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pH Dependence of Choline Esterase Activity at Various Substrate and Inhibitor Concentrations

EDITH HEILBRONN

Research Institute of National Defence, Department 1, Sundbyberg 4, Sweden

The pH dependence of choline esterase activity has been studied in several investigations[1-6,13]. This dependence was also shown in our laboratory by the electrometric method for determinations of choline esterase activity, which seems especially applicable for determinations involving pH variations. It was also of interest to analyse the inhibitory effect of a chemically stable reversible and competitive inhibitor by means of pH variations. 1,5-bis-(*p*-N-dimethyl-N-allyl-ammonium phenyl)-pentanon-3 [7] was the inhibitor used.

EXPERIMENTAL

The following experiments were performed:

1. Measurements of the dependence of enzyme activity on pH at constant substrate concentration. The enzymes used were a) acetylcholine esterase (AChE) and b) choline esterase (ChE).
2. Measurements of activity-pS curves at different pH values. (AChE).
3. Measurements using 1,5-bis-(*p*-N-dimethyl-N-allyl-ammonium phenyl)-pentanon-3, synthesized by Tammelin [8] according to Copp [9], as the inhibitor. I_{50} was determined at different pH values. (AChE and ChE.)

For all determinations of enzyme activity the electrometric method described by Tammelin [10] was applied. The temperature in all experiments was 25° C. No determinations were run above pH 9 owing to the nature of the glass electrodes used.

Buffer. To avoid unnecessary change of ionic media at different pH values, a buffer solution principally composed as described by Britten and Robinson [11] was used. 100 ml of their stock solution plus different amounts of 0.2 *M* NaOH were made up to 400 ml with 0.2 *M* NaCl, thus maintaining the concentration of Na^+ constant.

Enzymes. For determinations on AChE cobra venom was used. A 0.005 % cobra venom preparation in the following solution was made: 0.1 *M* $MgCl_2$, 0.05 *M* KCl, 0.05 *M* NaCl. The solution was kept in a refrigerator and was stable during the course of the experiments.

For determinations on ChE blood serum was used. 15 ml of serum were diluted to 100 ml with the salt solution described above and kept in a refrigerator.

Substrates. For measurements of pH-activity curves the following substrates were used (final concentrations): acetylcholine iodide (ACh) $7.3 \cdot 10^{-3}$ *M*, acetyl-β-methylcholine iodide (MeCh) $1.8 \cdot 10^{-2}$ *M*, butyrylcholine iodide (BuCh) $6.5 \cdot 10^{-3}$ *M*. The same concentration of acetylcholine iodide was used in I_{50} determinations. It was controlled

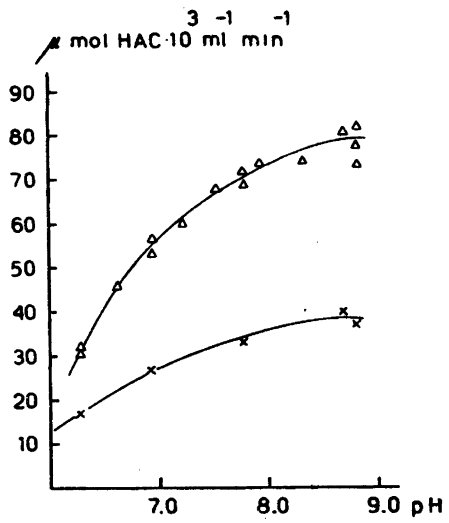

Fig. 1. pH-activity curves for AChE at constant substrate concentration. △—△ *ACh;* ×—× *MeCh.*

that the spontaneous hydrolysis of the substrates in the pH region used nowhere exceeded 3 % of the total hydrolysis [8,12].

Calibration. As the pH variations arising from evolution of acid vary at different pH values, each buffer solution was calibrated by infusion of 3 × 0.02 ml of 0.15 *M* acetic acid. An Agla syringe was used in the experiments. Serum and cobra venom gave different calibration values. The calculations used were those described by Tammelin [10].

Inhibitor. A 10^{-2} *M* solution of the inhibitor in distilled water was prepared and kept in a refrigerator. The solution was stable during the course of the experiments.

Reaction mixtures. For pH-activity and pS-activity curves the reaction mixture in the vessels had the following composition: 3 ml buffer solution, 3 ml enzyme solution and after stabilization of the E.M.F. value, 0.6 ml of substrate solution.

For I_{50} curves the enzyme solution was three times stronger and only 1 ml was used in each vessel. The vessels contained 2 ml of varying amounts of inhibitor.

The pH was determined in the final reaction mixtures.

RESULTS

pH-activity curves. a) *AChE.* The pH-activity curves for the systems AChE-acetylcholine and AChE-acetyl-β-methylcholine were measured. Fig. 1 shows the two curves. b) *ChE.* Fig. 2 shows the pH-activity curves for the systems ChE-acetylcholine and ChE-butyrylcholine.

pS-activity curves. Fig. 3 shows curves where the varying acetylcholine concentrations are plotted against the activities.

I_{50}-curves. Fig. 4 shows the curves obtained when pI_{50}-values for AChE and ChE are plotted against pH-values.

DISCUSSION

Table 1 shows that AChE splits acetylcholine and acetyl-β-methylcholine and ChE splits acetylcholine and butyrylcholine at a constant ratio. This ratio seems to be independent of the pH.

