ULTRAFILTERABLE PEPTIDES FROM RAT LIVER AND *E. COLI* HOMOGENATES

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1. Introduction

Downs and Pigman [1] reported the isolation of glycopeptides from bovine and ovine submaxillary mucin (BSM and OSM) after alkaline β-elimination and mild acid hydrolysis. The peptide chains are composed of 20 and 28 amino acids, respectively, with the longer chain as the main product. Weiss and Pigman [2] obtained similar glycopeptides in high yields by the action of trypsin on BSM. For reasons described elsewhere [3], a twostage synthesis of the protein core has been proposed. According to this concept, small peptides are formed at the ribosomes, and the protein is assembled by polymerases at the Golgi membranes. As a confirmation of this mechanism and to test its generality, efforts were made to isolate the peptide pool directly from several types of submaxillary glands, *E. coli* cells, and rat liver. The results obtained for submaxillary glands will be reported elsewhere [4]. Those for *E. coli* and rat liver are reported in the present paper.

2. Methods

Liver from two male rats was frozen in dry ice, immediately suspended in 0.1 M NaCl and homogenized for about two minutes. The homogenate was boiled for 15 min. Further work was performed on the supernatant obtained after centrifugation of the boiled suspension at 35,000 *g* for 20 min at 4°C.

E. coli B was grown in a culture medium containing D-glucose as the sole carbon source and ammonium sulfate as nitrogen source. Incubation of the microorganism was carried out at 30°C for a period of 18 hr. The cells were harvested by centrifugation in a Sharples continuous flow centrifuge, boiled in 100 ml of a 0.008 M phosphate buffer, pH 7.7 for ten min, and submitted to sonic oscillation for 20 min in an ice bath using a Branson sonicator.

The disrupted cell-suspension and the hepatic saline extract were each separately ultrafiltered at 4°C, *in vacuo*, using a cellophane tubing (0.62 cm or 1/4 inches in diameter). Both ultrafiltrates (crude extracts) were lyophilized and dried *in vacuo* over phosphorus pentaoxide. The dry material (202 mg from 15 l of growth medium from *E. coli* and 3.25 g from 40 g of wet liver) was dissolved in 5 ml of buffer (pH 7.7), and for each of the two ultrafiltrates, an aliquot of 3 ml was applied to Sephadex G-50 columns (coarse, 2 × 100 cm). The conditions of gelfiltration and the pattern of elution of both liver and *E. coli* extracts are outlined in fig. 1. The effluents representing individual peaks were combined as indicated in fig. 1 and submitted to two-dimensional high-voltage electrophoresis (pH 1.9 and 4.7) and thin layer chromatography. In both instances, several well-defined ninhydrin reactive spots were detected (fig. 2).

No autodigestion of either extract was observed, when followed by ninhydrin reaction over a period of 48 hr, at room temperature.

Each combined fraction (see fig. 1) was submitted to acid hydrolysis (6 N HCl at 110°C for 22 hr). Aliquots before and after hydrolysis were analyzed by the ninhydrin method. These results are reported in table 1. The amino acid compositions of all fractions hydrolyzed as above for both the *E. coli* and liver ultrafiltrates are shown in tables 2 and 3. In neither material was any hydroxyproline detectable.

3. Results

Boiled rat liver *E. coli* cells were sonicated briefly,

Table 1
Effect of hydrolysis on ultrafiltrate and sephadex G-50 treated fractions from *E. coli* B and rat liver. Results are expressed as μ-moles mg^{-1} of leucine of dry material.

Fraction	*E. coli* B			Liver		
	Unhydrolyzed	Hydrolyzed	Ratio	Unhydrolyzed	Hydrolyzed	Ratio
Ultrafiltrate	0.43	1.46	3.4	.70	7.6	10.8
A	0.33	6.81	20.7	.25	5.05	20.2
B	0.5	3.39	6.8	.25	1.24	5.0
C	0.5	1.00	2.0	.55	1.13	2.1
D	0.18	1.35	7.5	.45	2.23	4.9

Hydrolysis was done in 6 N HCl for 22 hr at 110°C.

Table 2
Amino acid composition of *E. coli* and rat liver peptides ($\mu g\ mg^{-1}$).

Amino acid	*E. coli*				Rat liver			
	A	B	C	D	A	B	C	D
Lysine	152	93	83	14	109	88	84	39
Histidine	55	19	23	19	21	14	21	69
Amonia	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
Arginine	36	58	32	19	37	26	tr	–
Aspartic Acid	109	115	55	14	158	109	61	59
Threonine	92	40	28	12	60	34	22	21
Serine	35	33	37	19	46	36	46	42
Glutamic Acid	157	245	170	14	227	363	441	298
Proline	55	276	32	tr	50	37	tr	tr
Glycine	55	94	189	833	57	131	139	204
Alanine	117	84	194	31	35	42	89	89
Half Cystine	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
Valine	47	55	65	24	66	48	46	31
Methionine	Pres	26	46	Pres	Pres	Pres	–	Pres
Isoleucine	29	37	14	tr	45	21	16	15
Leucine	41	52	18	tr	58	38	34	29
Tyrosine	–	–	–	–	–	–	–	42
Phenylalanine	19	22	14	tr	34	13	tr	63

Pres = Present in appreciable amounts.
Tr = trace amounts

in water, ultrafiltered and eluted with water from a Sephadex G-50 column. The elution was monitored by the Lowry method [5] for proteins. The results are shown in fig. 1.

