STUDIES ON THE ACTIVE SITE OF SHEEP TRYPSIN

James Travis
Department of Biochemistry
University of Georgia
Athens, Georgia 30601

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In a continuing effort to obtain information regarding the active site of trypsin and chymotrypsin, studies have been extended to both pork and dogfish pancreas (Rovery et al., 1960; Prahl and Neurath, 1962). The results of the studies on pork trypsin suggest that it is a molecule with physical properties somewhat comparable to the bovine species but with large changes in its primary structure (Travis and Liener, 1965). These differences do not, apparently, extend to the active centers of these molecules since it has been shown that amino acid sequences are highly conserved in these areas (Travis and Liener, 1965a; Smith and Liener, 1967).

This report shows that at least some of these structural similarities extend to the sheep species, and also suggests a possible reason for the autolytic nature of both bovine and sheep trypsins.

EXPERIMENTAL

Crystalline sheep trypsin was prepared from activated pancreatic extracts and chromatographically purified by ion-exchange chromatography on CM-cellulose (Travis, to be published). The enzyme has a specific esterase activity on BAEE[1] of 21 units/min./mg. of protein at pH 8.1 and 37°. Homogeneity was

[1]Abbreviations: BAEE, N-benzoyl-L-arginine ethyl ester; DFP, Di-isopropyl fluorophosphate; DIP, Di-isopropyl phosphoryl; TPCK, L-(1-tosylamido-2-phenyl)-ethyl chloromethyl ketone.

shown both in the ultracentrifuge at pH 3.2 and by disc electrophoresis (Reisfeld et al., 1962).

Pork trypsin was prepared by the method of Travis and Liener (1965) while bovine trypsin was a commercial product (Worthington). DFP^{32} was obtained from New England Nuclear Corporation.

Samples of oxidized DIP^{32} sheep, pork and bovine trypsins (100 mg. each) (Travis and Liener, 1965a) were digested with TPCK-treated bovine trypsin (0.04 μmoles) for 5 hours at pH 8.1 and 37°. Fingerprints were prepared of each digest by the procedure of Katz et al. (1959) with electrophoresis at pH 3.7 for one hour, followed by chromatography in n-butanol-acetic acid-water (4:1:5). Staining of the papers was performed with 0.2% ninhydrin in acetone.

Partial acid hydrolysis of radioactive peptides eluted from unstained chromatograms was performed in 6N HCl at 105° for ten minutes. The digests were then subjected to high voltage electrophoresis at pH 3.7, 2000 V, for 60 minutes and the electropherograms stained with ninhydrin.

RESULTS AND DISCUSSION

The amino acid composition of sheep trypsin, based on a molecular weight of 25,500, is given in Table I along with previously published data for bovine and pork trypsins. From comparison of these compositions it would appear that the sheep enzyme has no strong relationship to either of the other two enzymes. However, the fact that it undergoes an autolytic degradation which can be inhibited by Ca^{++} (Buck et al, 1962) suggests that structurally it is probably more like the bovine enzyme.

When fingerprints of tryptic digests of the three inhibited, oxidized enzymes are compared, it becomes more obvious that sheep trypsin and bovine trypsin do, indeed, have a more similar structure (Fig. 1). Furthermore, the radio activity appears to be associated with a common peptide in these species which is more acidic than that occurring in the pork enzyme.

Although the actual structure of the sheep peptide has not yet been deter-

TABLE I

Amino Acid Composition of Sheep, Pork, and Bovine Trypsins

	Sheep*	Pork**	Bovine***
Alanine	17	16	14
Arginine	4	4	2
Aspartic Acid	20	18	22
Half-Cystine	12	12	12
Glutamic Acid	14	17	14
Glycine	19	26	25
Histidine	3	4	3
Isoleucine	10	15	15
Leucine	14	16	14
Lysine	12	10	14
Methionine	2	2	2
Phenylalanine	5	4	3
Proline	9	10	9
Serine	26	24	33
Threonine	15	11	10
Tyrosine	6	8	10
Tryptophan	-	6	4
Valine	17	16	17
Amide	-	23	27

*Travis, data to be published
**Travis and Liener (1965)
***Walsh and Neurath (1964)

mined, electrophoresis patterns of partially hydrolyzed radioactive peptides give identical patterns with the sheep and bovine peptides, suggesting a homologous sequence. One can then, tentatively, write the sequence of all three active site peptides as follows:

Pork[1]: Asn-ser-cys-gln-gly-asp-s*er-gly-gly-pro-val-val-cys-gly-gln-gln-leu

Bovine: Asn-ser-cys-gln-gly-asp-s*er-gly-gly-pro-val-val-cys-ser-gly-lys

Sheep: Asn-ser-cys-gln-gly-asp-s*er-gly-gly-pro-val-val-cys-ser-gly-lys

It is interesting to speculate on the replacement of a lysyl residue in sheep and bovine trypsins by a glutaminyl residue in the pork enzyme. The latter enzyme undergoes autolysis only very slowly, presumably because of the absence

[1]The original amino acid sequence of this peptide (Travis and Liener, 1965a) has been re-examined and is correctly rewritten here.

