ANALOGY BETWEEN ACTIVE SITES OF CATHEPSIN B_1 AND PAPAIN

H. KEILOVÁ and J. TURKOVÁ

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia

Received 16 October 1970

1. Introduction

Cathepsin B_1^*, a tissue protease, belongs to enzymes in which the SH-group of cysteine plays a key role at the active site. While the mechanism of catalysis by proteases of the serine type has been elucidated in considerable detail, our information on the mechanism of catalysis by SH-proteases is rather scarse. The mechanism of action of SH-proteases has so far been studied most intensively on papain [1–4]. It remains to be shown, however, which amino acid residues in addition to cysteine participate at the active site of this enzyme, i.e. whether it is a carboxyl or an imidazole group of histidine.

Since capthepsin B_1 has a number of enzymatic characteristics very similar to those of papain (such as the pH optimum, the specificity of cleavage of the B-chain of oxidized insulin [5], we decided to determine in kinetic experiments whether its active site is also formed of active groups similar to those in papain.

2. Materials and methods

Cathepsin B_1^* was isolated from bovine spleen in this laboratory [5]. The homogeneity of the preparation was assayed by disc-electrophoresis. A preparation of cathepsin B_1 isolated by Otto [6] from the same source served as a comparison standard**. Papain was a commercial preparation of Lachema, Prague.

N-Benzoyl-L-arginine ethyl ester hydrochloride (BAEE) was a commercial preparation of Fluka.

* The designation B_1 was accepted at the Symposium on Tissue Proteinase, Cambridge, England, 1969.

** We wish to thank Dr. Otto (Bonn) for supplying us with the preparation.

2.1. *Determination of enzymatic activity*

The enzymatic reaction was carried out in Model TTT1a pH-stat (Radiometer, Copenhagen, Denmark) equipped with Model TT A 31 titration assembly and a jacketed reaction vessel. The measurement was performed at 37° and the reaction vessel was temperature-controlled with an accuracy of ± 0.1°. The volume of the reaction mixture was 2 ml. The reaction medium contained 0.3 M KCl, 0.001 M EDTA, and 0.01 M dithiothreitol, and 0.02 N NaOH ($f = 0.9978$) served as titrant. The kinetic constants were evaluated graphically using the Lineweaver–Burk plot [7].

For the investigation of the pH dependence of the reaction velocity, the method used by Glazer [8] in this study on the subtilisin cleavage oi BAEE was employed.

3. Results and discussion

The mechanism of action of proteases containing a serine or a SH-group at their active sites ("serine- and SH-types") share many features. While the other active amino acid of the serine-type proteases has been shown in all cases to be histidine of $pK = 5.6–7$, this is not the case for all SH-type proteases. In the SH-protease from streptococci the presence of a histidine residue of $pK = 6$ has been also established [9,10] at the active site, yet in the group of plant SH-proteases (papin, ficin, bromelain) an ionizable group of $pK = 3.9–4.3$ was found. This finding seems to indicate the presence of a carboxyl group at the active site; in the case of papain an aspartic acid residue was suggested [3, 11]. On the other hand, Husein and Lowe using a ditopic derivative of dibromoacetone were able to find a histidine molecule 5 Å distant from the cys-

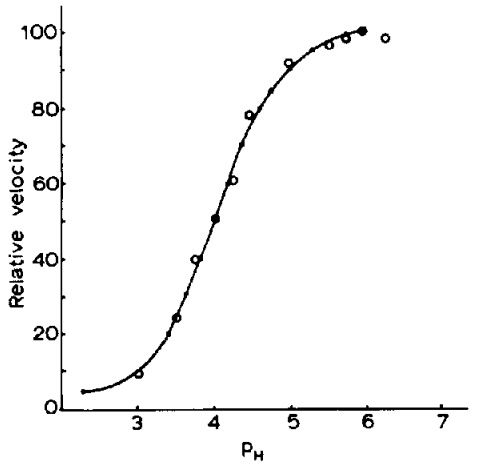

Fig. 1. Values of relative velocity as function of pH for hydrolysis of 0.25 M BAEE by capthesin B_1 at 37°. —●—●— theoretical curve calculated for an titratable group of pK = 4.0, ○ experimental values.

teine residue. They have proposed a scheme of catalysis involving the participation of histidine and explain the decreased pK value by assuming the existence of a hydrogen bond which links the imidazole group to the thiol group in the active enzyme.

We found that the results of our experiments with cathepsin B_1 are similar to those obtained with plant proteases. For both cathepsins B_1, i.e. for the preparation isolated in our laboratory and for enzyme isolated by K. Otto, the same K_m value 5×10^{-2} M was determined. The K_m value for papain determined under identical conditions was 2×10^{-2} M in accordance with the data reported by other authors [1].

Since the participation of the SH-group at the active site of cathepsin B_1 lies beyond any doubt, we focused our attention to the determination of the pK value of the group ionizable in the acidic range. The values of relative reaction velocities were obtained by measuring the decomposition of the substrate at a concentration approximately 5 times higher than the K_m value; these reaction velocities therefore approximate V_{max}. We interpreted our results by the method used by Glazer [8]. From the measured values we obtained a curve which is entirely in agreement with the theoretical curve calculated for pK = 4 (fig. 1). By an identical approach we checked the value of pK = 4.2 for papain which is in agreement with the data obtained by other authors [1].

The conclusive answer to the question which groups participate at the active site of papain can most probably be expected from results of X-ray diffraction analysis. We assume though that the analogous behaviour of cathepsin B_1 and papain seems to suggest a very similar mechanism of action of these two SH-proteases.

References

[1] E.L. Smith and M.J. Parker, J. Biol. Chem. 233 (1958) 1387.
[2] E.L. Smith, J. Biol. Chem. 233 (1958) 1392.
[3] D.C. Williams and J.R. Whitaker, Biochemistry 7 (1968) 2562.
[4] S.S. Husain and G. Lowe, Biochem. J. 108 (1968) 855.
[5] H. Keilová and B. Keil, FEBS Letters 4 (1969) 295.
[6] K. Otto and S. Bhakdi, Z. Physiol. Chem. 350 (1969) 1577.
[7] H. Lineweaver and D. Burk, J. Am. Chem. Soc. 56 (1934) 658.
[8] A.N. Glazer, J. Biol. Chem. 242 (1967) 433.
[9] B.I. Gerwin, W.H. Stein and S. Moore, J. Biol. Chem. 241 (1966) 3331.
[10] Teh-Yung Liu, J. Biol. Chem. 242 (1967) 4029.
[11] A. Light, R. Frater, J.R. Kimmel and E.L. Smith, Proc. Natl. Acad. Sci. U.S. 52 (1964) 1276.