The nearest anionic site is, of course, the catalytic anionic site which is approx. 5 Å away. So, the catalytic anionic site should be the site of acceleration. In the second place, all or at least many cations should accelerate the methanesulfonylation in the same manner; in doing so, they should compete with each other. Consequently, in order to check our hypothesis of the electrostatic nature of the acceleration, the competition between various cationic accelerators in accelerating methanesulfonylation of acetylcholinesterase was investigated.

Methods and Materials

Methanesulfonylation of acetylcholinesterase takes place at the esteratic site of the enzyme [6]. The acceleration with a cationic reversible accelerator arises presumably from the binding of the accelerator at an independent anionic site [1]. So the methanesulfonylation in the presence of one accelerator follows the scheme:

$$\mathrm{E} + \mathrm{I} \xrightarrow{k} \mathrm{E}' \tag{1}$$

$$\mathrm{E} + \mathrm{A} \overset{K_\mathrm{A}}{\rightleftharpoons} \mathrm{EA} \tag{2}$$

$$\mathrm{EA} + \mathrm{I} \xrightarrow{k_\mathrm{A}} \mathrm{E}' \tag{3}$$

where E is the enzyme, I the irreversible inhibitor methanesulfonylfluoride, E′ the acylated enzyme, A the accelerator, EA the complex enzyme · accelerator, k the second-order rate constant for methanesulfonylation, K_A the dissociation constant for the binding of accelerator A to the enzyme and k_A the second-order rate constant for methanesulfonylation of the enzyme · accelerator complex.

The rate equation for methanesulfonylation without accelerator is [1]

$$\ln \frac{[\mathrm{E}]}{[\mathrm{E}]_0} = -k[\mathrm{I}]t = -k_\mathrm{app}t \tag{4}$$

and the rate equation for the reaction with accelerator A in a concentration which does not saturate the enzyme [1]

$$\ln \frac{[\mathrm{E}]}{[\mathrm{E}]_0} = -k \frac{1 + \dfrac{k_\mathrm{A}}{k}\dfrac{[\mathrm{A}]}{K_\mathrm{A}}}{1 + \dfrac{[\mathrm{A}]}{K_\mathrm{A}}}[\mathrm{I}]t = -k_\mathrm{a}[\mathrm{I}]t \tag{5}$$

where [E] is the concentration of the uninhibited enzyme, $[E]_0$ the total concentration of the enzyme, t time, k_{app} the apparent first-order rate constant, [A] the concentration of accelerator A and k_a the second-order rate constant for methanesulfonylation in the presence of accelerator A in concentration [A]; the values of k_a, of course, are lower than that of k_A but they approach k_A with higher concentrations of A.

It follows from Eqn. 5 that

$$\frac{k_a - k_A}{k - k_a} = K_A \frac{1}{[A]} \tag{6}$$

which makes possible a graphical determination of K_A from experiments with and without a single accelerator.

For methanesulfonylation in the presence of two accelerators A and B, the reaction scheme represented in Eqns. 1—3 was extended with equations:

$$E + B \overset{K_B}{\rightleftharpoons} EB \tag{7}$$

$$EB + I \xrightarrow{k_B} E' \tag{8}$$

where EB is the complex between the enzyme and accelerator B, K_B the dissociation constant for B, and k_B the second-order rate constant for methanesulfonylation of the enzyme saturated with B. The scheme characterized by Eqns. 1—3, 7, 8 applies to a system where the binding of the irreversible inhibitor methanesulfonylfluoride takes place at one active site and the binding of the accelerator at another independent site, which is the same for both accelerators.

A derivation of the rate equation for the Scheme 1—3, 7, 8 yielded the following expression:

$$\ln \frac{[E]}{[E]_0} = -k \frac{1 + \frac{k_A}{k}\frac{[A]}{K_A} + \frac{k_B}{k}\frac{[B]}{K_B}}{1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}} [I]t = -k_{ab}[I]t \tag{9}$$

in which k_{ab} is the second-order rate constant for methanesulfonylation in the presence of two competing accelerators A and B in concentrations [A] and [B], respectively.

Eqn. 9 makes it possible to check whether or not two accelerators compete with each other in accelerating the methanesulfonylation of acetylcholinesterase.

