LIMITED PROTEOLYSIS OF RIBONUCLEASE - ACTIVE COMPONENTS*

John A. Rupley,** Tatsuo Ooi, and Harold A. Scheraga
Department of Chemistry, Cornell University, Ithaca, N. Y.

Received May 7, 1962

Recently it was shown by Rupley and Scheraga (1960) that, whereas chymotrypsin and trypsin do not digest ribonuclease at room temperature, they do so at elevated temperature where ribonuclease is partially unfolded (Hermans and Scheraga, 1961). In this communication we report the results of the purification of the digestion products, the isolation of active components from the tryptic digest, and the location of the chymotryptic and tryptic splits.

The digestion of ribonuclease A by either chymotrypsin or trypsin was carried out at pH 6.5, at 60°C, and the products were separated on an IRC-50 column. Further purification was carried out on sephadex to remove small peptides from the digestion products. Amino acid composition, N-terminal group (with FDNB and leucine aminopeptidase), and C-terminal group (with carboxypeptidase A and B, respectively) analyses were carried out on the separated fragments and on their performic acid oxidation products to locate the sites of proteolytic attack.

---

* This work was supported by research grant No. E-1473 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service, and by grant No. G-14792 from the National Science Foundation.

** Present address: Department of Chemistry, University of Arizona, Tucson, Arizona.

The most plentiful of the chymotryptic digestion products was an enzymically inactive fragment in which the N-terminal tail was missing (hydrolysis of tyr 25-cys 26 bond). This component, even though chromatographically homogeneous, is apparently a mixture of several species, since appreciable but partial hydrolysis was observed for bonds met 79-ser 80, tyr 97-lys 98, leu 35-thr 36, and tyr 76-ser 77 (listed in order of decreasing extent of hydrolysis). Slight (approximately 10%) splitting of the phe 46-val 47 bond was observed. In addition, N-terminal glutamic acid or glutamine was present, suggesting a split of the tyr 73-glu(NH_2) 74 bond.

The tryptic digest was chromatographed to yield three major components I-III. Component I was unreacted ribonuclease, while components II and III had hydrolyzed peptide bonds in the following locations. Components II and III each had 30% of the activity of ribonuclease.

Component	Bond Split
II	lys 31-ser 32 and arg 33-asp(NH_2) 34 (i.e. ser 32-arg 33 dipeptide missing*)
III	a mixture of two components with lys 31-ser 32 split in one and arg 33-asp(NH_2) 34 split in the other

These results indicate that the regions which unfold in the thermal transition are:

(A) near residues 31-36 (splitting of 31-32, 33-34, and 35-36 bonds),

* Allende and Richards (1962) found that trypsin digests the S-protein. From the amino acid composition of one of the digestion products, they found that a seryl and an arginyl group were missing, and suggested that the ser 32-arg 33 dipeptide had been removed.

(B) near residues 76-77, 79-80 and 97-98,

(C) near the first disulfide bridge (splitting of 25-26 bond).

Since the N-terminal tail is required for activity, it is not surprising that the chymotryptic fragment is inactive. The site of attachment of this tail cannot be too buried since subtilisin splits the ala 20-ser 21 bond (Richards, 1955); chymotrypsin splits the 25-26 bond after heating. However, the tail itself must be buried since trypsin does not split the lys 7-phe 8 bond, even upon heating. Also, the failure of chymotrypsin to split bonds tyr 115-val 116 and phe 120-asp 121 suggests that the C-terminal tail remains buried in the thermal unfolding at neutral pH. This is in contrast to its probable unfolding in the thermal transition at low pH (Anfinsen, 1956). Presumably, components II and III of the tryptic digest would not have been active if the lys 7-phe 8 bond had been split. Their activity, despite the splitting of bonds 31-32 or 33-34, indicates that these bonds are not completely essential for activity.

Further details on the action of chymotrypsin (Rupley and Scheraga, 1962) and trypsin (Ooi et al, 1962) on ribonuclease will be reported later. Also, the physical chemistry of the active component from the tryptic hydrolysis is now under investigation.

REFERENCES

Allende, J. E., and Richards, F. M., Biochem., 1, 295 (1962).

Anfinsen, C. B., J. Biol. Chem., 221, 405 (1956).

Hermans, J., Jr. and Scheraga, H. A., J. Am. Chem. Soc., 83, 3283, 3293 (1961).

Ooi, T., Rupley, J. A., and Scheraga, H. A., to be submitted (1962).

Richards, F. M., Compt. rend. trav. lab. Carlsberg, Ser. Chim., 29, 329 (1955).

Rupley, J. A., and Scheraga, H. A., Biochim. Biophys. Acta, 44, 191 (1960).

Rupley, J. A., and Scheraga, H. A., to be submitted (1962).