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Salt Effects on Cholinesterase-Catalyzed Hydrolysis of Acetylcholine

TÓNU KESVATERA, AIN LANGEL, AND JAAK JÄRV

Laboratory of Bioorganic Chemistry, Institute of Chemical Physics and Biophysics of the Estonian Academy of Sciences, P.O. Box 670, Tallinn 200026, and Laboratory of Bioorganic Chemistry, Tartu State University, Tartu 202400, Estonian SSR, USSR

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Quantitative description of salt effects on the kinetics of acetylcholine hydrolysis catalyzed by acetylcholinesterase and butyrylcholinesterase is given over the wide range of salt concentrations.

INTRODUCTION

The influence of inorganic salts on reactions of cholinesterases with cationic substrates has been shown to include the electrostatic effect, which is revealed in the binding step of these reactions (*1–3*). The analysis of the electrostatic effect with a Brønsted–Debye–Hückel expression has led to the estimate of the effective charge number of acetylcholinesterase of -6 to -9 (*2*).

The high value of the effective charge of the enzyme suggested that acetylcholinesterase could be considered as a polyelectrolyte. Accordingly, the electrostatic effect on the acetylcholinesterase-catalyzed hydrolysis of a cationic substrate has been described by the term $\psi_{z+} \log[M^{z+}]$ (*4*), which has been originally used for the salt effect on the ligand binding by linear polyelectrolytes (*5–7*). The parameter ψ depends on the charge density of the polyelectrolyte and on the charge number of salt cation, z, $[M^{z+}]$ is the concentration of salt cation. In addition, a recent study (*3*) has pointed to the significance of salting effects in acetylcholinesterase reactions, which can be taken into account by the term $\Delta\kappa c$. The resulting equation for the salt effects on the binding step of cholinesterase-catalyzed hydrolysis of cationic substrates can be written as

$$pK_s = pK_s^0 - \psi_{z+} \log[M^{z+}] + \sum_{j=1}^{m} \Delta\kappa_{b,j} c_j, \qquad [1]$$

where $\Delta\kappa_b = \kappa_E + \kappa_S - \kappa_{ES}$ (*3*) is a characteristic of the salt, denoted by j, and c is the salt concentration.

It was thought of interest to extend the verification of the above approach (i.e., Eq. [1]) to the salt effect on the acetylcholinesterase-catalyzed hydrolysis of the physiological substrate, acetylcholine, and, perhaps more important, to the case of another enzyme, butyrylcholinesterase.

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EXPERIMENTAL

Acetylcholinesterase was the preparation from cobra *Naja naja oxiana* venom (*3*). Butyrylcholinesterase from horse serum was purchased from Mechnikov Institute of Sera and Vaccine, Moscow. Acetylcholine iodide was from Sigma and inorganic salts were purchased from Reakhim (USSR). Enzyme stock solutions were made 0.1–1 μM in 0.15 M KCl. The concentration of acetylcholinesterase was determined as described in (*3*) and that of butyrylcholinesterase as described in (*8*).

The kinetics of cholinesterase-catalyzed hydrolysis of acetylcholine were followed titrimetrically (Radiometer Titrigraph TTT2/SBR3/ABU12, Denmark) at 25°C and pH 7.5. The 0.01 M KOH solution was used in titration, which was performed in a closed vessel with an argon atmosphere. Initial rates were followed during 5–10 min. The first-order kinetic curves were registered for at least three half-life periods. Data processing was based on the reaction scheme

$$E + S \overset{K_s}{\rightleftharpoons} ES \xrightarrow{k_2} EA + P_1 \xrightarrow{k_3} E + P_2. \qquad [2]$$

The first-order rate constants $k_{obs} = (k_2/K_s)[E]_0$ were calculated from the kinetic curves obtained at low substrate concentration, where the ratio $[S]/K_m$ was less than 0.2. The second-order rate constants were calculated as $k_{II} = k_{obs}/[E]_0$. Values of $K_m = K_s/(k_2/k_3 + 1)$ and $k_{cat} = k_2/(k_2/k_3 + 1)$ and parameters of salt effect equations were calculated by iteration, using the nonlinear regression method (*9*).