The first step in our investigations was the determination of the second-order rate constants k_a for methanesulfonylation of acetylcholinesterase in the presence of one accelerator. Each second-order rate constant was determined essentially as already described [3,6]. The enzyme was incubated for various lengths of time with methanesulfonylfluoride in various concentrations, with added accelerators or without them, and the remaining activity determined according to the method of Ellman et al. [7]. Subsequently, the first-order rate constant was calculated for each concentration of methanesulfonylfluoride from the time dependence of methanesulfonylation; three first-order rate constants were always determined for three different concentrations. Then, the second-order rate constant was determined from the dependence of the first-order rate constant on the concentration of methanesulfonylfluoride. Five to seven second-order rate constants were determined for the same number of different concentrations of each accelerator.

Three accelerators were tested: decamethonium, tetraethylammonium and tetramethylammonium. They were chosen because they are good accelerators and because they bind to different anionic sites (see Discussion). Additionally, sodium cation was tested, too, because this cation was supposed to act electrostatically, being a simple and also an "inert" (see refs. 8—11) ion. The first three cations were used as iodides, and Na^+ added as NaCl.

The concentrations of dedamethonium, tetraethylammonium, tetramethylammonium and sodium ranged from 0 to $2 \cdot 10^{-4}$, 0 to $1 \cdot 10^{-2}$, 0 to $1 \cdot 10^{-1}$ and 0 to 2 M, respectively. The concentration of methanesulfonylfluoride ranged from 0.2 to 2 mM; in experiments with the strong accelerators decamethonium and tetratehylammonium the concentrations were ten times lower.

From the dependence of k_a on the concentration of accelerator, the second-order rate constant for methanesulfonylation of the enzyme · accelerator complex, k_A, was determined by extrapolation to saturating concentrations of accelerator.

The dissociation constants, K_A, were determined graphically by means of Eqn. 6.

Competition between accelerators was tested by following methanesulfonylation in the presence of two accelerators simultaneously. The concentration of the first accelerator in each pair was kept constant while the concentration of the second one was varied, and vice versa. The second-order rate constants obtained for methanesulfonylation in the presence of each pair of accelerators were compared to the corresponding second-order rate constants k_{ab} obtained by means of Eqn. 9. All possible pairs from decamethonium, tetraethylammonium and tetramethylammonium and the pair decamethonium-sodium were used.

All experiments were carried out at 25°C. The universal buffer solution of Britten and Robinson [12—14] was used; its ionic strength was adjusted to 0.2 M by addition of NaCl. The pH of the buffer was 8.4, which is the optimal value for methanesulfonylation of acetylcholinesterase at 25°C [6].

The enzyme used was acetylcholinesterase from the electric organ of *Electrophorus electricus*, Worthington, ECHP 55C469, 4000 units/mg. The stock solution of the enzyme contained 0.1 mg of the preparation in 1 ml of the universal buffer solution. Methanesulfonylfluoride was purchased from Eastman Organic chemicals. The stock solution of methanesulfonylfluoride was a 50 mM solution of the inhibitor in spectroquality acetone.

Results

The effect of decamethonium on methanesulfonylation of acetylcholinesterase is shown in Fig. 1. The second-order rate constant for methanesulfonylation of enzyme · decamethonium complex, k_A, determined from Fig. 1 by extrapolation, and the dissociation constant for decamethonium, K_A, deter-

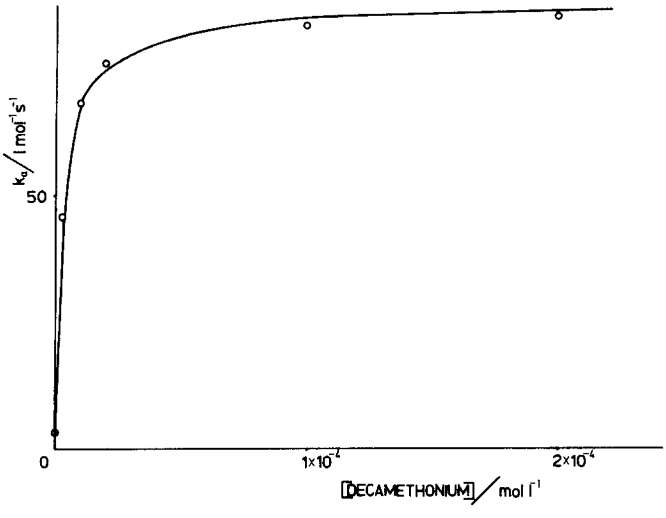


Fig. 1. Effect of decamethonium on methanesulfonylation of acetylcholinesterase. k_a is the second-order rate constant for methanesulfonylation of acetylcholinesterase in the presence of accelerator. Each point represents the mean value from three determinations. The curve in the figure is theoretical, calculated by means of Eqn. 5 (see text) and using the corresponding constant from Table I.

mined graphically by means of Eqn. 6 are given in Table I, together with the corresponding maximal acceleration, k_A/k.

