# ULTRAFILTERABLE PEPTIDES FROM HOMOGENATES OF BOVINE AND PORCINE SUBMAXILLARY GLANDS

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## 1. Introduction

Evidence has been provided [1] that peptides are intermediates in the biosynthesis of proteins. To test this, we report attemps to isolate the peptides directly from homogenates of bovine and porcine submaxillary glands.

## 2. Experimental

### 2.1. *Peptide extraction*

Frozen porcine and bovine submaxillary glands (35–60 g wet weight) were cut into small pieces and homogenized in 100–300 ml of water in a Waring Blender. The homogenate was boiled for 15 min, and the precipitate and cellular debris removed by centrifugation at 22,500 *g*. The supernatant was ultrafiltered and the clear ultrafiltrate lyophilized.

### 2.2. *Gel filtration*

The product was subjected to gel filtration on Sephadex G-50 (fine), and the eluent fractions were monitored by Lowry protein determination*. The pattern obtained is shown in fig. 1. Fractions Pre-A, A, B for bovine and A′ and B′ for porcine glands were separated for analysis. Fractions C and C′ were too small for extensive analysis.

### 2.3. *Amino acid analysis*

The amino acid analysis of fractions corresponding to those indicated on the gel filtration patterns was carried out by the method of Moore, Stein and Spackman*. Hydrolyses for this analysis were conducted in 6 N HCl in sealed tubes at 110°C for 22 hr.

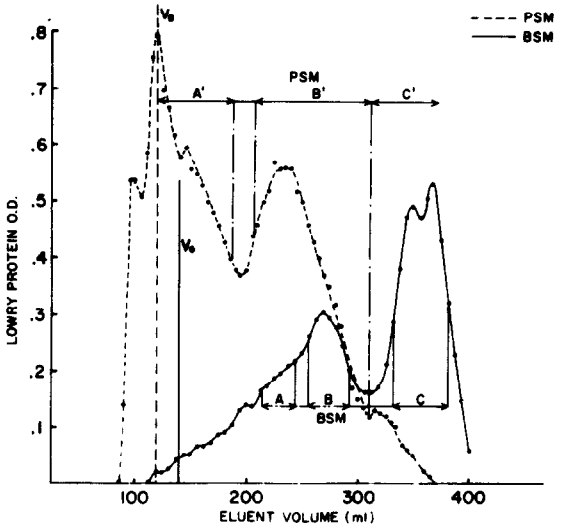


Fig. 1. The Sephadex G-50 (fine) gel filtration of the peptides from homogenized bovine and porcine submaxillary glands: A Sephadex G-50-fine column (2 X 100 cm) previously equilibrated with water was used. A flow rate of 20 ml/hr was maintained with a polystaltic pump. Three ml containing about 100 mg was placed on the coulmns and the elution was with water.

### 2.4. *Ninhydrin value*

Ninhydrin values* were determined on peptide fractions before and after acid hydrolyses. Leucine was used as the reference standard for these analyses.

* References are in preceeding paper.

## 3. Results

The extracts from homogenized and ultrafiltered bovine and procine submaxillary glands were passed through a Sephadex G-50 column using water as eluate. The elutions were monitored by the Lowry method,* and three or four fractions were formed for each tissue as shown (A, B, C) in fig. 1. Fractions C and C′ had a bright yellow color. The positions of these peptides in the column are similar to those for the 22 and 28 amino acid peptides obtained after treatment of BSM with trypsin [2].

The size of the peptides was estimated on these fractions by the ninhydrin method before and after acid hydrolysis. The results are given in table 1. Fractions A and A′ were the largest and had about 12 to 15 component amino acids. Fractions B and B′ averaged as tri- and penta-peptides. Fractions C and C′ were even smaller. The actual values for fractions A and A′ are probably low since the values for the unhydrolyzed materials increased on standing.