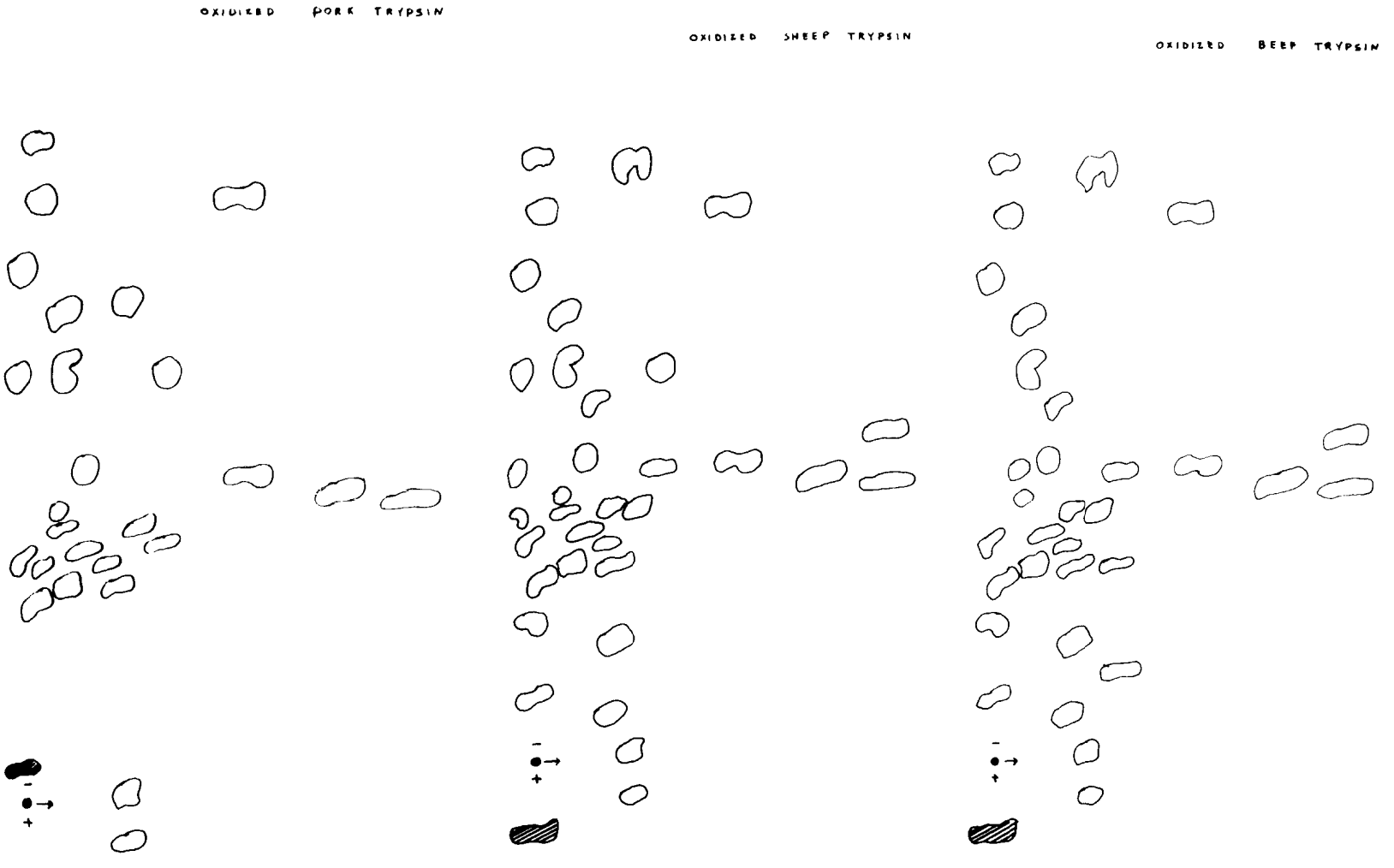

Figure 1. Fingerprint patterns of tryptic digests of oxidized, DIP^{32} labelled, pork (left), sheep (center), and bovine (right) trypsins. Cross-hatched areas were radioactive as well as ninhydrin positive.

of an easily accessible lysyl or arginyl residue. It is quite possible that this mutation may have occurred near the active site of the enzyme since activity is so rapidly lost during the autolysis of bovine and sheep trypsins. Experiments to determine the major point or points of autolytic cleavage are currently under investigation.

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