The values of these constants for tetraethylammonium, tetramethylammonium and sodium, obtained in the same manner from analogous diagrams (not shown) are also presented in Table I.

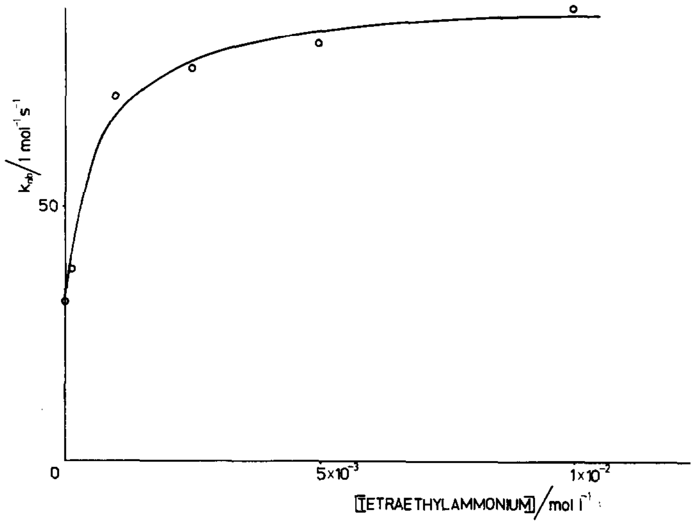
Methanesulfonylation in the presence of pairs of accelerators decamethonium plus tetraethylammonium, tetramethylammonium or sodium, respectively, is shown in Figs. 2—7.

TABLE I

CHARACTERISTIC CONSTANTS FOR METHANESULFONYLATION OF ACETYLCHOLINESTERASE IN THE ABSENCE AND PRESENCE OF SOME CATIONIC ACCELERATORS

k is the second-order rate constant for methanesulfonylation, k_A the second-order rate constant for methanesulfonylation of enzyme · accelerator complex, K_A the dissociation constant for the binding of the cation to the acceleration site of the enzyme, obtained as explained in the text, and K_I the dissociation constant for the binding of the cation to the inhibition site of the enzyme, obtained from the inhibition of the hydrolysis of acetylcholine [15—17].

Methanesulfonylation in the presence of	k ($l \cdot mol^{-1} \cdot s^{-1}$)	k_A ($l \cdot mol^{-1} \cdot s^{-1}$)	$\frac{k_A}{k}$	K_A ($mol \cdot l^{-1}$)	K_I ($mol \cdot l^{-1}$)
No accelerator	3.1				
Decamethonium		90	29	$3.7 \cdot 10^{-6}$	$1.8 \cdot 10^{-6}$
Tetraethylammonium		88	28	$3.2 \cdot 10^{-4}$	$2.5 \cdot 10^{-4}$
Tetramethylammonium		17	5.5	$1.4 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$
Na^+		10	3.2	1.3	



Figs. 2—7 Methanesulfonylation of acetylcholinesterase in the presence of pairs of accelerators. k_{ab} is the second-order rate constant for methanesulfonylation in the presence of a pair of accelerators. Each point represents the mean value from three determinations. The curves in the figures are theoretical, calculated by means of Eqn. 9 (see text) and using the corresponding constants from Table I.

Fig. 2. (decamethonium) constant, $2 \cdot 10^{-6}$ M, (tetraethylammonium) varied.

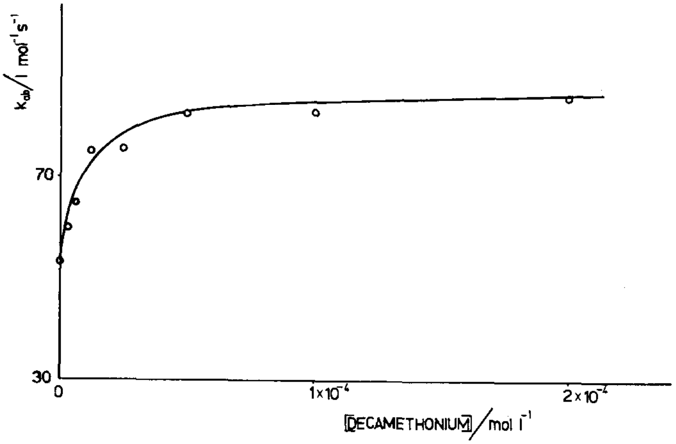


Fig. 3. (tetraethylammonium) constant, $5 \cdot 10^{-4}$ M, (decamethonium) varied.