Table 1
Ninhydrin values of the sephadex fractions of bovine and porcine peptides before and after hydrolysis.

| | Leucine (μmole/mg sample) | | |
|---|---|---|---|
| Fraction | Unhydrolyzed | Hydrolyzed | Ratio |
| PS-A′ | 0.45 | 6.8 | 15.0 |
| PS-B′ | 1.05 | 2.9 | 2.7 |
| PS-C′ | 1.30 | 2.4 | 1.8 |
| BS-A | 0.600 | 7.2 | 12.0 |
| BS-B | 0.710 | 3.5 | 5.0 |
| BS-C | 0.972 | 2.3 | 2.3 |

The amino acid composition of the peptides fractions A, A′, B, B′ and Pre-A are given in table 2. Qualitatively, the amino acid compositions are similar to the ones reported for bovine and porcine glycoproteins before separation of the minor and major fractions on hydroxyapatite [3]. Aromatic and sulfur amino acids are absent or only present in small amounts. Large proportions of glycine, alanine, threonine, serine, and proline are present. Unexpectedly large proportions of the dicarboxylic acids were also found. In unpublished work on the minor fraction of bovine submaxillary mucin, Dr. T.Ericsson has found that this material is associated with a protein fraction rich in basic and dicarboxylic amino acids.

Bovine fraction A and procine A′ and B′ showed traces of methionine. Bovine fraction Pre-A,B and procine A′ and B′ showed the presence of small amounts of hexosamines. Porcine B′ had an unknown peak located after arginine on the trace from the short column; this fraction also showed traces of cysteine.

All fractions showed absorption peaks in the ultraviolet at 250 to 280 nm. The absorption peaks were as follows: A, 275 (broad); B, 262; Pre-A, 275 (broad); A′, 260; B′, 252; C′, 252 nm. Fractions C and C′ which were strongly yellow colored were relatively insoluble in water but dissolved in acids and water-acetone mixtures.

Each of the three peptide fractions from the bovine and porcine materials was subjected to high voltage electrophoresis. The results are shown in figs. 2 and 3. At pH 3.7, both cationic and anionic spots were present, which stained with the ninhydrin reagent. The bovine peptides (BSP) showed 16 spots and the porcine peptides (PSP) shows 18 spots, but some spots probably contained several peptides since their size was large.

In fig. 3, both ninhydrin staining for peptides and benzidine-periodate staining for carboxydrates were used on the PSP fractions B and C. Only some of the anionic materials showed the presence of carbohydrate components. Fraction A did not show any staining for carbohydrate components.

As reported in the previous paper for *E. coli* and rat liver cells, a limited number of small peptides occur in the ultrafiltrates of bovine and porcine submaxillary glands. The ultraviolet absorptions at 260 to 280 nm possibly arise from the presence of activators attached to the peptides, since the proportion of aromatic amino acids is small.

Further work on the isolation and characterization of the fractions is in progress.

## Acknowledgements

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Table 2
Amino acid composition of peptides from bovine and porcine submaxillary glands.

| | PSM A′ | | PSM B′ | | BSM A | | BSM B | | BSM (Pre-A) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *moles* 100 moles | *g* 100 g[a] | *moles* 100 moles | *g* 100 g[a] | *moles* 100 moles | *g* 100 g[a] | *moles* 100 moles | *g* 100 g[a] | *moles* 100 moles | *g* 100 g[a] |
| Lysine | 5.8 | 4.3 | 5.1 | 4.6 | 6.7 | 8.3 | 4.3 | 5.1 | 4.9 | 6.0 |
| Histidine | 1.8 | 1.3 | 1.4 | 1.2 | tr | tr | 3.5 | 5.4 | 1.1 | 1.5 |
| Arginine | 2.1 | 1.3 | 2.6 | 1.9 | 2.8 | 4.3 | 8.1 | 10.9 | 3.8 | 5.7 |
| Aspartic acid | 10.8 | 9.0 | 6.3 | 6.3 | 9.7 | 10.9 | 11.8 | 12.4 | 11.5 | 12.7 |
| Threonine[b] | 6.8 | 6.4 | 4.5 | 5.2 | 9.3 | 9.1 | 6.6 | 6.1 | 8.0 | 7.7 |
| Serine[b] | 10.5 | 11.6 | 9.6 | 12.8 | 12.5 | 10.6 | 12.8 | 10.1 | 11.9 | 9.9 |
| Glutamic acid | 12.8 | 9.5 | 24.1 | 2.2 | 15.9 | 20.0 | 18.7 | 21.9 | 15.8 | 19.6 |
| Proline | 7.8 | 7.7 | 5.6 | 6.8 | 8.7 | 8.2 | 8.6 | 7.6 | 10.8 | 10.0 |
| Glycine | 11.6 | 19.5 | 8.0 | 16.2 | 9.1 | 5.0 | 9.2 | 4.8 | 9.9 | 5.4 |
| Alanine | 6.9 | 9.3 | 13.7 | 22.5 | 1.0 | 7.0 | 5.9 | 3.9 | 9.2 | 6.3 |
| Valine | 6.5 | 6.2 | 6.0 | 7.0 | 4.3 | 4.1 | 2.7 | 2.5 | 4.5 | 4,3 |
| Isoleucine | 2.7 | 2.3 | 2.3 | 2.4 | 3.2 | 3.5 | 2.4 | 2.5 | 3.0 | 3.2 |
| Leucine | 13.0 | 11.0 | 8.5 | 8.7 | 5.5 | 6.1 | 5.3 | 5.5 | 5.6 | 6.1 |
| Tyrosine | – | – | 0.8 | 0.6 | 1.0 | 1.7 | 0.4 | 0.6 | 0.3 | 0.5 |
| Phenylalanine | 1.0 | 0.7 | 2.2 | 1.7 | 0.8 | 1.2 | 0.3 | 0.4 | 0.2 | 0.3 |
| | 100.1 | | 100.1 | | 100.5 | 100.0 | 100.6 | 99.7 | 100.5 | 99.2 |

