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Acetylcholinesterase as polyelectrolyte in reaction with cationic substrates

V. Tóugu, A. Pedak, T. Kesvatera and A. Aaviksaar

Laboratory of Bioorganic Chemistry, Institute of Chemical Physics and Biophysics of the Estonian Academy of Sciences, PO Box 670, Tallinn 200026, USSR

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It is shown that the salt effect in acetylcholinesterase-catalyzed hydrolysis of 2-(*N*-methylmorpholinium)-ethylacetate can be quantitatively described by the equation $\log(k_2/K_S) = \log(k_2/K_S)^\circ - \psi\log[M^{+Z}]$ following from Manning's polyelectrolyte theory; the ψ values for salts with univalent and bivalent cations at different pH values of the reaction medium were in accordance with the conclusions of the theory. Manning's polyelectrolyte theory seems to be a useful framework for studying salt effects in the reactions of charged substrates with enzymes as globular polyions.

Acetylcholinesterase; Polyelectrolyte; Salt effect

1. INTRODUCTION

Effects of inorganic salts on the interactions of cholinesterases with cationic substrates and inhibitors have been extensively studied [1–5]. It is generally recognized that an increase in the concentration of electrolytes in the reaction medium reduces the affinity of cholinesterase towards cationic ligands. Usually the salt effect is explained as the influence of ionic strength on the electrostatic interaction between the enzyme and the substrate [1–3]. Accordingly, the changes in the activity coefficients of the reactants are interpreted in terms of the Debye-Hückel theory. However, the validity of such a treatment for the activity coefficient of an enzyme is not clear.

Alternatively, the influence of salts has been discussed in terms of cation binding of the acetylcholinesterase anionic site [4,5].

Correspondence address: A. Aaviksaar, Laboratory of Bioorganic Chemistry, Institute of Chemical Physics and Biophysics of the Estonian Academy of Sciences, PO Box 670, Tallinn 200026, USSR

Since acetylcholinesterase is a polyanion under working conditions at pH > 7, we have used the conclusions from Manning's polyelectrolyte theory [6,7] in an attempt to describe the salt influence on acetylcholinesterase reactions with cationic substrates, e.g. 2-(*N*-methylmorpholinium)ethylacetate. Although the theory was developed for linear polyelectrolytes we assume in our analysis that in certain cases it may also be applicable to globular proteins since the shape-dependent effects on the counterion distribution near the polyelectrolyte are small [8].

The applicability of Manning's approach to the salt effect in acetylcholinesterase reactions has been discussed earlier in [3] but on the basis of limited experimental data it has been concluded that the variation of the rate constants in these reactions at different salt concentrations was more similar to ionic strength dependence in terms of semi-empirical extentions of the Debye-Hückel expressions for the activity coefficients of the reactants.

According to Manning's theory, the dissociation constant of cationic ligand-polyanion complex

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Table 1

Influence of salts on the acetylcholinesterase-catalyzed hydrolysis of 2-(*N*-methylmorpholinium)ethylacetate at 25°C and pH 7.5

C_S (M)	$k_2/K_S \times 10^{-5}$ ($M^{-1} \cdot s^{-1}$)	C_S (M)	$k_2/K_S \times 10^{-5}$ ($M^{-1} \cdot s^{-1}$)	C_S (M)	$k_2/K_S \times 10^{-5}$ ($M^{-1} \cdot s^{-1}$)	C_S (M)	$k_2/K_S \times 10^{-5}$ ($M^{-1} \cdot s^{-1}$)
1	2	3	4	5	6	7	8
Cobra venom acetylcholinesterase							
KCl							
0.001	97.7	0.030	25.1	0.18	12.6	1.00	5.37
0.002	87.1	0.050	17.8	0.27	11.7	1.30	4.90
0.004	55.0	0.090	16.2	0.30	9.55	1.60	4.46
0.006	43.6	0.10	15.1	0.40	8.51	2.50	3.95
0.010	39.8	0.12	14.8	0.60	7.07	2.90	4.17
0.020	31.6	0.15	14.1	0.90	5.50		
NaCl							
0.004	61.7	0.10	15.8	0.50	7.76	1.60	4.90
0.010	34.6	0.20	12.9	0.70	6.60	2.10	3.98
0.030	24.0	0.30	10.0	1.05	5.62	3.10	4.90
0.050	20.4						
KNO_3							
0.10	13.5	0.60	6.78	1.00	5.25	1.50	4.68
0.20	10.5	0.80	5.89	1.20	5.13	2.00	4.17
0.40	7.41						
$MgCl_2$							
0.0017	34.7	0.015	19.1	0.090	10.7	0.32	7.41
0.0033	35.6	0.033	15.8	0.10	10.2	0.60	6.46
0.0050	30.9	0.040	12.2	0.20	8.71	0.90	5.37
0.0067	26.3	0.050	13.2	0.24	8.51	1.20	4.67
0.010	22.4						
$CaCl_2$							
0.0020	41.6	0.010	24.8	0.10	11.8	0.40	6.28
0.0025	40.8	0.025	19.8	0.20	9.04	0.60	5.48
0.0050	31.0	0.050	13.0				
Electric eel acetylcholinesterase							
KCl							
0.0045	63.1	0.015	99.9[a]	0.12	14.1	0.60	7.55[a]
0.0060	62.3	0.030	36.3	0.15	35.5[a]	1.50	4.16[a]
0.0060	131[a]	0.030	42.6[a]	0.30	12.0	1.90	4.65
0.0075	73.7	0.060	21.8	0.45	10.7[a]	3.00	3.78
0.0090	88.9[a]	0.060	36.3[a]	0.60	6.60	3.00	3.36[a]
0.15	42.4	0.090	17.9				
$MgCl_2$							
0.0032	36.3	0.032	12.9	0.16	8.51	0.64	5.88
0.0064	26.9	0.064	12.6	0.32	6.74	0.96	3.39
0.016	19.9	0.096	10.9				