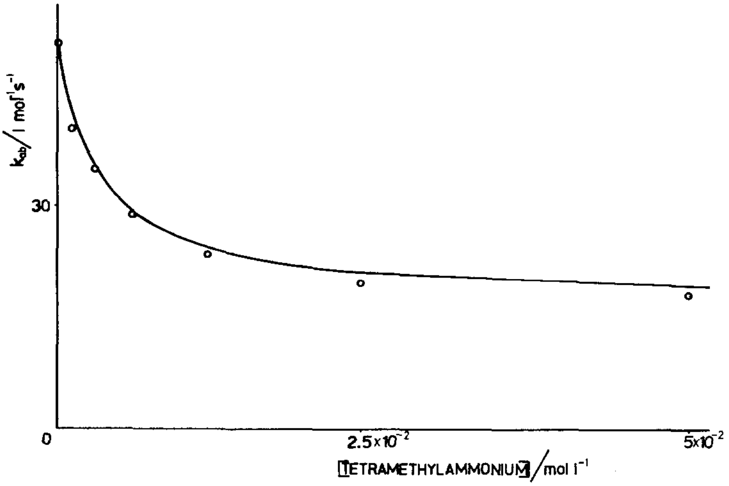


Fig. 4. (decamethonium) constant, $5 \cdot 10^{-6}$ M, (tetramethylammonium) varied.

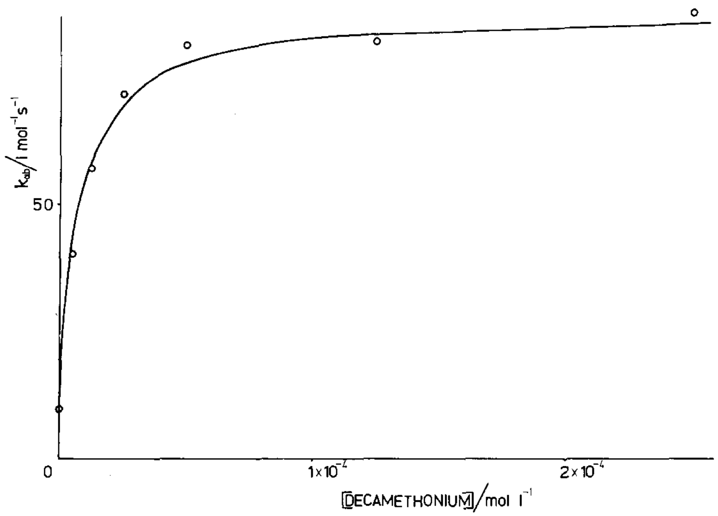


Fig. 5. (tetramethylammonium) constant, 1.5 · 10^{-3} M, (decamethonium) varied.

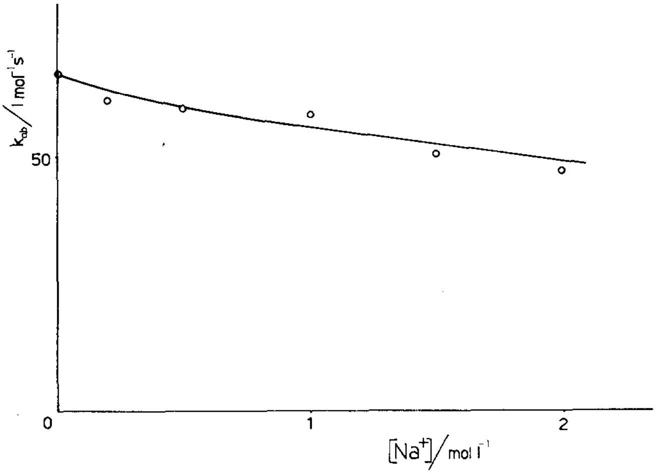


Fig. 6. (decamethonium) constant, 1 · 10^{-5} M, (Na^+) varied.