Considerable peptide material was present, only a small portion of which was found in the void volume.

The peptide mixtures were separated into four fractions (A, B, C, D) as indicated in the figure. Ninhydrin determinations [6] before and after acid hydrolysis were used to estimate the size of the peptides and are given in table 1. The ratios show the approximate number of amino acids present in each fraction. Both materials are composed of peptides having about 20 amino acids in fraction A and of smaller peptides in the later fractions.

The crude ultrafiltrates of both liver and *E. coli*

Table 3
Relative amino acid composition of *E. coli* and rat liver peptides (moles 100 moles^{-1}).

Amino acid	*E. coli*				Rat liver			
	A	B	C	D	A	B	C	D
Lysine	12.0	7.2	5.6	0.6	9.0	6.9	6.3	2.8
Histidine	4.2	1.4	1.6	0.9	1.6	1.0	1.5	4.8
Ammonia	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
Arginine	2.4	3.9	1.8	0.7	2.6	1.7	tr	tr
Aspartic Acid	9.8	10.3	4.3	0.8	14.7	9.6	5.3	4.9
Threonine	9.4	4.1	2.6	0.7	6.5	3.4	2.2	1.9
Serine	4.1	3.9	3.9	1.3	6.2	4.2	5.3	4.6
Glutamic Acid	12.6	19.5	17.6	0.7	18.8	28.5	33.7	21.9
Proline	5.9	3.0	3.0	tr	5.5	3.9	tr	tr
Glycine	10.0	17.0	29.5	90.2	10.6	23.5	24.3	34.2
Alanine	17.1	12.1	24.1	2.7	5.3	6.1	12.4	11.9
Half Cystine	Pres	Pres	Pres	Pres	Pres	Pres	Pres	Pres
Valine	4.9	5.8	6.0	1.4	7.1	5.0	4.6	2.9
Methionine	Pres	2.1	3.1	–	Pre	Pres	–	Pres
Isoleucine	2.6	3.4	0.9	tr	4.2	1.9	1.4	1.3
Leucine	3.7	4.6	1.3	tr	5.5	3.5	3.0	2.4
Tyrosine	–	–	–	–	–	–	–	2.4
Phenylalanine	1.4	1.6	0.8	tr	2.5	0.9	tr	4.1

Pres = Present in appreciable amounts.
Tr = trace amounts.

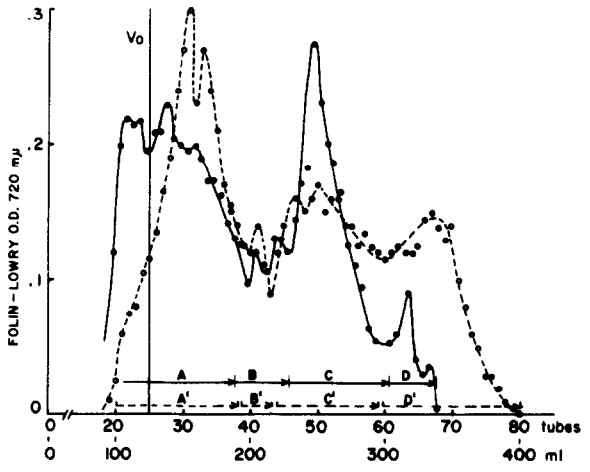

Fig. 1. Column chromatograpgy of ultrafiltrates from rat liver (———) and *E. coli* (- - - -) on Sephadex G-50 coarse (2 X 100 cm). Three ml aliquots containing 300 mg were put on the column and 5 ml fractions were collected using water as the eluent (20 ml/hr).

showed strong absorptions at 260 nm. Fraction D and, to lesser extent C were bright yellow in color and the liver fractions had a strong absorption band at 360 nm falling into a second smaller peak at 440 nm; little absorption was evident above 500 nm.

The amino acid compositions of the four fractions are given in tables 2 and 3. All of the common acids are present in some of the fractions, but tyrosine was found only in fraction D of the rat liver peptides. The most striking features are generally the large amounts of glycine, alanine, glutamic acid, and aspartic acid in most of the fractions. The *E. coli* fraction D was almost completely a polyglycine fraction. Cystein was present in all fractions but was not measured. Liver fractions A and D, and all *E. coli* fractions had large amounts of ammonia, which was not measured. Only traces or no hexosamine peaks were present.

Several unknown ninhydrin-reacting acids were evident in traces. These peaks fell between those for cysteic acid and aspartic acid. They were observed for liver fractions B, C, D and all *E. coli* fractions. One of the unknown materials showed its ninhydrin reaction product as having an absorption at 440 nm.