[a] pH 8.6

depends on the total cation concentration $[Me^{+Z}]$ as follows [9]:

$$pK_S = pK_S^0 - m'\psi \log[Me^{+Z}], \quad (1)$$

where K_S^0 is the dissociation constant at $[Me^{+Z}] = 1$ M and m', the number of ion pairs formed ($m' = 1$ in the case of univalent ligand); ψ is the fraction of counterions bound per polyion charge,

$$\psi = \frac{1}{Z}\left(1 - \frac{\alpha}{Z}\right), \quad (2)$$

where Z is the charge of the salt cation and α is a constant depending on the charge density of the polyelectrolyte and on the dielectric constant of the medium.

In order to check the validity of these conclusions in acetylcholinesterase-catalyzed hydrolysis of cationic substrates we have studied the influence of salts on the second-order rate constant k_2/K_S of the reactions of cobra venom and electric eel acetylcholinesterases with 2-(*N*-methylmorpholinium)ethylacetate (in these reactions salts change only K_S while k_2 remains constant in a wide range of salt concentrations; unpublished). The obtained values of k_2/K_S over the large range of salt concentrations are given in table 1. The data cannot be explained on the basis of the above mentioned cation binding models.

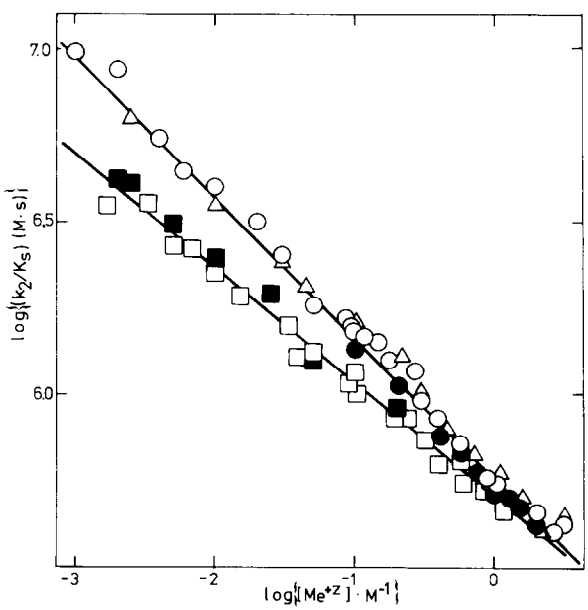

Fig.1. The $\log(k_2/K_S)$ versus $\log[Me^{+Z}]$ plots for the reaction of cobra venom acetylcholinesterase with 2-(*N*-methylmorpholinium)ethylacetate at 25°C and pH 7.5 for KCl (○), NaCl (△), KNO_3 (●), $MgCl_2$ (□) and $CaCl_2$ (■).

2. MATERIALS AND METHODS

Acetylcholinesterase from cobra *Naja naja oxiana* venom was used after purification by affinity chromatography [10]. The specific activity of the enzyme preparation was 5000 IU/mg at 25°C and pH 7.5 with acetylcholine as substrate. Acetylcholinesterase from electric eel was obtained from Serva and used without further purification.

Acetylcholinesterase stock solutions were made in 0.15 M KCl. The concentrations of the enzymes were calculated from the initial rates of the hydrolysis of acetylcholine (2.3 mM) at 25°C, pH 7.5, in 0.15 M KCl making use of the molecular activities $a_m = 6.33 \times 10^3\ s^{-1}$ for cobra venom acetylcholinesterase [11] and $a_m = 10.8 \times 10^3\ s^{-1}$ for electric eel acetylcholinesterase [12].

