Biochimica et Biophysica Acta, 327 (1973) 393–397

BBA 67092

ON THE NATURE OF THE ACCELERATION OF THE METHANESULFONYLATION OF ACETYLCHOLINESTERASE BY TETRAETHYLAMMONIUM*

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(Received July 25th, 1973)

SUMMARY

1. In order to obtain some information about the nature of the acceleration by tetraethylammonium of the methanesulfonylation of acetylcholinesterase (acetyel choline acetylhydrolase, EC 3.1.1.7), the sulfonylation of the enzyme by methanus sulfonylfluoride, with and without added tetraethylammonium, was studied at various temperatures.

2. The results show that tetraethylammonium increases the activation entropy of the methanesulfonylation of acetylcholinesterase, which more than compensates for the higher activation enthalpy in the presence of tetraethylammonium. The findings indicate that the acceleration is due to structural changes in the esteratic site of the enzyme under the influence of tetraethylammonium.

Myers and Kemp[1] showed that methanesulfonylfluoride is an irreversible inhibitor of acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7); they suggested that this compound might act in the same manner as diisopropylfluorophosphate. Kitz and Wilson[2], and Alexander *et al.*[3], showed that some other methanesulfonates form methanesulfonyl derivatives. Their experiments also indicated[2,3] that the site in the enzyme that is sulfonylated is possibly the same site which is acetylated during the normal enzymic activity. We have recently obtained[4,5] some more indications that the site that is sulfonylated by methanesulfonylfluoride is the esteratic site.

The rate of the reaction of the enzyme with methanesulfonates is in general lower when competitive inhibitors of the enzymic acetylcholine hydrolysis, such as tetraethylammonium, are present[2,3]. In the case of methanesulfonylfluoride, however, Kitz and Wilson[6] observed that the influence of the competitive inhibitors dimethylammonium, trimethylammonium, tetramethylammonium and tetraethylammonium is quite different: they greatly enhance the rate of sulfonylation of acetylcholinesterase

* The work was presented in part at the 9th International Congress of Biochemistry, Stockholm, July 1–7, 1973.

by methanesulfonylfluoride; tetraethylammonium increases the reaction rate by more than 30 times.

We have tried to obtain some indications regarding the nature of the acceleration by studying the methanesulfonylation of acetylcholinesterase, both in the presence and absence of tetraethylammonium, at different temperatures.

The sulfonylation of the esteratic site of acetylcholinesterase by methanesulfonylfluoride can be described by the following scheme[2,4]:

$$EH + I \xrightarrow{k_a} E' + HF \qquad (1)$$

with EH, the esteratic site of acetylcholinesterase; I, the inhibitor methanesulfonylfluoride; E', the sulfonylated esteratic site; HF, hydrogen fluoride; and k_a, the apparent second-order rate constant for the methanesulfonylation.

Experiments were carried out as follows (*cf.* ref. 4): the enzyme was incubated with an excess of methanesulfonylfluoride for 0–7 min. After the incubation and extensive dilution of 100 times, the remaining activity of the enzyme was measured according to the method of Hestrin[7]. From the time dependence of the sulfonylation, the apparent first-order rate constant was determined; the determination was always made at three different concentrations of methanesulfonylfluoride ranging from 0.2–2 mM in the experiments without tetraethylammonium. The second-order rate constant was then determined graphically, from the dependence of the first-order constant on the concentration of methanesulfonylfluoride. Experiments were made at different temperatures, from 5 °C–25 °C. In experiments with tetraethylammonium, the concentration of this substance was 50 mM, which is about 100 times greater than the dissociation constant for the binding of tetraethylammonium to acetylcholinesterase[8]; thus, the enzyme was practically saturated with tetraethylammonium during the incubation while during the determinations of the enzymatic activity the solutions were sufficiently diluted that tetraethylammonium did not interfere with these assays. Since the sulfonylation, in the presence of tetraethylammonium, was about 30 times faster than without it, the concentration of methanesulfonylfluoride in those experiments was lowered correspondingly.

All experiments were carried out in a thermostat. The universal buffer solution of Britten and Robinson[9–11] was used; NaCl was added to a total ionic strength of 0.2. The buffer solution had a pH of 8.3, which is approximately the optimum for the methanesulfonylation of acetylcholinesterase at 25 °C (ref. 4). The enzyme used was acetylcholinesterase, Worthington, ECHP 1 JA, 1.097 units/mg. The stock solution of the enzyme contained 0.15 mg of the preparation in 1 ml of 0.1 M phosphate buffer solution, pH 7.0, with 0.1% gelatin. Methanesulfonylfluoride was of Eastman Organic Chemicals. The stock solution of methanesulfonylfluoride was a 50 mM solution of methanesulfonylfluoride in spectroquality acetone; in experiments in which tetraethylammonium was added, the methanesulfonylfluoride stock solution was 1 mM. Incubation solutions were always prepared immediately before each experiment.

The experimental results are summarized in Fig. 1.

