HUMAN PANCREATIC ENZYMES: INTERACTION OF HUMAN TRYPSIN WITH CHICKEN OVOMUCOID

James Travis

Department of Biochemistry
University of Georgia, Athens, Georgia 30601

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SUMMARY: The enzymatic activity of human trypsin is not affected by complexing with chicken ovomucoid, an inhibitor of bovine trypsin. However, at least four peptide bonds are broken to produce a modified inhibitor during incubation of the two proteins. This cleavage does not, in itself, cause any decrease in the inhibitory properties of chicken ovomucoid towards bovine trypsin. However, subsequent digestion of the modified ovomucoid with carboxypeptidase B causes rapid loss of its inhibitory properties suggesting that at least one of the peptide bonds broken by human trypsin is at the active center of this inhibitor. Evidently, bond cleavage at the active center of naturally occurring proteinase inhibitors by proteolytic enzymes can occur without inactivation of either the inhibitor or the proteinase.

Chicken ovomucoid is a naturally occurring proteinase inhibitor which stoichiometrically inhibits the enzymatic action of bovine trypsin. Studies of the effects of this protein on trypsins from other species indicate that porcine and ovine trypsins are also inactivated (Buck et al, 1962); however, human trypsin is unaffected even in the presence of high concentrations of inhibitor (Feeney et al, 1969; Coan and Travis, 1971). This latter finding, while of obvious nutritional significance in man, has raised the question as to whether any interaction occurs at all between the human enzyme and chicken ovomucoid. The present communication describes experiments which indicate that human trypsin does react with chicken ovomucoid, resulting in several bond cleavages one of which probably occurs at the active site of the inhibitor.

MATERIALS AND METHODS

Bovine trypsin (2x crystallized), bovine α-chymotrypsin (3x crystallized) and porcine carboxypeptidase B were obtained from the Worthington Biochemical

Company. The trypsin was treated with TPCK prior to use. Human trypsin and human δ-chymotrypsin were prepared by the method of Coan et al, (1971).

Enzyme esterase activity was followed by the procedure of Schwert and Takenaka (1955) using BAEE and ATEE as substrates for trypsin and chymotrypsin respectively. Assays performed in the presence of ovomucoid were made by preincubation of enzyme and inhibitor for 5 minutes at 25^{o} in 0.05M Tris-HCl, 0.05M $CaCl_2$, pH 8.0, followed by addition of substrate.

Modified chicken ovomucoids were prepared by the procedure of Ozawa and Laskowski, Jr. (1966). In a typical experiment, 50 mg of virgin inhibitor was incubated with 1 mg of either human trypsin, human δ-chymotrypsin, bovine trypsin, or bovine α-chymotrypsin in 0.1M HOAc, 0.02M $CaCl_2$, pH 3.75 at 25^{o} for 24 hours. The pH was then raised to 8.1 by addition of 1M Tris and the samples chromatographed on a column of Sephadex G-75 (1.9 x 60 cm) equilibrated with 0.05M Tris-HCl, pH 8.1. In each case, including a control sample of untreated ovomucoid, the applied material was resolved into one major and two minor components. It was found that only the major fraction had inhibitory activity towards bovine trypsin and this material was dialyzed against distilled water and lyophilized. In general, the yield of modified inhibitors obtained, by weight, were 60% of that originally incubated. Each was fully active against bovine trypsin and had identical amino acid compositions consistent with that reported for the native inhibitor.

Carboxypeptidase B (CPB) digestion of each of the modified ovomucoids was performed by dissolving the protein in 0.05M Tris-HCl, pH 8.0, adding DFP treated CPB at a ratio of 5 units per mg of ovomucoid, and incubating at 25^{o}. Aliquots were removed at various times for the determination of inhibitory activity, as well as for examination of free amino acids released. In the latter case CPB digestion was stopped by the addition of 1M HOAc and protein was precipitated with 10% TCA. The supernatants were then dried and analyzed directly with a Beckman Model 120C amino acid analyzer.

Table 1

Digestion of Modified Chicken Ovomucoids with Carboxypeptidase B*

Modifying enzyme	Amino acids released (moles/mole ovomucoid)				Activity retained (%)
	Lys	Arg	Leu	Others	
None	0.002	0.003	0.000	0.010	100
Bovine trypsin	0.008	0.720	0.003	0.011	31
Human trypsin	1.800	1.430	0.004	0.015	25
Bovine α-chymotrypsin	0.005	0.005	0.700	0.020	100
Human δ-chymotrypsin	0.007	0.005	0.030	0.022	100

*Aliquots were removed at 0, 2, 5, 15, 30, 60 and 120 minutes for analysis. Maximum release in each sample was obtained in not more than 30 minutes.

RESULTS AND DISCUSSION

Amino acids released after CPB digestion of the various ovomucoids are given in Table 1. Although aliquots were taken for analysis for times ranging from 2 minutes to 2 hours, essentially total release of all amino acids occurred within 30 minutes.

In the case of bovine trypsin modified chicken ovomucoid only arginine was released in quantities considered to be important. This is in contrast to the results of Feinstein et al, (1966) who also detected the release of tyrosine and leucine in their digestion mixtures. The differences may be due to chymotrypsin contamination in trypsin preparations despite precautions taken to reduce this possibility. Significantly, the greatest release of amino acids was obtained with ovomucoid which had been incubated with human trypsin. Because only 70% of the theoretical arginine was freed from bovine trypsin modified ovomucoid it is likely that two arginine and two or three lysine bonds were cleaved by the human enzyme. The results with chymotrypsin modification of ovomucoid were also interesting in that a leucine bond was cleaved by the

bovine enzyme. In contrast, human δ-chymotrypsin did not, apparently, interact with the inhibitor.

It is well known that the des-arginine derivative of bovine trypsin modified chicken ovomucoid is no longer capable of acting as an inhibitor (Feinstein et al, 1966). In these experiments it was found that even though several bonds were cleaved in human trypsin modified chicken ovomucoid all of its inhibitory activity towards bovine trypsin was retained. However, digestion with CPB resulted in the rapid loss of this function until only 25% of the inhibitory activity remained. This is in good agreement with the data for amino acids released indicating that not all of the ovomucoid was digested or modified during the incubation time. The inhibitory properties of human and bovine chymotrypsin modified ovomucoids were not affected by CPB digestion.

The above results suggest that one of the two arginine bonds of chicken ovomucoid that is cleaved by human trypsin is at the active center of the inhibitor. Because human trypsin is not inactivated by chicken ovomucoid it is apparent that some proteolytic enzymes of pancreatic origin can hydrolyze critical peptide bonds in proteinase inhibitors without being inactivated themselves.

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