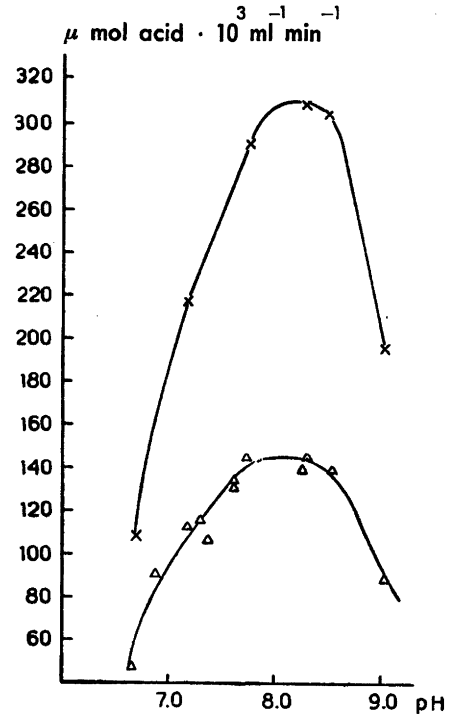

Fig. 2. pH-activity curves for ChE at constant substrate concentration. △—△ *ACh;* ×—× *BuCh.*

The curves of hydrolysis obtained with AChE seem to reach a plateau at pH 8.6. This has earlier [3,13] been shown to be part of an activity optimum reaching from pH 8.3 to pH 9. The activity of ChE reaches its optimum between pH 7.7 and 8.3 in agreement with the values found by Bergman and Wurzel [5]. Below pH 6 the activity of the enzyme decreases rapidly to zero.

The inhibitory effect of 1,5-bis-(*p*-N-dimethyl-N-allyl ammonium phenyl)-pentanon-3 decreases with decreasing pH. For both AChE and ChE the pI_{50}-pH curve starts to fall at pH 7.9.

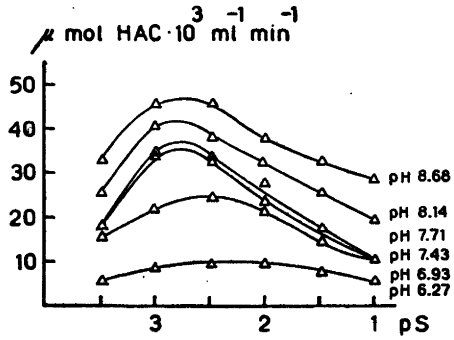

Fig. 3. pS-activity curves for AChE at different pH-values. Substrate ACh.

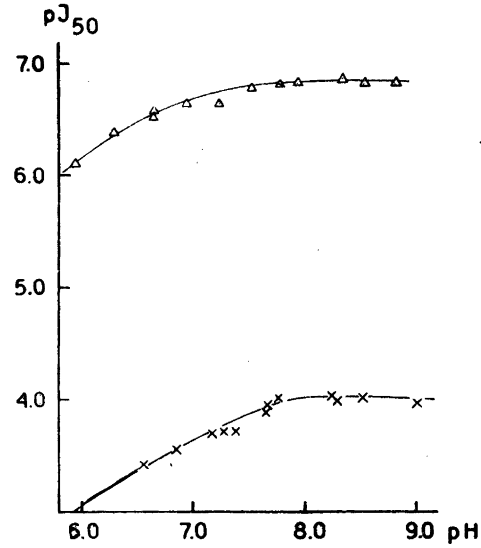

Fig. 4. pI_{50}—pH curves. △—△ AChE; ×—× ChE. Inhibitor: 1,5-(p-N-dimethyl-N-allyl-ammonium phenyl)-pentanon-3. Substrate ACh.

The pS-activity curves show, that the optimum substrate concentration increases below pH 7.43. The pH-dependence of the enzyme-substrate complex seems to be similar to the pH dependence of the enzyme-inhibitor complex. This may be reasonable, as both the substrate and the inhibitor are quaternary ammonium compounds.

From the pI_{50}-pH curves it can be seen that care must be taken in comparing I_{50} values obtained when working at a different pH. Thus the pH in Warburg

Table 1.

pH	ChE		
	μmoles acid · 10^3 ml^{-1} min^{-1}		ratio
	ACh	BuCh	ACh/BuCh
9.01	90	196	0.46
8.47	140	305	0.46
8.27	145	308	0.47
7.73	145	290	0.50
7.17	114	218	0.46
6.65	49	109	0.45
	AChE		
	μmoles acid . 10^3 ml^{-1} min^{-1}		ratio
	ACh	MeCh	ACh/MeCh
8.80	78	37	2.1
8.68	81	40	2.1
7.77	70	33	2.1
6.93	55	27	2.0
6.27	32	17	1.9

determinations is about 7.4 and in electrometric determinations usually 8.0, so that I_{50} values determined by these two methods are not exactly comparable.

Experience during this investigation shows that the electrometric method for determinations of choline esterase activity is very well suited for determinations of I_{50} values at different pH values, as in this case the relative activity values obtained by the apparatus can be used directly. As soon as it is necessary to compare true activity values, calibrations of the solutions must be performed. This is a rather time-consuming procedure, which has to be repeated for each enzyme, buffer solution and pH. If the calibrations are done with great care, the method is exact.

SUMMARY

Using an electrometric method for determinations of choline esterase activity, the activity-pH dependence for AChE and ChE has been measured. AChE activity was found to reach a plateau at pH 8.6 and was found not to decrease again before pH 9. An optimum for ChE activity was found between pH 7.7—8.3.

By the same method the inhibition-pH dependence has been measured using 1,5-bis-(*p*-N-dimethyl-N-allyl-ammonium phenyl)-pentanon-3 as an inhibitor. The inhibition was found to decrease with decreasing pH below pH 7.9. (AChE and ChE).

The pS maximum at different pH-values has been measured for AChE. It was found that the pS maximum increases with decreasing pH below pH 7.43.

The value of the electrometric method for determinations of activity-pH dependence is discussed.

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