exchange must have taken place in the diazonium compound at + 25°. The kinetics of this and similar reactions will be investigated.

All melting points were determined on the Kofler bank.

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## Butyrylcholine Esterase: Influence of pH on Enzyme Activity and Irreversible Denaturation

EDITH HEILBRONN

*Research Institute of National Defence, Dept. 1, Sundbyberg 4, Sweden*

There is much confusion in the literature concerning the shape of the pH-activity curves of choline esterases. It is obvious that there are several factors which together form the shape of these curves. Assuming that the substrate used does not change its charge in the examined pH-area, the following factors have to be considered. Concerning the substrate: The concentration of the substrate is important, as the substrate may compete with hydrogen or hydroxyl ions for an active group in the enzyme. Concerning the medium: In using buffers for maintenance of different pH-values from, *e.g.*, 5—10, both the charge of the ions (*e.g.* phosphate), and thus the ionic strength, and the kind of substance present has to be changed. Concerning the protein: A change in enzyme activity from one pH to another may depend on true reversible changes in one or more of the active centres in the enzyme. On the other hand, the irreversible denaturation of the protein has to be considered. This depends on the temperature used in the experiment, the incubation time, the buffer ions, the ionic strength, the kind of enzyme and the state of purity of the enzyme used. Also, the procedure used during the experiment influences the enzyme activity; in this laboratory (unpublished work) it has thus been observed that the shaking rate in a Warburg apparatus influences the activity. Vigorous shaking causes a lowering of the activity.

During experiments on the purification of choline esterases we had reason to look into the stability of serum fraction IV-6-3 from human retroplacentar serum (BuChE). The studies were performed by means of an automatic recording titrator [1]. The sample containing the enzyme was kept, under stirring, in 0.10 M potassium chloride at 25°C during 5 — 120 min and at pH-values between 6.00 and 10.00. These pH-values were obtained by the addition of minute amounts of sodium hydroxide from the syringe of an automatic titrator. Afterwards, the pH was adjusted to 8.00 and the enzyme activity was determined. The substrate was a $10^{-2}$ M solution of bu-

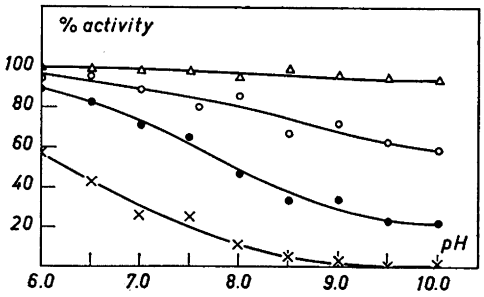


*Fig. 1.* Butyrylcholine esterase activity measured at pH 8.00 after incubation at different pH-values, expressed in percent of activity obtained at pH 8.00 with neglectible incubation time.
Incubation times: △ = 5 minutes, ○ = 30 minutes, ● = 60 minutes, × = 120 minutes. Temperature 25°C.

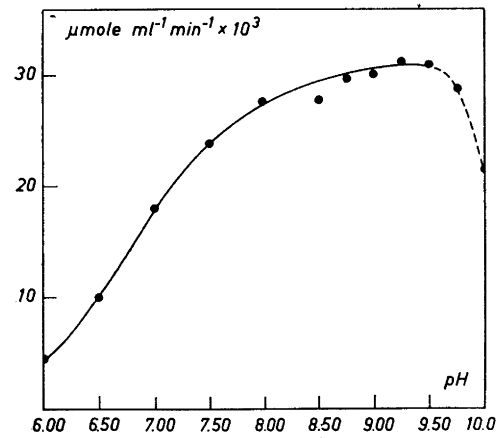


*Fig. 2.* pH-activity curve for butyrylcholine esterase at 25°C. Substrate: $10^{-2}$ M acetylcholine iodide.

tyrylcholine iodide (final concentration). The activity was recorded during a 5 min period immediately after addition of the substrate and after a small correction of the pH had been made. (This correction was necessary since the substrate was somewhat acid.) Fig. 1 shows the result. The activities are expressed in per cent of an initial enzyme activity obtained at pH 8.00. Irreversible denaturation was found to be slowest around pH 6.00 and increases with the pH and with the incubation time.

The automatic recording titrator was then used to reinvestigate the pH-activity curve for BuChE. A $10^{-2}$ M solution of acetylcholine iodide was used as substrate. The obtained values of the activity were corrected for spontaneous hydrolysis as described earlier [2]. Fig. 2 shows the curve, which agrees well with the experimental data published earlier by Wilson [3] and a curve published by Hase [4]. (Hase, however, used equine serum as source for the enzyme). The curve obtained in this work is, however, different from curves published by many others and is also different from two curves obtained in this laboratory. [5,6] The present curve and those published by Wilson and Hase were obtained with an automatic titrator, thus without buffer solutions, and in two cases without incubation times for the enzyme (Hase seems to have incubated for 15 min). The curves were determined with a $10^{-2}$ M or $10^{-2.4}$ M (Hase) concentration of the substrate. Wilson, furthermore, used a 0.1 % solution of gelatine to protect the enzyme. An earlier published curve by Heilbronn [5] was obtained in buffer solutions and at a lower substrate concentration. The different buffer ions may have caused denaturation of the enzyme during the incubation time of 30 min and may also have enhanced hydrolysis in different ways. Tammelin [6] used unpurified human serum, and also a lower substrate concentration. Obviously, the shape of the pH-activity curve depends on the method used.

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## Fractionation of Snake Venom by the Gel-Filtration Method

WALTER BJÖRK and JERKER PORATH

*Institute of Biochemistry, University of Uppsala, Uppsala, Sweden*

The use of gel-filtration as a fractionation method has recently been reported [1]. Attempts to apply the procedure to the fractionation of crude biological extracts have been successful [2,3], but in these cases only for separating proteins and protein-complexes from low-molecular weight material. It was shown, however [2], that proteins of low molecular weight like ribonuclease and cytochrome c could be retarded on a gel of suitable crosslinking, and Gelotte has recently obtained a separation of enzymes from pancreatic juice (private communication). In earlier work carried out at this institute [4] most of the UV-absorbing components of the venom from the ringhals cobra, *Hemachatus haemachates*, including lecithinase A, had been found to have a rather low molecular weight, and thus we thought it would be of great value to in-