Effect of Salt on the Kinetics of Butyrylcholinesterase

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It has previously been reported that the presence of common salts, *e.g.*, NaCl, affects the activity of both acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (EC 3.1.1.8)[1] and also causes a change in the relationship of activity to substrate concentration, particularly for the former enzyme.[2] Recent studies on the effect of NaCl on the kinetics of acetylcholinesterase (from electric eel and bovine erythrocytes) [3,4] demonstrated that these kinetics deviate from the hyperbolic Michaelis-Menten equation at low ionic strength, but not in the presence of high concentrations of NaCl.

In the present paper we describe the effect of different salts on the kinetics of butyrylcholinesterase, particularly with respect to the non-hyperbolic kinetics of the hyrolysis of butyrylthiocholine and thiophenyl acetate demonstrated recently.[5,6]

A partly purified butyrylcholinesterase preparation from Organon (Holland) was used without further purification. Butyrylcholinesterase activity was determined at 30 ± 0.1 °C according to a modified Ellman procedure [7,8] on a Beckman 25 spectrophotometer with a Beckman recorder. The enzyme was incubated 1 – 2 h at 30 °C in 50 mM tris-HCl buffer, pH 7.4, containing 0.25 mM DTNB * and the modifier. The reaction mixture consisted of 2.9 – 3.0 ml of the enzyme solution and 0.1 – 0.005 ml of the substrate solution. The protein concentration in the cuvette was 0.03 mg/ml.

The ratio of the intercept on the abscissa and the intercept extrapolated from high substrate concentrations on the same axis, visualized in a $v/(v/S)$ plot in Fig. 1, was used as a measure of the non-linear kinetic behaviour. These intercept values, I_1 and I_2, respectively, were obtained graphically from the reciprocal slope (when $1/S \rightarrow \infty$) of the Lineweaver-Burk plot $(1/v)/(1/S)$ and from the reciprocal intercept on the ordinate (extrapolated from the slope at high S of the Woolf-Hanes plot $(S/v)/S$, respectively. All values of slopes and intercepts were calculated by linear regression on a Compucorp 327 Scientist.

The intercept ratios, summarized in Table 1, were calculated for the enzymatic hydrolysis of butyrylthiocholine in the presence and absence of different concentrations of salt.

It is obvious that the monovalent cations Na^+ and K^+ produced very similar monotonic decreases in non-linearity up to a concentration of 4 M salt, where the ratio became less than one. In kinetic terms this phenomenon is described as a change from negative to positive co-operativity in the hydrolysis of butyrylthiocholine (Fig. 2). The divalent cation Mg^{2+} did not change these kinetics in the same manner, even though the initial decrease in non-linearity in the presence of Mg^{2+} was faster than that seen with corresponding concentrations of Na^+ and K^+.

The use of salt concentrations as high as 4 M must be accompanied by controls designed to determine whether any irreversible effects or large conformational changes due to a changed

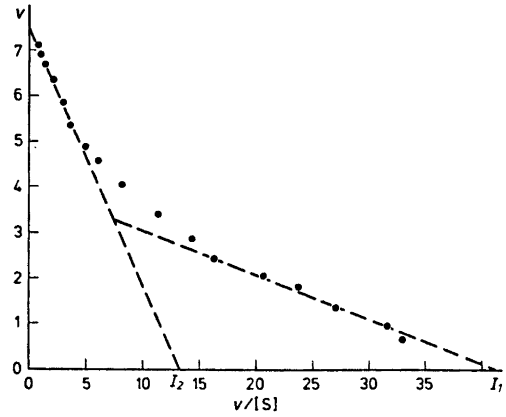

Fig. 1. The EAH plot of butyrylcholinesterase activity with butyrylthiocholine as substrate in the absence of salt. For further details see the text.

* Abbreviations: DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EAH, Eadie-Augustinsson-Hoffstee plot or $v/(v/S)$; S, substrate concentration.