RESULTS

The influence of salts on the second-order rate constant of acetylcholinesterase-catalyzed hydrolysis of acetylcholine is shown in Fig. 1. For the butyrylcholines-

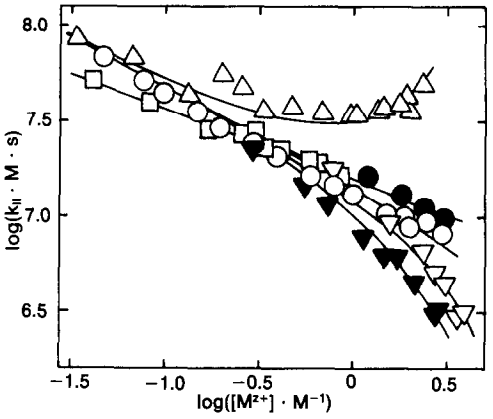

FIG. 1. The log k_{II} versus log[M^{z+}] plots for the reaction of acetylcholinesterase with acetylcholine at 25°C and pH 7.5. Theoretical curves are calculated according to Eq. [3] by using the values of log k^0_{II}, ψ and $\Delta\kappa_j$ from Table 1. NaCl (●), KCl (○), LiCl (▽), CsCl (▼), Na_2SO_4 (△), and $MgCl_2$ (□).

TABLE 1

Parameters of Eq. [3] for the Influence of Inorganic Salts on the Second-Order Rate Constants of Hydrolysis of Acetylcholine Catalyzed by Acetylcholinesterase and Butyrylcholinesterase at 25°C and pH 7.5

Salt	$\log(k_{II}^0 \cdot M \cdot s)$	ψ	$\Delta\kappa$ (M^{-1})	r	SD	n
Acetylcholinesterase						
	7.18 ± 0.03	0.50 ± 0.04		0.991	0.0575	52
LiCl			−0.10 ± 0.02			
NaCl			0.02 ± 0.02			
KCl			−0.03 ± 0.02			
CsCl			−0.16 ± 0.02			
Na_2SO_4			0.60 ± 0.05			
$MgCl_2$	7.22 ± 0.04	0.37 ± 0.03	0	0.994	0.0231	11
Butyrylcholinesterase						
	5.40 ± 0.03	0.47 ± 0.04		0.994	0.0615	64
LiCl			−0.54 ± 0.02			
NaCl			−0.25 ± 0.02			
KCl			−0.20 ± 0.02			
RbCl			−0.28 ± 0.02			
$BaCl_2$	5.15 ± 0.38	0.38 ± 0.07	−1.7 ± 0.5	0.983	0.1059	10

terase-catalyzed hydrolysis of the substrate the influence of salts was similar to that in Fig. 1. The data were described by the equation

$$\log k_{II} = \log k_{II}^0 - \psi_{z+} \log[M^{z+}] + \sum_{j=1}^{m} \Delta\kappa_j c_j, \qquad [3]$$

where $\Delta\kappa = \Delta\kappa_a + \Delta\kappa_b$ (*3*), and $\log k_{II}^0$ was indifferent to the nature of salt. The electrostatic effect, $\psi_{z+} \log[M^{z+}]$, was characterized by uniform ψ_+ for various salts with univalent cations, while the ψ_{2+} values for bivalent cations were in quantitative agreement with the relation $\psi_{2+} = (1 + \psi_+)/4$ following from the Manning theory (*7*). The reason for the divergence observed in the influence of salts at their high concentrations (Fig. 1) lies in the term for the salting effect, $\Delta\kappa c$ (*3*). The results of data processing according to Eq. [3] are presented in Table 1.

Further, we studied the influence of KCl on K_m and k_{cat} of butyrylcholinesterase-catalyzed hydrolysis of acetylcholine (Table 2). The dependence of k_{cat} upon the salt concentration suggested that both k_2 and k_3 may be affected by the salting effect ($k_2 = k_2^0 \times 10^{\Delta\kappa_2 c}$ and $k_3 = k_3^0 \times 10^{\Delta\kappa_3 c}$, $\Delta\kappa_2$ and $\Delta\kappa_3$ are salting coefficients). The value of k_3, equal to 2600 s^{-1} (*11*), which was estimated in the presence of 0.15 M KCl, was used as an approximation for k_3^0. Accordingly, the values of k_{cat} from Table 2 were fitted to the equation

$$\log[k_{cat}k_3^0/(k_3^0 - k_{cat})] = \log k_2^0 + (\Delta\kappa_2 - \Delta\kappa_3)c_{KCl}. \qquad [4]$$

