Peptides obtained by tryptic digestion of clupeine

Peptides in a tryptic digest of clupeine* have been separated quantitatively by elution analysis on an Amberlite IRC-50 (XE-64) column, in the manner similar to that reported previously[2]. The elution diagram in Fig. 1 shows that this digest was fractionated into about 16 chromatographic peaks. Although some peaks were still mixtures of 2 to 4 peptides, they could be successfully separated as the DNP derivatives by paper chromatography or paper electrophoresis. The structure of the isolated peptides was examined by means of the DNP method and carboxypeptidase-B digestion. Leucine aminopeptidase digestion and hydrazinolysis were also used to ascertain the amino acid sequences of rather complicated hexapeptides (4d and 7, Table I). The nature and the amount of almost all of the fragments in the digest were thus determined. The results summarized in Table I give nearly complete information on the sequences in which the monoamino-monocarboxylic acid residues (including proline) of clupeine participate. It is of considerable interest that many common sequences can be found between the peptides in Table I and those obtained by MONIER AND JUTISZ from partial hydrolysates of salmine[3], in spite of a considerable difference in monoamino acid composition between both protamines.

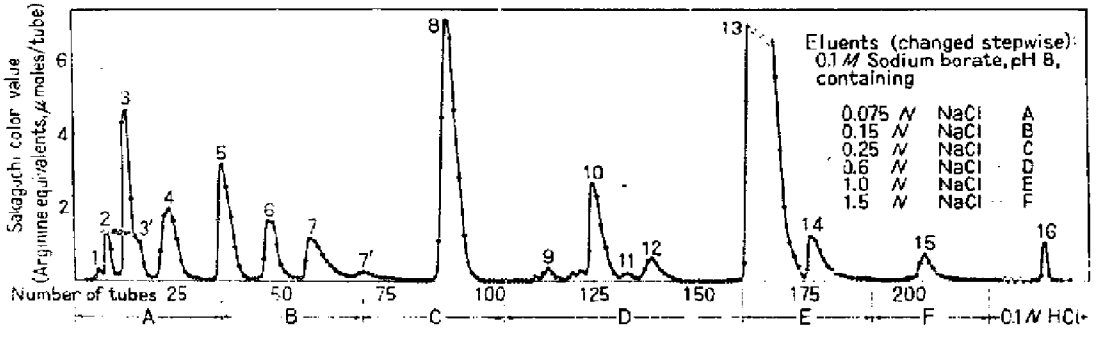

Fig. 1. Chromatographic separation of a tryptic digest of clupeine. 60 mg clupeine sulfate in 3.0 ml 0.067 *M* phosphate, pH 7.81, were incubated with 0.34 mg trypsin (Nutritional Biochem. Co., twice crystallized) for 20 h at 30°, and then the whole digest was chromatographed at 30° on a 1.0 × 30 cm column packed with Amberlite IRC-50 (XE-64) in equilibrium with the starting eluent. Six eluents of progressively increasing concentrations of Na^+ were used. Finally, all the peptides remaining in the column were eluted by 0.1 *N* HCl as a single peak (No. 16), after the column was washed with 0.2 *N* acetic acid to remove Na^+. The effluent was collected in 3.1-ml fractions with a flow rate of about 4 ml/h. Aliquots (0.2–0.1 ml) were removed for analysis by the Sakaguchi colorimetry.

It has been well-known since KOSSEL's days that both protamines contain arginine (A) and monoamino acids (M) in the molar ratio of approximately 2 to 1. For this reason, a repeating structure with a unit of MA_2 has been proposed by KOSSEL[4] and WALDSCHMIDT-LEITZ *et al.*[5] for clupeine, and one with a unit of M_2A_4 by FELIX *et al.*[6]. On the other hand, a mixed formula containing two units, M_2A_m and MA_n, has been suggested by MONIER *et al.*[3] for the structure of salmine. No quantitative studies, however, have been performed to decide the ratio of the two units.

Abbreviation: DNP, dinitrophenyl.

* The less soluble specimen prepared from mature milts of Japanese herring (*Clupea pallasii*)[1].

TABLE I

PEPTIDES OBTAINED FROM A 20-H TRYPTIC DIGEST OF 90 mg (APPROX. 11 μMOLES) CLUPEINE SULFATE

Fraction		Structure	Amount μmoles	Fraction		Structure	Amount μmoles
2		Thr·Thr·Arg	2.4	8		Arg	28.6
3 (+ 3′)	a	Val·Ser·Arg	7.7	9	a	Arg·(Val,Ser)·Arg	0.4
	b	Thr·Arg	4.5		b	Val·Ser·Arg·Arg	0.2
	c	Pro·Val·Arg	2.1		c	Arg·Thr·Thr·Arg	0.1
	d	Pro·Ileu·Arg	0.2	10		Arg·Pro·Arg	6.0
4	a	Ala·Gly·Arg	1.9	11	a	Arg·Ala·Arg	0.3
	b	Ala·Ser·Arg	2.0		b	Arg·Thr·Arg	0.2
	c	Ser·Arg	2.5	12	a	Ala·Arg·Arg	1.3
	d	Ser·Ser·Arg·–Pro·Ileu·Arg	1.7		b	Pro·Arg·Arg	0.6
5		Ala·Arg	10.9	13		Arg·Arg	42.1
6		Pro·Arg	5.5	14		Arg·Arg·Pro·Arg	2.8
7 (+ 7′)		Ala·Ser·Arg·–Pro·Val·Arg	5.0	15		Arg·Arg·Arg	1.5

The results in Table I show that a formula similar to that of MONIER *et al.* seems more adequate for clupeine also. Analytical data further indicate that two-thirds* of the monoamino acid residues in the molecule exist in the form of –AMMA–, and the rest in the forms of –AMA– and MA– (N-terminus). It is also worth noticing that a peculiar sequence**, –AMMAMMA–, was found for the first time in protamines (see 4d and 7, Table I). Such a sequence shows distinctly the presence of an arginine residue separated singly by monoamino acids, while peptides Nos. 13, 14 and 15 suggest the occurrence of considerable amount of poly(tri- or more)-arginine sequences in clupeine.

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* These figures should be regarded as average values, because clupeine has an intrinsically heterogeneous nature. The present specimen of clupeine is an approx. equimolar mixture of molecules with N-terminal sequences of Ala·Arg·Arg- and Pro·Arg·Arg- respectively[7].

** This sequence amounts to approx. 0.8 mole/mole clupeine, if it is assumed that peptides Nos. 3c and 4b are derived from the peptide No. 7. Actually, DNP-Ala·Ser·Arg·Pro·Val·Arg was slowly but completely split into DNP–Ala·Ser·Arg and Pro·Val·Arg by prolonged tryptic digestion.