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Isoelectric focusing of clostridiopeptidase B

Clostridiopeptidase B (clostripain, EC 3.4.4.20) has been recently purified to apparent homogeneity from a commercially available collagenase preparation[1]. Specificity studies against natural polypeptides as well as synthetic esters and amides have revealed a marked specificity for arginine residues. Further specificity studies[2] on a synthetic methionyllysyl-bradykinin clearly demonstrate hydrolysis at the arginine–proline bond, one that is highly resistant to tryptic digestion. The unusual limited specificity lends special significance to this enzyme as a tool for primary structure analysis in proteins. In addition, detailed structural studies may be of unusual significance as to the origin of this sulfhydryl enzyme's remarkable specificity. Although detailed physicochemical data have been described earlier[1,3], the purpose of this communication is to report the isoelectric point of clostridiopeptidase B, a property which may have significant influence on any success of attempts to crystallize the enzyme.

The isoelectric point was determined at 8° by the isoelectric focusing technique[4] using the commercially available LKB column and ampholyte. Clostridiopeptidase B was prepared by the method of MITCHELL AND HARRINGTON[1] and introduced onto the column in the reduced state after prior incubation with dithiothreitol. The isoelectric point was determined over two pH ranges, 3–10 and 3–6, allowing 2 days for equilibrium to be established. Enzymatic activity was determined by the initial hydrolysis of benzoyl arginine ethyl ester ($2.5 \cdot 10^{-4}$ M) in 0.1 M sodium phosphate

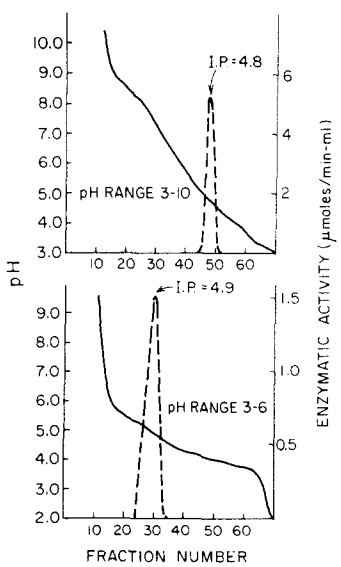

Fig. 1. Isoelectric focusing of clostridiopeptidase B at 8°. Each fraction contains a volume of 3.1 ml. The left ordinate represents the pH gradient (— — —) while the right ordinate indicates the activity against benzoyl arginine ethyl ester (— — —). Top: A pH gradient of 3–10; bottom: A pH gradient of 3–6. I.P. = isoelectric point.

(pH 7.8) containing $2.5 \cdot 10^{-3}$ M dithiothreitol. pH was determined on a TTT 1 Radiometer titrimeter standardized against pH 7 and pH 4 buffers. Fig. 1 illustrates the elution of activity as a function of pH over two different pH ranges with equivalent results of 4.8 and 4.9 for the apparent isoelectric points, respectively. This observed value is consistent with the enzyme's behavior in disc electrophoresis[1,5]. In each case there was a small amount of protein precipitation at the isoelectric point. However, the latter can be easily solubilized by raising the pH to 7 with sodium phosphate buffer.

The observed acid isoelectric point of clostridiopeptidase B is markedly different from the alkaline value of 11 reported for trypsin[6] although each have similar pH optima between 7 and 8. This marked difference in charge at the pH optima may partially explain the observed difference in specificity, especially the inhibition of clostridiopeptidase B by polyarginine, whereas the latter is digested to di- and tripeptides by trypsin[1]. In this laboratory the newly developed technique of isoelectric focusing[4] has given consistently reproducible results, not only in the present study, but with lysozyme Ch (ref. 7) and rabbit muscle phosphoglucomutase (unpublished observation). It is of value not only as an analytical tool but as a preparative procedure as well.

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