DIFFERENTIATION OF THE TYROSYL GROUPS OF RIBONUCLEASE A BY IODINATION*

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Three of the six tyrosyl residues of ribonuclease are abnormal (Shugar, 1952; Tanford et al, 1955). Since the amino acid sequence of this protein is known (Hirs et al, 1960), iodination offers the possibility of locating the positions of the three abnormal groups in the polypeptide chain, thereby providing information about the conformation of the molecule in solution.

EXPERIMENTAL

Ribonuclease A was iodinated at 10°C at pH 9.4 according to the recommendations of Hughes and Straessle (1950) to produce diiodotyrosyl groups without affecting other amino acid residues. Several derivatives were prepared with different degrees of iodination.**

RESULTS

Alkaline Titration Data:- Spectrophotometric titrations were carried out at alkaline pH at 312 mμ and 295 mμ (10°C) to determine the number of diiodotyrosyl and tyrosyl residues (Table I).

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**The tyrosyl groups react with iodine at different rates; three groups react rapidly and the remaining ones more slowly. For example, three groups reacted completely in 1.5 hrs. The fourth group was not completely iodinated after 3 hrs. When an amount of iodine equivalent to six tyrosyl groups was added, two groups remained un-iodinated even after 25 hrs., the iodine color persisting for this length of time.

TABLE I

TITRATION OF DIIODOTYROSYL GROUPS

Derivative	Number of tyrosyl groups equivalent to the amount of iodine added	Change in molar extinction coefficient, E, at 312 mμ	Number of tyrosyls iodinated*	pK**
A	3	15,000	3.1	8.0
B	4	18,000	3.8	7.8
C	6	18,800	3.9	7.6

*Based on a value of 4800 per single group of diiodotyrosine in insulin (Gruen et al, 1959).

**The observed pK's are somewhat higher than that of diiodotyrosine, 6.5, (Gruen et al, 1959) and close to that of monoiodotyrosine, 8.2, (Herriott, 1948). However, the appearance of the U.V. absorption spectrum indicates that monoiodotyrosine is absent. The high pK's may be due to local interactions in the iodinated protein.

The titration at 295 mμ showed an increase in E of about 3000 between pH 8 and 12.5 in derivatives A, B, and C. However, this contribution is due to the diiodotyrosyl groups rather than to the ionization of the un-iodinated abnormal tyrosyl groups. At pH 12 and 12.5 the abnormal tyrosyl groups ionize irreversibly on standing at room temperature for several hours. This behavior is similar to that of native ribonuclease.

U.V. Difference Spectra at Low pH:- U.V. difference spectra were obtained at low pH and compared with those for native ribonuclease (Hermans and Scheraga, 1961). The difference spectra of derivatives A, B, C are similar to each other and to that of the uniodinated molecule. Since the native protein contains two tyrosyl-carboxyl hydrogen bonds imbedded in hydrophobic regions (Hermans and Scheraga, 1961), it appears that these interactions still exist in derivatives A, B, and C. Precipitation occurs below pH 1 in all 3 derivatives; it seems that the solubility of ribonuclease is decreased at low pH by iodination.

U.V. Absorption Spectra:- The U.V. absorption spectra of derivatives A and C were examined between 270 mμ and 320 mμ at pH 10.5 and 11.5, respectively, to search for monoiodotyrosine (peak at 305 mμ according to Herriott, 1948) and the un-iodinated, abnormal tyrosines (peak at 276 mμ, since the abnormal tyrosines would still be protonated at these pH's). Two peaks were observed, one at 287 mμ and one between 310 mμ and 311 mμ. The former is due to the un-iodinated tyrosyl groups (3 in derivative A and 2 in derivative C) and the latter to diiodotyrosyl groups (3 in derivative A and 4 in derivative C). It can be shown, by addition of separate curves for tyrosyl and diiodotyrosyl groups, that the tyrosyl peak will appear at 287 mμ because of the contribution from the diiodotyrosyl spectrum. It can also be concluded from these absorption curves that monoiodotyrosine is absent.

CONCLUSIONS

1. Two tyrosyl groups of ribonuclease are not iodinated under the conditions used here (derivatives B and C). The three rapidly iodinated tyrosyl groups are the three normal ones (derivative A). In addition, one of the abnormal tyrosyl groups can be iodinated (derivatives B and C), but at a slower rate.

2. Since derivatives A, B, and C, and native ribonuclease have the same low pH U.V. difference spectrum, and since all these compounds have un-iodinated abnormal tyrosyl groups, the low pH difference spectrum must arise from two of the tyrosyl groups which ionize irreversibly at alkaline pH.

3. Since one of the abnormal tyrosyl groups can be iodinated without changing the environment of the other two abnormal tyrosyl groups, the iodinated abnormal group may be a reversibly ionizable one, but with high pK. Presumably, it cannot be distinguished

from the two other abnormal groups in an alkaline titration of the native molecule because of the irreversible change in the conformation of the native molecule at high pH. It is also reasonable to conclude that this tyrosyl, unlike the other two abnormal tyrosyls, is not near a carboxyl group since it does not contribute to the U.V. difference spectrum at low pH. When it is iodinated it titrates like the three other diiodotyrosyls, suggesting a possible small conformational change in the vicinity of this tyrosyl upon iodination.

4. The conformations of derivatives A, B, C and the native molecule appear to be similar since all have abnormal tyrosyls.

Enzymatic hydrolysis experiments are in progress to locate the 3 and 2 un-iodinated, abnormal tyrosyl groups of derivatives A and B, respectively. Such studies should permit the distinction between diiodotyrosine and monoiodotyrosine, if any of the latter is present. Similar experiments are being carried out with methylated ribonuclease (Broomfield and Scheraga, 1961) to identify the abnormal carboxyl groups which are interacting with the abnormal tyrosyl groups.

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