It is seen from Fig. 1 that logarithms of the k_a values are linearly dependent on $1/T$. Thus, the apparent thermodynamic quantities $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta G^{\ddagger}$ can be calculated by means of the equations

$$\Delta H^{\ddagger} = -2.3\, R \frac{\mathrm{d}(\log k_a)}{\mathrm{d}(1/T)} - RT \tag{2}$$

$$\Delta S^{\ddagger} = 4.58 \left(\log k_a - 10.32 - \log T + \frac{\Delta H^{\ddagger}}{4.58\, T}\right. \tag{3}$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{4}$$

with $\mathrm{d}(\log k_a)/\mathrm{d}(1/T)$ obtained graphically from Fig. 1.

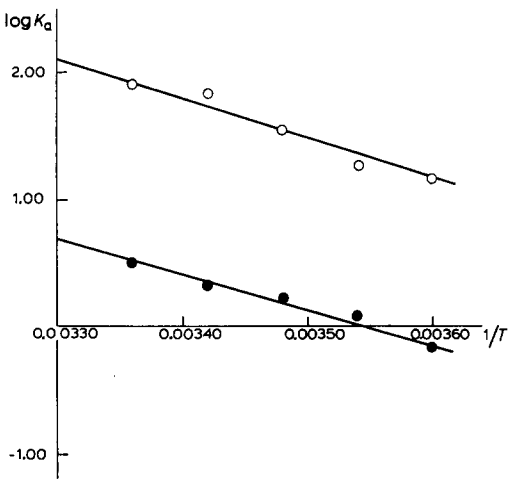

Fig. 1. The dependence of the logarithm of the second-order rate constant, k_a, for methanesulfonylation of acetylcholinesterase on the reciprocal of the absolute temperature, T, in the presence (○) and absence (●) of tetraethylammonium. Each point in the diagram represents the average of three determinations.

The values calculated thus are given in Table I.

It is evident from Table I that the lower activation free enthalpy of the methanesulfonylation of acetylcholinesterase, in the presence of tetraethylammonium, is due solely to an increase of the activation entropy; the increase of about 13 e.u. more than compensates (*cf.* ref. 12) a higher activation enthalpy in the presence of tetraethylammonium. Consequently, the acceleration by tetraethylammonium of the methanesulfonylation of acetylcholinesterase is due to an increase

TABLE I

The thermodynamic quantities $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for methanesulfonylation of acetylcholinesterase in the presence and absence of tetraethylammonium at 25 °C, pH 8.3.

	$\Delta G^{\ddagger}$ (kcal·mole^{-1})	$\Delta H^{\ddagger}$ (kcal·mole^{-1})	$\Delta S^{\ddagger}$ (cal·mole^{-1}·deg^{-1})
Methanesulfonylation with tetraethylammonium	14.7	13.9	−2.7
Methanesulfonylation without tetraethylammonium	16.8	12.1	−15.8

in the activation entropy for the overall reaction. A direct participation of the tetraethylammonium molecule, in the reaction between acetylcholinesterase and methanesulfonylfluoride, is not likely; a direct electrostatic (*cf.* ref. 13) effect of the bound tetraethylammonium is also unlikely, since the methanesulfonylfluoride molecule is not charged. Another possible effect of tetraethylammonium on the methanesulfonylation, namely a favorable restriction of the orientation of the approaching methanesulfonylfluoride molecule, does not seem likely either[14]. An effect of tetraethylammonium on the dissociation constants of the active groups in the esteratic site of acetylcholinesterase (*cf.* ref. 4) would probably result only in a change of the activation enthalpy. Thus, the increased activation entropy in the presence of tetraethylammonium strongly indicates a change in the structure of one or both reactants, acetylcholinesterase and methanesulfonylfluoride, or the solvent, under the influence of tetraethylammonium. The value of the difference in the activation entropies, for the methanesulfonylation in the presence and absence of tetraethylammonium, is consistent with small structural changes. Methanesulfonylfluoride, being a small and uncharged molecule, does not presumably change its structure essentially when bound to the enzyme. Thus, it is acetylcholinesterase that must be changed under the influence of tetraethylammonium. Since the reaction between acetylcholinesterase and methanesulfonylfluoride takes place at the esteratic site of the enzyme only[2–5], the change in structure probably occurs in or near the esteratic site. Many mechanisms of the supposed structural change are possible (*cf.* refs 14–17), including a change in the flexibility of the esteratic site. However, it is difficult at present to conceive a concrete mechanism.

Nevertheless, the conclusion seems warranted that tetraethylammonium, when bound to a site distinct from the esteratic site of acetylcholinesterase, changes the structure of the esteratic site. Since the structure of tetraethylammonium is similar to the quaternary ammonium part of the natural substrate of acetylcholinesterase, acetylcholine, the present findings are interesting especially in connection with the hypothesis of the receptor role of acetylcholineserase[18] and with the induced fit theory[19].

ACKNOWLEDGEMENTS

This work was supported by Boris Kidrič Foundation, Ljubljana, Yugoslavia, and by National Institutes of Health, Bethesda, U.S.A., Grant P.L. 480 Agr. No. 02, 027-1. The author thanks Mrs Sonja Žitko-Trček for her valuable technical assistance.

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