DIFFERENCE IN REACTIVITY OF DISULPHIDE BONDS OF CHYMOTRYPSINOGEN AND CHYMOTRYPSIN WITH β-MERCAPTOETHANOL

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1. Introduction

The difference in the chemical reactivity of the disulphide bonds of trypsin and chymotrypsin, and of their zymogens toward reducing agents may indicate different three-dimensional arrangement of their polypeptide chains. Mesrob and Holeyšovský [1] have found that the disulphide bond VII–VIII (Cys 154–168) is reduced by β-mercaptoethanol in the presence of 8 M urea, with an irreversible loss in activity.

Light and Sinha [2] have shown that treatment of trypsinogen and trypsin with sodium borohydride results in selective reduction of two disulphide bonds, while under similar conditions, chymotrypsinogen and chymotrypsin were not reduced. In a recent study, we found a striking difference in the reactivity of chymotrypsinogen and chymotrypsin towards β-mercaptoethanol under mild conditions.

2. Methods

A 0.5% solution of the protein in 0.1 sodium acetate buffer, pH 6.5, was kept under nitrogen for 2–16 hr at 20°C in the presence of β-mercaptoethanol (0.1–7.5%). The solution was then acidified to pH 3.5 by acetic acid and the protein isolated on a column of Sephadex G-25 (fine). Free SH-groups in the sample of the desalted protein were determined either directly by reaction with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB)** [3] or *p*-chloro-mercuribenzoate (*p*-CMB) [4], or indirectly; in the latter case, the reduced sample was labelled with a calculated amount of DTNB (after adjustment of the solution to pH 7.0 by a phosphate buffer), desalted on a column of Sephadex G-25, treated with dithiothreitol, and free 5-mercapto-2-nitrobenzoic acid was determined photometrically according to Butterworth et al. [5]. The zymogen was activated in the usual way at pH 7.5 with trypsin, 7 hr in the presence of 0.002 M $CaCl_2$, the zymogen - trypsin ratio being 40:1. The chymotryptic activity was measured using ATEE [6] and BTEE [7] as substrates.

3. Results and discussion

Up to a concentration of β-mercaptoethanol of 7.5%, chymotrypsinogen was resistant to the reduction. The protein was recovered from the Sephadex column, in a 85–90% yield in the first peak. In all experiments run in parallel, the regenerated zymogen contained less than 0.2 mole of sulphydryl-groups per mole of protein. After the activation, its specific activity was 106–108% that of the untreated zymogen. This increase in specific activity was obviously due to a purification of the zymogen during the gel chromatography in which partly denatured zymogen was separated on the column. In a control experiment with a zymogen sample which had not been treated with the reducing agent, the same result was obtained. The potential activity of chymotrypsinogen was not

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** Abbreviations used: DTNB, 5,5′-dithio-bis-(2-nitrobenzoic acid); ATEE, acetyl-tyrosine-ethylester; BTEE, benzoyl-tyrosine-ethylester; *p*-CMB, *p*-chlor-mercuribenzoic acid.

affected after labelling with DTNB. In contrast, iodoacetic acid [8] or *p*-CMB were unsuitable because a 10-min treatment of chymotrypsinogen with these reagents at pH 6.5–8.5 fully removed its potential activity.

When chymotrypsin was treated with β-mercaptoethanol in a similar manner, the results were entirely different. In the presence of 0.1–0.2% β-mercaptoethanol, the chymotryptic activity of the reaction mixture decreased to 50–60% after 6 hr, and to 10% when a 2% reagent was used. The protein emerging from the gel column in a volume corresponding to the elution volume of the intact enzyme was fully active in all experiments and contained only traces of free SH-groups. The activity of this fraction was equal to the residual activity of the parent reaction mixture. After the protein-containing peak had been eluted, reduced polypeptide material with no activity emerged from the column. This extensive drop in molecular weight was not observed with a parallel sample of chymotrypsin which had not been reduced.

These results show a striking difference in the action of sodium borohydride [2] and β-mercaptoethanol. While neither chymotrypsinogen nor chymotrypsin are reduced to a noticeable degree by the former, treatment with β-mercaptoethanol leaves chymotrypsinogen intact while chymotrypsin is degraded to polypeptides of low molecular weight. The difference in conformation in the region of the disulphide bond VII–VIII pointed out by Light and Sinha [2] is therefore true only for zymogens; in the case of active enzymes, this problem requires further experimental investigation.

References

[1] B.Mesrob and V.Holeyšovský, Collection Czech. Chem. Commun. 33 (1968) 1359.
[2] A.Light and N.Sinha, J. Biol. Chem. 242 (1967) 1358.
[3] G.L.Ellman, Arch. Biochem. Biophys. 82 (1959) 70.
[4] R.Boyer, J. Am. Chem. Soc. 76 (1954) 4331.
[5] P.H.W.Butterworth, H.Baum and J.W.Porter, Arch. Biochem. Biophys. 118 (1967) 716.
[6] B.Hummel, Can. J. Biochem. Physiol. 37 (1959) 1393.
[7] G.Schwert and Y.Takenaka, Biochim. Biophys. Acta 16 (1955) 570.
[8] A.M.Crestfield, S.Moore and W.H.Stein, J. Biol. Chem. 238 (1963) 622.