Table 1. Non-linearity index (I_1/I_2) in dependence of the salt concentration.

Salt conc./M	I_1/I_2 NaCl	KCl	$MgCl_2$[a]
0	2.9	2.9	2.9
0.02	–	–	2.1
0.2	2.3	–	1.3
1	1.7	1.6	–
2	1.3	1.4	–
3.4	1.0	–	–
4	0.47	0.98	1.5

[a] The concentration values of $MgCl_2$ are expressed as 2 × M.

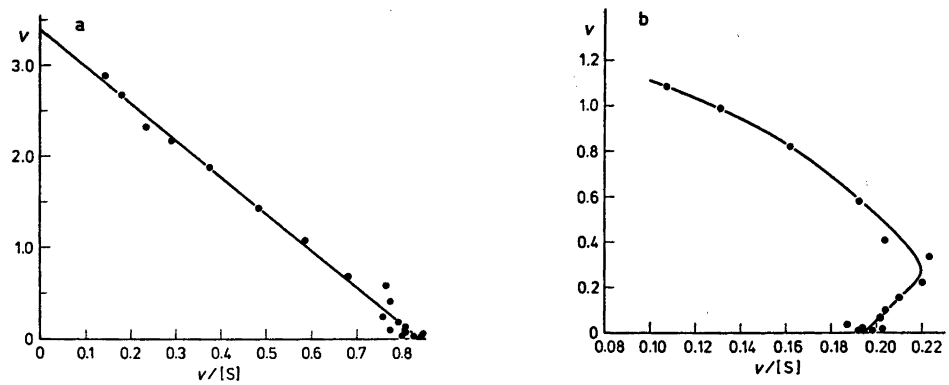

Fig. 2. The EAH plot of butyrylcholinesterase activity with butyrylthiocholine as substrate. (a) Catalysis in the presence of 3.4 M NaCl. The correlation coefficient was 0.995. (b) Catalysis in the presence of 4 M NaCl.

hydratisation of the enzyme, *e.g.* association or dissociation of subunits, did occur. No such irreversible effects could be observed, not even at the highest ionic strength used. After incubating the enzyme for 24 h in the presence of salt, its activity was not changed. Upon dilution to a moderate ionic strength (0.2 M) the specific activity was the same as for the non-incubated enzyme at the same salt concentration.

That the tetrameric nature and the shape of the enzyme are preserved in NaCl at concentrations as high as 2.5 M has been established previously.[9]

One indication that no gross conformational change occurs is the observation that in the presence of 4 M NaCl the enzymatic hydrolysis of thiophenyl acetate was changed very little. The maximum reaction rate was almost identical to that obtained in the absence of salt. At thiophenyl acetate concentrations as small as 0.025 mM the rate in the presence of salt was decreased by only 30 % compared to that in the absence of salt. With butyrylthiocholine as substrate the highest rate was reduced by 80 %; and with 0.025 mM butyrylthiocholine the rate was 0.6 % of the rate in the absence of salt.

This difference in kinetic behaviour might be interpreted in terms of hydrophobic interactions. As the salt concentration increases, the thiocholine moiety of the substrate might be transferred from the charged part of the "anionic site" to a more hydrophobic region.[10,11] Such a hydrophobic effect on the hydrolysis rate would be expected to be more pronounced with butyrylthiocholine than with thiophenyl acetate, of which the thiophenyl part would be expected to be already situated in a hydrophobic region.

It is possible to analyze the shapes of the graphs in Figs. 1 and 2a in terms of a co-operative or non-co-operative type of action of the butyrylcholinesterase upon butyrylthiocholine. However, the plot in Fig. 2b, which illustrates the catalysis of butyrylthiocholine in the presence of 4 M NaCl, is *not* in agreement with a non-co-operative system. In other words, the positive co-operativity observed excludes the possibility of independent binding (or catalytic) sites.

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