The results of the curve fitting according to Eq. [4] are given in Table 3. For $\Delta\kappa_3$ the obtained value was equal to zero pointing to the fact that k_3 was not affected

TABLE 2

Influence of KCl on K_m and k_{cat} for Butyrylcholinesterase-Catalyzed Hydrolysis of Acetylcholine at 25°C and pH 7.5

c_{KCl} (M)	K_m (mM)	k_{cat} (s^{-1})	c_{KCl} (M)	K_m (mM)	k_{cat} (s^{-1})
0.05	0.71 ± 0.08	1173 ± 157	0.63	5.04 ± 0.80	812 ± 98
0.075	1.30 ± 0.13	873 ± 22	1.42	4.71 ± 0.18	490 ± 11
0.10	2.04 ± 0.24	907 ± 23	1.82	4.67 ± 0.23	502 ± 30
0.15	1.21 ± 0.08	728 ± 13	2.30	8.55 ± 1.16	370 ± 13
0.31	2.48 ± 0.33	700 ± 24	2.90	8.91 ± 0.09	242 ± 12
0.38	1.58 ± 0.05	672 ± 7	3.60	15.1 ± 0.24	170 ± 18
0.50	2.48 ± 0.33	700 ± 23			

Note. A limited range of substrate concentrations has been used where the hydrolysis could be described by the Michaelis–Menten equation (*10*).

by KCl. Thus, Eq. [4] actually describes the salt effect on k_2 (the acylation step), illustrated in Fig. 2a.

Subsequently we calculated the values of $K_s = K_m k_3/(k_3 - k_{cat})$, which are presented in Fig. 2b to illustrate the salt effect on the binding step of butyrylcholinesterase-catalyzed hydrolysis of acetylcholine. Figure 2b shows that the influence of KCl on the binding step, according to Eq. [1], can be ascribed to the electrostatic effect, while $\Delta\kappa_b$ equals zero. Table 3 shows that the value of ψ obtained for pK_s coincides with the respective value of ψ for the second-order rate constant (see also Table 1).

DISCUSSION

The results presented here complement previous salt effect studies (*3*, *4*). They reveal a similar electrostatic effect in the binding step of cationic substrate hydro-

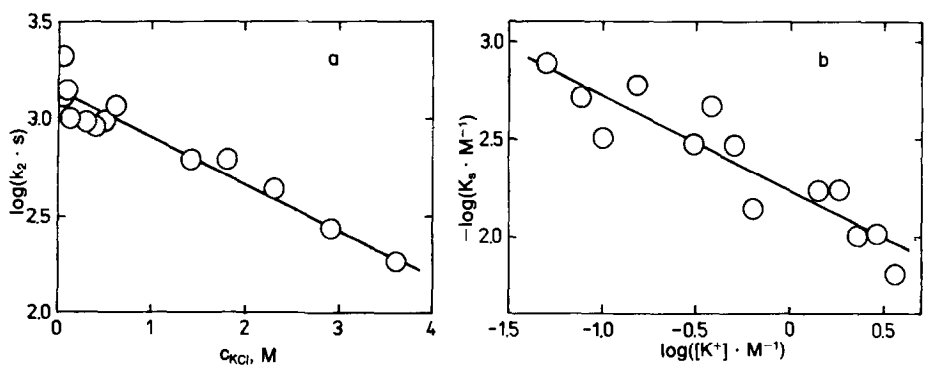

FIG. 2. Influence of KCl on the acylation (a) and binding (b) steps of the reaction of butyrylcholinesterase with acetylcholine at 25°C and pH 7.5. Solid lines are in accordance with the results of data processing in Table 3.

