LOCATION OF THE THREE BURIED TYROSYL GROUPS OF RIBONUCLEASE A*

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Cha and Scheraga (1961) described the iodination of ribonuclease A to form derivative A in which three of the six tyrosyl groups were iodinated. In the present communication we report initial data on the location of the positions of the three iodinated groups in the amino acid sequence.

EXPERIMENTAL

Derivative A was analyzed on an IRC-50 column by elution at a pH and ionic strength gradient of pH 6.40, μ = 0.15 to pH 6.47, μ = 1.0. The chromatographic pattern is shown in Fig. 1 together with that of derivative C, the latter having an average of four iodinated tyrosyl groups (Cha and Scheraga, 1961). The protein concentrations differed in the two chromatographic runs. The main peak from derivative A is composed of 74% of a slow-moving component and 26% of a fast one. Increasing the degree of iodination leads to an increase in the fast-moving component at the expense of the slow-moving one (upper curve of Fig. 1). Therefore, the fast-moving component of derivative A is more highly iodinated and will not interfere (i.e. will not contribute spurious non-iodinated tyrosyl peptides) in the fingerprint experiments to be described below.

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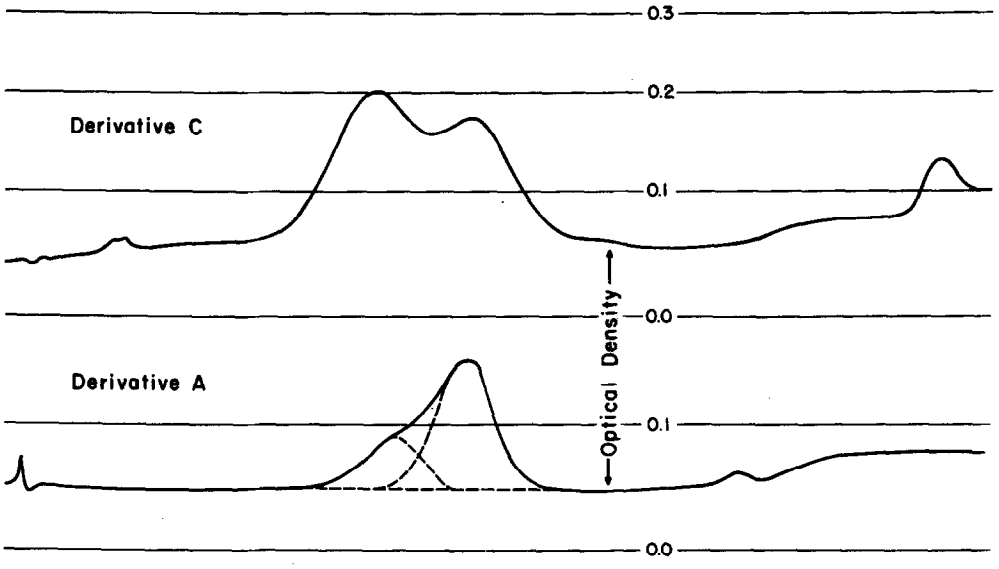

Fig. 1

Derivative A was used in the following experiments without chromatographic purification.

Equal amounts of both derivative A and the native protein were oxidized with performic acid at -10°C by the method of Hirs (1956)**. The reaction products were lyophilized, then digested with trypsin and chymotrypsin, and fingerprinted** by the procedure of Anfinsen et al (1959) and Katz et al (1959)***. Some of the fingerprints

** Under the same conditions diiodotyrosine is destroyed; neither mono-iodotyrosine nor tyrosine are formed in the performic acid oxidation of diiodotyrosine. Therefore, any diiodotyrosyl-containing peptides obtained from the oxidation of derivative A will be modified and will have different mobilities on paper than the corresponding peptides from ribonuclease A. Any iodinated peptides from the fast-moving, more highly-iodinated impurity in derivative A will also have different mobilities, and will not interfere with the interpretation of the fingerprint.

*** Modification of tyrosyl groups during oxidation in the presence of halide ion is minimized at low temperature (-10°C) (Thompson, 1954; Hirs, 1956). A possible small amount of modification of un-iodinated tyrosyl groups at -10°C could arise from the presence of iodide ion produced by the destruction of diiodotyrosine. However, this effect did not seem to arise to any significant extent.

were stained with ninhydrin and some with Pauly reagent. The fingerprints of derivative A were compared with those of the native protein.

RESULTS AND DISCUSSION

The results of the fingerprinting experiments are shown in Table I.

Table I

IDENTIFICATION OF IODINATED AND UN-IODINATED TYROSYL PEPTIDES

Peptide No.[a]	Composition[b]	Appearance in fingerprint of derivative A[c]	Position of tyrosyl in sequence
7	glu·ser·tyr	No	76
8	tyr·pro·asp·ala·cys·tyr·lys	Yes	92, 97
20	asp·gly·thr·asp·glu·cys·tyr	No	73
28	his·ileu·ileu·val·ala·cys·glu·gly·asp·pro·tyr	Yes	115

(a) Notation of peptides (Anfinsen et al, 1959).

(b) Composition of peptides determined by Anfinsen et al (1959).

(c) While equal amounts of both derivative A and the native protein were used, the intensities of the spots of peptides 8 and 28 from derivative A were about 1/3 to 1/2 those from native ribonuclease. This decrease in intensity could arise either from some further iodination (i.e. from the small amount of fast-moving component shown in Fig. 1) or from a modification of uniodinated tyrosyl groups by iodide ion during the performic acid oxidation at -10°C. The intensities of the ninhydrin spots from the non-tyrosyl-containing peptides were qualitatively identical in the fingerprints from derivative A and from native ribonuclease.

As also reported by Anfinsen et al (1959), the fingerprint does not show an identifiable peptide containing the sixth tyrosyl group at position 25. However, it is clear from the absence of peptides 7 and 20, and the presence of peptides 8 and 28, that tyrosyl

residues 92, 97 and 115 were not iodinated;**** these three are, therefore, presumed to be the "buried" tyrosyl groups of ribonuclease.

Further work on derivative A, and also on derivative C, is in progress.

REFERENCES

Anfinsen, C. B., Åqvist, S. E. G., Cooke, J. P. and Jönsson, B., J. Biol. Chem., 234, 1118 (1959).

Cha, C. Y. and Scheraga, H. A., Biochem. and Biophys. Res. Comm., 5, 67 (1961).

Hirs, C. H. W., J. Biol. Chem., 219, 611 (1956).

Katz, A., Dreyer, W. D., and Anfinsen, C. B., J. Biol. Chem., 234, 2897 (1959).

Thompson, E. O. P., Biochim. et Biophys. Acta, 15, 440 (1954).

**** The question might arise as to whether chymotrypsin will digest the chain in locations following a destroyed diiodotyrosyl group. Since peptide 12 (Anfinsen et al, 1959), ser·thr·met, (following iodinated tyr 76) appears in the fingerprint, and since derivative A contains three iodinated tyrosyl groups, it seems that chymotrypsin was able to split peptide bonds after tyr 73 and tyr 76 in derivative A.