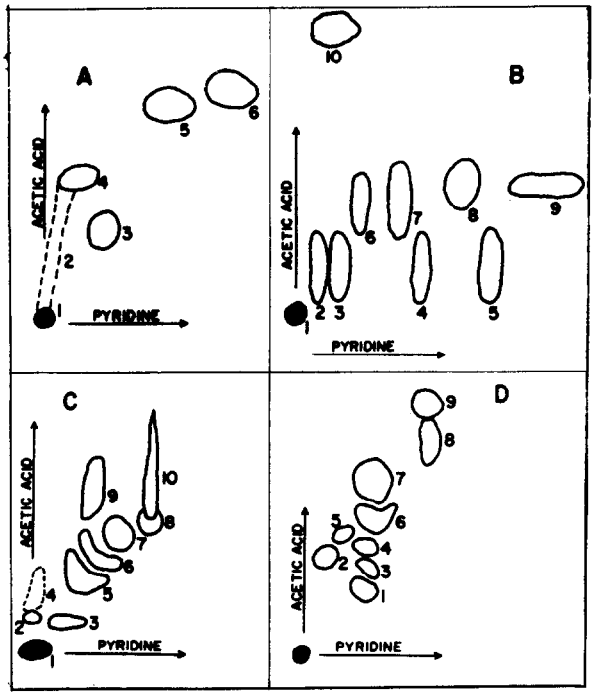

Fig. 2. Peptide maps of Sephadex G-50 (coarse) fractions of rat liver performed on TLC plates (Q 1, Quantum Industries, Hanover, N. J.) The first dimension was run in a system containing butan-1-ol, acetic acid and water (3:1:1). The second dimension was run in iso-amyl alcohol, pyridine, water (30:30:35). At the completion of the run, the plates were dried and stained with 0.4% ninhydrin in acetone.
Abbreviations: (A, B, C, D) correpond to Sephadex G-50 patterns shown in fig. 1.

Two-dimensional peptide "maps" on thin layer plates are shown in fig. 2 for the four liver peptides and in fig. 3 for the *E. coli* peptides. Liver fraction A showed six spots, B nine spots and D nine spots, a total of 34 spots. *E. coli* peptides had for fraction A six spots, B seven spots, C twelve spots and D nine spots, a total of 34 spots.

4. Discussion

It is clear that rat liver and *E. coli* cells contain a pool of small peptides, averaging 2 to 20 amino acids. About 34 ninhydrin reacting spots were found in the

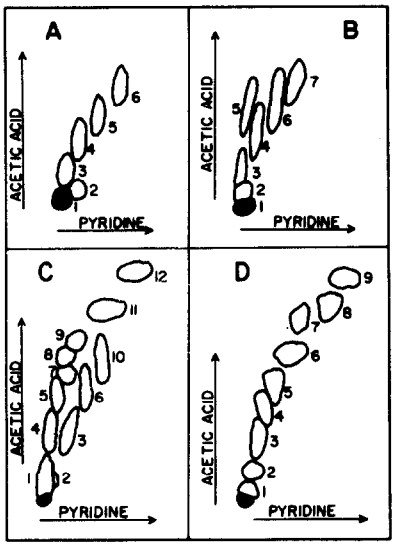

Fig. 3. Peptide maps of Sephadex G-50 (coarse) fractions of *E. coli* B ultrafiltrate. For conditions, consult fig. 2.

peptides maps of the four peptide fractions of both materials. The high ultraviolet absorptions at 260 nm suggests that they may contain an "activator" such as a pyrimidine base or a nucleotide.

It is unlikely that these peptides were formed by autolysis or by cathepsins, since boiled tissues were used and the crude ultrafiltrates did not show any increase in ninhydrin value upon long standing. In addition all operations were carried out as quickly as possible at 4°C.

One of the fractions from each material had a sequence composed of about twenty amino acids. This size is similar to the 20 and 28 amino acid repeating units occurring in BSM and OSM, and to the 22 amino acid unit in PSM [2, 3]. However, the presence of dipeptides was unexpected. The dipeptide fraction is presumably attached to other materials since it was eluted ahead of fractions having 5 to 8 amino acids.

The liver fraction eluted last had a bright yellow color with two fairly sharp absorption peaks at 360 and 440 nm.

J.A.Ramsey [7] writes, "it appears that whereas carbohydrate polymers can be built up gradually, by successive addition of units, this does not seem to be true of proteins; analyses show the presence in organ-

isms of proteins and of free amino acids, but peptides of intermediate size are conspiciously absent. This suggests that protein molecules are not built up piecemeal but are assembled in their completed form in a single operation." Similarly, R.W.Hendler [8] also writes, "Today, no one speaks of peptide intermediates", although he suggests that they may exist.

The existence of a pool of peptides in widely different biological proteins as shown in the current and the accompanying paper must then be considered as consistent with the two-step concept of protein synthesis as a general mechanism [3].

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