SELECTIVE DEGRADATION BY BROMINE WATER OF THE POLYPEPTIDE CHAINS OF OXIDIZED INSULIN*

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In degradative work on oxytocin (Mueller, Pierce, and du Vigneaud 1953; Ressler, Trippett, and du Vigneaud 1953; du Vigneaud *et al.* 1954) it was found that whereas oxidation with performic acid gave a single product (I), oxidation with

$$H.Cy(SO_3H).Tyr.Ileu.Glu(NH_2).Asp(NH_2).Cy(SO_3H).Pro.Leu.Gly.NH_2$$
(I)

bromine water or treatment of (I) with bromine water gave two peptide fragments, resulting from cleavage of a dibromotyrosylisoleucyl bond. The reaction did not depend on the isoleucyl residue, since in vasopressin, where the sequence is –tyrosyl-phenylalanyl–, a similar cleavage with bromine water was noted (Popenoe and du

* Manuscript received October 27, 1959.

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Vigneaud 1953). This fragmentation is one of the few known selective degradations (Thompson 1959*b*) by a chemical method and it was of interest to apply the method to other polypeptides to determine whether the reaction was general.

Experimental

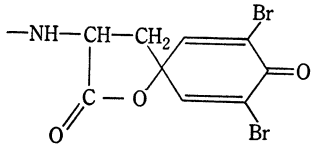
The glycyl chain of oxidized insulin contains a -Tyr.Cy(SO_3H).Asp(NH_2).OH sequence (Sanger and Thompson 1953) and treatment of this polypeptide with bromine water in aqueous methanol (4: 1 v/v) at −5 to −10°C gave rise to the peptide H.Cy(SO_3H).Asp(NH_2).OH which was readily isolated by paper chromatography (Sanger and Thompson 1953). To obtain a quantitative estimate of the extent of the cleavage the reaction mixture was dinitrophenylated (Sanger 1945) and after acid hydrolysis the dinitrophenyl (DNP)-amino acids were separated and estimated. The ether extract of the hydrolysate contained DNP-glycine, arising from the original *N*-terminal residue, and DNP-glutamic acid which were separated by paper chromatography and estimated by the method of Levy (1954). The aqueous phase contained DNP-cysteic acid which was estimated after separation by paper ionophoresis (Thompson 1959*a*). The yields of DNP-glutamic acid and DNP-cysteic acid varied from 17 to 32 per cent. and 34 to 42 per cent., respectively, of the amount of DNP-glycine after applying a correction (Porter and Sanger 1948) for the destruction of DNP-amino acids during hydrolysis.

The treatment with bromine water (1 hr, −5 to −10°C) was next applied to the phenylalanyl chain of oxidized insulin followed by dinitrophenylation, hydrolysis, and separation and estimation of the DNP-amino acids. In addition to the original phenylalanyl *N*-terminal residue, DNP-derivatives detected were those of threonine and leucine in yields of 13 per cent. and 19 per cent., respectively, of the DNP-phenylalanine.

With both these polypeptides the new *N*-terminal residue corresponded with those which are linked by their amino groups to tyrosyl groups, thus demonstrating the wide specificity of the reaction. The yields obtained in these experiments were much lower than those obtained during selective degradation by enzymes, but reaction conditions for the glycyl chain of oxidized insulin were varied only between periods of 1 hr at −5 to −10°C followed by acid treatments ranging from 0·01N to 1N HCl at −5°C or room temperature for 1 hr; or treatment with 0·1N ammonia for 1 hr at room temperature.

Discussion

Further work on this method has recently been published by Corey and Haefele (1959) and Schmir, Cohen, and Witkop (1959). These workers have elucidated the course of the reaction, which involves an oxidation during which the phenolic ring of the tyrosyl residue is converted to a dienone–lactone structure (II) with cleavage



(II)

of the dibromotyrosyl peptide bond. Near quantitative yields were obtained with simple model compounds while *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucine was found by Schmir, Cohen, and Witkop (1959) to release 40 per cent. of isoleucine after oxidative bromination.

Low yields in this selective oxidation may limit the usefulness of this reaction in protein chemistry to the rapid determination of residues linked to the carboxyl group of tyrosine. Bromine oxidation of cystine residues in proteins such as insulin and papain has previously been found to be non-quantitative (Thompson 1956) but the possibility that denaturation by agents such as urea or detergent would increase the extent of reaction, as is the case during reduction (Thompson 1959*b*), was not investigated.

For proteins containing tryptophan Patchornik, Lawson, and Witkop (1958) have shown that tryptophyl peptide bonds are also readily cleaved by the action of bromine or *N*-bromosuccinimide but Ramachandran and Witkop (1959) have found conditions where trytophyl bonds are attacked without affecting tyrosyl peptide bonds. Ramanchandran and Witkop (1959) found that tryptophyl bond cleavages in proteins treated with *N*-bromosuccinimide in the presence of urea only averaged 20–40 per cent. and were often very low.

Acknowledgment

The author wishes to thank Dr. S. J. Leach for samples of the chains of oxidized insulin purified by countercurrent distribution.

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