2-(Methylmorpholinium)ethylacetate iodide and inorganic salts NaCl, KCl, KNO_3, $MgCl_2$ and $CaCl_2$ were of analytical grade from the USSR.

The rates of the enzymic hydrolysis of the

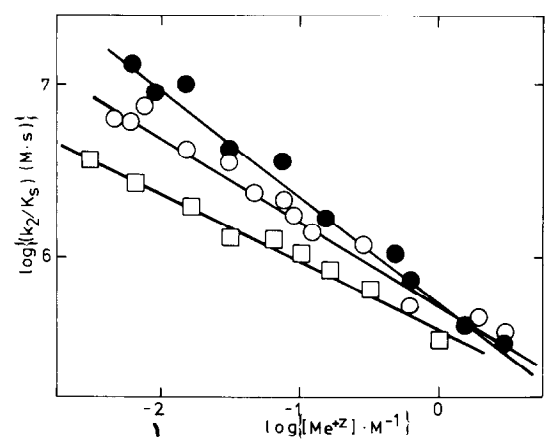

Fig.2. The $\log(k_2/K_S)$ versus $\log[Me^{+Z}]$ plots for the reaction of electric eel acetylcholinesterase with 2-(*N*-methylmorpholinium)ethylacetate at 25°C for KCl (○) and $MgCl_2$ (□) at pH 7.5, and for KCl (●) at pH 8.6.

substrates were determined titrimetrically on a Radiometer pH-stat (TTT2/SBR3/ABU12, Denmark) at 25°C. 0.01 M KOH was used in titration. The second-order rate constants k_2/K_S, in accordance with the reaction scheme

$$E + S \overset{K_S}{\rightleftharpoons} ES \xrightarrow{k_2} EA + P_1 \xrightarrow{k_3} E + P_2 \qquad (3)$$

were calculated from the pseudo-first order rate constants $k_{obs} = (k_2/K_S)[E]_o$ obtained by the differential method of Rudakov [13] directly from the first-order kinetic curves at low substrate concentration, where $[S] \ll K_S k_3/(k_2 + k_3)$.

3. RESULTS AND DISCUSSION

The results of the treatment of the experimental data in table 1 are summarized in table 2. As k_2 in acetylcholinesterase-catalyzed hydrolysis of 2-(*N*-methylmorpholinium)ethylacetate is salt independent, we have used eqn 1, with $m' = 1$, in the form of

$$\log(k_2/K_S) = \log(k_2/K_S)^o - \psi\log[Me^{+Z}] \qquad (4)$$

The correlations according to eqn 4 are illustrated in figs 1 and 2.

The ψ and $\log(k_2/K_S)^o$ values for various salts with univalent cations coincide within the 95% confidence interval. The same (with different ψ) is valid for $CaCl_2$ and $MgCl_2$.

The ψ values for univalent and divalent cations are related to each other according to eqn 2 and the slopes of $\log(k_2/K_S)$ versus $\log[Me^{2+}]$ plots can be predicted using the ψ values obtained for univalent cations. Introducing the number values into eqn 2 we find $\psi = 0.35$ for the cobra venom enzyme and $\psi = 0.37$ for the electric eel enzyme, which are in agreement with the experimental ψ values in table 2.

In the case of the electric eel enzyme the slopes of $\log(k_2/K_S)$ versus $\log[Me^{+Z}]$ dependences are larger than for the cobra venom acetylcholinesterase. This corresponds to the fact that p*I* value for the eel enzyme is lower and, accordingly, this enzyme has somewhat larger negative surface charge density at pH 7.5 than the cobra venom enzyme. Similarly, the salt effect is larger at pH 8.6 where the charge density of the enzyme is higher than it is at pH 7.5.

On the basis of the analysis presented it is obvious that the proposed approach provides a good description of the experimental results. Manning's polyelectrolyte theory seems to be a useful framework for studying salt effects in systems containing enzymes as globular polyions.

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Table 2

Parameters of eqn 4 for the salt influence in the reactions of acetylcholinesterases with 2-(*N*-methylmorpholinium)ethylacetate at 25°C and pH 7.5

Enzyme source	Salts	$\log\{(k_2/K_S)^o(M \cdot s)\}$	ψ	r	s	n
Cobra venom	KCl, NaCl, KNO_3	5.756 ± 0.007	0.418 ± 0.006	0.996	0.035	45
	$MgCl_2$, $CaCl_2$	5.703 ± 0.012	0.331 ± 0.008	0.993	0.034	27
Electric eel	KCl	5.74 ± 0.03	0.481 ± 0.039	0.992	0.062	13
	KCl[a]	5.75 ± 0.04	0.606 ± 0.024	0.991	0.073	10
	$MgCl_2$	5.59 ± 0.03	0.345 ± 0.016	0.992	0.065	10

[a] pH 8.6

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