TABLE 3

The Influence of KCl on the Kinetic Parameters of Butyrylcholinesterase-Catalyzed Hydrolysis of Acetylcholine at 25°C and pH 7.5

A^a	A^0	ψ	$\Delta\kappa$ (M^{-1})	r	SD	n
$\log(k_{II} \cdot M \cdot s)^b$	5.46 ± 0.06	0.47 ± 0.07	−0.24 ± 0.04	0.983	0.107	36
$-\log(K_m \cdot M^{-1})$	2.50 ± 0.11	0.45 ± 0.13	−0.10 ± 0.06	0.964	0.120	12
$-\log(K_m \cdot M^{-1})^c$	3.38 ± 0.02	0.49 ± 0.04	0	0.991	0.032	5
$-\log(K_s \cdot M^{-1})$	2.23 ± 0.04	0.50 ± 0.06	0	0.992	0.137	13
$\log(k_2 \cdot s)$	3.14 ± 0.03	—	−0.24 ± 0.04	0.959	0.088	13

[a] Data have been fitted to the equation $A = A^0 - \psi \log[K^+] + \Delta\kappa c_{KCl}$.

[b] k_{II} calculated from the values of k_{cat} and K_m in Table 2 and those obtained directly from the first-order kinetic curves at low substrate concentration were simultaneously used in data processing.

[c] Acetylcholinesterase-catalyzed hydrolysis of acetylcholine at 25°C and pH 7.5. Data from Ref. (*3*).

lysis for both acetylcholinesterase and butyrylcholinesterase (Tables 1 and 3). This is not surprising, since the content of (negatively) charged amino acids in the structure of butyrylcholinesterase is similar to that for acetylcholinesterases from various sources (*12*). It should be noted that Eq. [1] also provides a good quantitative description of the data on the influence of inorganic salts on the binding step of the reaction of horse serum butyrylcholinesterase with *N*-methylcarbamylcholine at 25°C and pH 7.4 (*1*). The curve fitting of the data from Ref. (*1*) by Eq. [1] yields $\psi_+ = 0.56 \pm 0.08$, $pK_s^0 = 2.10 \pm 0.04$, and $\Delta\kappa_{b,K_2SO_4} = 0.23 \pm 0.12$ and the $\Delta\kappa_b$ values are equal to zero for NaCl, KCl, CsCl, KBr ($r = 0.989$, SD = 0.077, $n = 34$), and $\psi_{2+} = 0.32 \pm 0.06$, $pK_s^0 = 2.23 \pm 0.10$, $\Delta\kappa_{b,CaCl_2} = -1.03 \pm 0.23$ ($r = 0.998$, SD = 0.0335, $n = 5$).

On the other hand, manifestation of the salting effect in the acylation step of butyrylcholinesterase-catalyzed hydrolysis of acetylcholine points to the difference in the catalytic mechanism of the two enzymes, since the absence of the salting effect in the acylation step of acetylcholinesterase-catalyzed hydrolysis of substrates is well-documented (*3*). Again, our observation concerning the influence of salt on the acylation of butyrylcholinesterase is supported by the data in (*1*), which reveal the salting-out effect on the carbamylation of butyrylcholinesterase according to the equation (*3*)

$$\log k_2 = \log k_2^0 + \sum_{j=1}^{m} \Delta\kappa_{a,j} c_j, \qquad [5]$$

where $\log(k_2^0 \cdot s) = -1.91 \pm 0.02$ and $\Delta\kappa_a$ values are -0.09 ± 0.02 for NaCl, -0.06 ± 0.01 for KCl, -0.58 ± 0.06 for CsCl, -0.21 ± 0.04 for KBr, and 0.08 ± 0.06 for K_2SO_4 ($r = 0.962$, SD = 0.045, $n = 20$; the data at $c > 0.1$ M). The deacylation step appears to be salt-independent for both enzymes (for butyrylcholinesterase see also Ref. (*1*) and for acetylcholinesterase Ref. (*3*)).

The results presented here show that salt effects observed in the kinetics of cholinesterase-catalyzed hydrolysis of acetylcholine can be quantitatively de-

scribed for the wide range of salt concentrations. It is significant that the analysis of the electrostatic effect in terms of the Manning polyelectrolyte theory has been proved to be applicable not only for acetylcholinesterase (*4*), but also for another enzyme, butyrylcholinesterase. Moreover, a glance at the data for the salt effect on reactions of superoxide dismutase (*13, 14*), cytochrome *c* oxidase, and c-type cytochromes (*15*), etc., suggests that the approach proposed here would find application for several enzymes. It is to be anticipated that consideration of the polyelectrolyte character of proteins will find general use in enzyme chemistry.

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