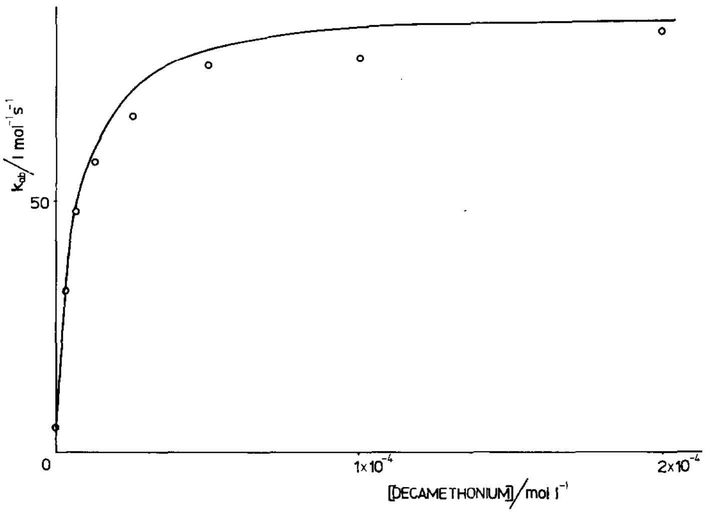


Fig. 7. (Na^+) constant, 3.75 · 10^{-1} M, (decamethonium) varied.

Discussion

It can be seen from Fig. 1 and Table I that decamethonium, tetraethylammonium and tetramethylammonium, as known (see refs. 1 and 4), are good accelerators of methanesulfonylation of acetylcholinesterase. Table I shows that sodium is also an accelerator, although rather weak and with a very high dissociation constant. It seems unlikely that sodium in high concentrations, as used in our experiments, would have no indirect effect on acetylcholinesterase, e.g. through ionic strength. It should be noted here, however, that almost the same dependence of the rate of methanesulfonylation on the concentration of sodium was found also in experiments, not described in the previous sections, where sodium was used in the form of sulfate. This indicates that ionic strength has no great influence on the acceleration with sodium cation.

The values of the dissociation constants for the binding of decamethonium, tetraethylammonium and tetramethylammonium to the acceleration site of the enzyme, are approximately the same as the dissociation constants for the binding of these cations to the inhibition site (Table I, refs. 15—17). The agreement is very good if we consider that our materials and experimental conditions were different from those used by other authors and that, besides, the values for the constants obtained from inhibition experiments cited in the literature differ considerably from each other [18]. The agreement indicates that the acceleration site is the same as the inhibition site, probably the catalytic anionic site. The same conclusion was reached by Rosenberry [19] as a result of similar considerations based on the results of different authors. It should be mentioned here that some of the constants shown in Table I have been determined already (see refs. 1 and 4) and that the corresponding results agree well with those in Table I. Our determinations, however, were obtained from many sets of experiments and by means of a graphical method, so they should be more reliable.

It is evident from Figs. 2—5 that the values for the second-order rate constants k_{ab} approach the values for the second-order rate constants k_a when the concentration of one accelerator in each pair is increasing. This means that each accelerator, when sufficiently concentrated, is able to displace the other accelerator from the acceleration site. Moreover, the agreement between the experimental data in Figs. 2—5 and the corresponding theoretical curves based on the assumption of pure competition between accelerators is very good. The same is true of the pairs tetraethylammonium and tetramethylamonium (figures not shown). This proves that decamethonium, tetraethylammonium and tetramethylammonium compete with each other for the acceleration site on the enzyme and, accordingly, that they accelerate from the same site. Since tetramethylammonium probably binds only to the catalytic anionic site of acetylcholinesterase, decamethonium and tetraethylammonium (though also to an outer site [20]) the acceleration site must be the catalytic anionic site and not an outer one, as was proposed for some cases of deacetylation of acetylcholinesterase [21]. Figs. 6 and 7 show that sodium, too, competes in the acceleration with decamethonium for the catalytic anionic site. The same mode of action, therefore, seems to be involved in the acceleration of methanesulfonylation of acetylcholinesterase with sodium cation, as in the case of decamethonium, tetraethylammonium and tetramethylammonium.

In conclusion, it seems that accelerators of methanesulfonylation of acetylcholinesterase accelerate the reaction from the catalytic anionic site. Since this site is close to the esteratic site where methanesulfonylation takes place, this finding corroborates our hypothesis on the electrostatic nature of the acceleration effect.

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