[a] Per 100 g of protein as determined by sum of anhydro amino acid residues.
[b] Corrections of 2.7 and 10.0% were made for acid destruction of threonine and serine, respectively.

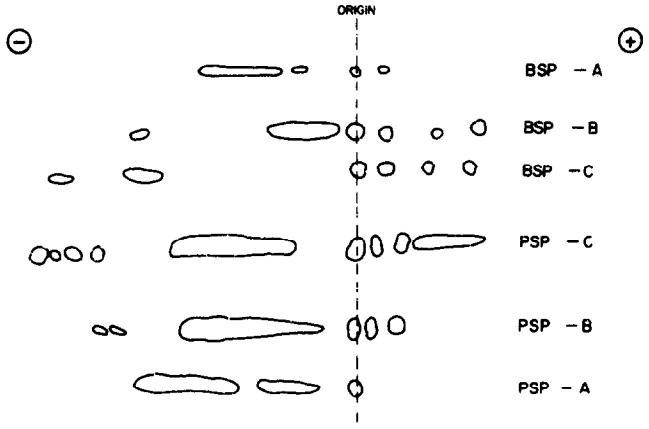


Fig. 2. Electrophoresis: Each of the sephadex G-50 fractions was subjected to electrophoresis. For each fraction 20 to 45 μg of material was spotted on sheets of Whatman No. 1 paper. A pyridine acetate buffer at pH 3.7 was used as well as a borate buffer at pH 8.5 (1500 V was applied for 1 hr). With both buffer systems, several distinct spots were located for each fraction. The spots were visualized with ninhydrin and with a benzidine periodate stain.

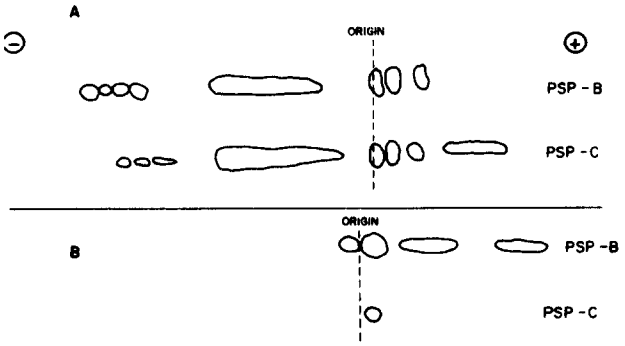


Fig. 3. A. The electrophoresis of PSM peptides B and C. These spots were developed with a ninhydrin stain. B. The benzidine periodate stain was used. 1000 V was applied for 60 min using a pyridine acetate buffer at pH 3.7.

## References

[1] W.Pigman, F.Downs, J.Moschera and M.Weiss, Proceedings of the international conference on blood and tissue antigens (Academic Press, 1970).
[2] M.Weiss and W.Pigman, J. Biol. Chem., submitted for publication.
[3] G.Tettamanti and W.Pigman, Arch. Biochem. Biophys. 124 (1968) 41.