On the Constituent Amino Acids, *N*-Terminal Residues and the Molecular Weights of Protamines[1]

By Toshio ANDO, Shin-ichi ISHII, Chieko HASHIMOTO, Makoto YAMASAKI and Koichi IWAI

(Received March 8, 1952)

Each sample of crude protamines, clupein and salmine, was tentatively fractionated into two parts, namely the more and the less soluble parts, making use of the different solubilities of their picrates in 67% aqueous acetone, and they were then purified to yield colorless crystal-like sulfates respectively[2].

The following amino acids were found as the constituents in the purified specimens of protamines by means of paper chromatography of their hydrolysates respectively: Arg, Pro, Ala, Val, Ser, Thr, Iso (or Leu) and Gly in both the more and the less soluble clupein; Arg, Pro, Ser, Val, Gly, Ala and Iso (or Leu) in the less soluble salmine. Thus, while no other amino acid than those heretofore known[3,4,5] was found in salmine, glycine was now found for the first time, besides the other constituents[4,6,7], in each specimen of our clupein. The probability of an occurrence of glycine from the contaminated nucleic acid can be excluded, as the ultraviolet absorption of the specimens in solution showed no presence of the above acid. Between the less and the more soluble clupein, some differences could be found in the relative contents of the constituent amino acids. Studies are now in progress with respect to the nature as well as the homogeneity of the soluble substances.

Further results were obtained, showing that salmine (the less soluble) has indeed proline[8,5] as the only *N*-terminal residue of the peptide molecule, being proved by means of the DNP-method[9] followed by paper chromatographic and spectrophotometric methods, and has a molecular weight of *ca.* 7,300 as the free base estimated tentatively[10] from the methoxyl content (0.34%) of the methyl ester sulfate, which was prepared under a mild condition of esterification.[11] In the case of the less soluble specimen of our clupein, it has, contrary to the recent results of German researchers,[6] alanine but not proline as the *N*-terminal residue, being confirmed on paper chromatograms not only as the DNP-alanine[12] produced but also as the free alanine regenerated[13] therefrom, and it is assumed[10] to have a molecular weight of *ca.* 10,800 as the free base from the methoxyl content (0.23%) of the ester sulfate. Contrary to the old view that salmine is about twice as large as clupein, both protamines seemed thus to have the molecular weight of nearly the same order.

The electrometric titration of the protamines and the ultraviolet absorption of the DNP-clupein (λ_{max}: 358-360 and 257-260 mμ at pH=9.1) and of the DNP-salmine (λ_{max}: 372-375 mμ at pH=9.1) seemed to give further results consistent with those obtained above for their *N*-terminal residues. Details of these experiments will be reported later.

Institute of Science and Technology, Tokyo University, Komaba-machi, Meguro-ku, Tokyo

(1) The present paper was read by one of the authors (T. A.) before the symposiums on protein structure held on May 20, 1951 in Osaka University and on Febr. 16, 1952 in Tokyo University.

(2) T. Ando, *et al.*, *Repts. Radiation Chem. Res. Inst. Tokyo Univ.*, **3**, 14 (1948); **4**, 31 (1949); **5**, 56 (1950).

(3) G. R. Tristram, *Nature*, **160**, 637 (1947); D. Hamer, *et al.*, *ibid.*, **163**, 689 (1949).

(4) R. J. Block, *et* al., *Proc. Soc. Expt. Biol. Med.*, **70**, 494 (1949).

(5) S. F. Velick, *et al.*, *J. Biol. Chem.*, **191**, 233 (1951).

(6) K. Felix, *et al.*, *Z. physiol. Chem.*, **286**, 67 (1950); E. Waldschmidt-Leitz, *et al.*, *Experientia*, **7**, 183 (1951).

(7) Y. Kuroda, *J. Biochem.* (Japan), **38**, 115 (1951).

(8) R. R. Porter, *et al.*, *Biochem. J.*, **42**, 287 (1948).

(9) F. Sanger, *Biochem. J.*, **39**, 507 (1945).

(10) The assumption is made that the purification of the ester is accomplished.

(11) H. Fraenkel-Conrat, *et al.*, *J. Biol. Chem.*, **161**, 259 (1945).

(12) *Cf.* G. Biserte, *et al.*, *Bull. soc. chim. biol.*, **33**, 50 (1951).

(13) *Cf.* A. G. Lowther, *Nature*, **167**, 767 (1951).