Fig. 1. All differences between the isotope effects except that between the *ortho* and *para* positions in toluene are believed to be significant. Differences in solubility between isotopic molecules are believed to be negligible and without importance for the present conclusions.

From the work privately communicated by Gold and Satchell it is evident that the *meta* position in toluene is somewhat more reactive than a single position in benzene, just as with the common electrophilic substitutions. Thus, it appears that the ratio k_r/k_D increases with decreasing reactivity, but whether this is a general rule cannot, of course, be inferred from the present very limited experimental material.

The Swedish Natural Science Research Council has contributed to the infrared spectrographic equipment used in this investigation.

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Received July 2, 1956.

Open Peptide Chains in Chymotrypsinogen and α -Chymotrypsin

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The principle of breaking S—S linkages in proteins by oxidation has been used extensively and with considerable success in the study of the chemical constitution of the peptide chains in these substances¹.

Upon the suggestion of M. Ottesen the author has applied this method to the study of α -chymotrypsin and chymotrypsinogen. Part of this work was reported in a paper read at a meeting in Uppsala in January 1956² and in the following the further development of these investigations is briefly described.

The opening of the disulfide bridges by oxidation³ causes the α -chymotrypsin molecule to fall apart essentially into three peptides which were separated as follows:

The oxidized enzyme was dissolved in alkali and pH brought down to 6 whereby a precipitate (fraction B) was formed. The supernatant was placed on a small Dowex 50 column (H^+ form). By elution with water, fraction A was collected, and by subsequent elution with NH_4OH , fraction C was obtained.

Fractions A, B, and C could also be separated by dialysis (removal of A) followed by free electrophoresis at pH-values higher than 7.5 (separation of B and C).

Amino acid analysis and end-group determinations were carried out on the fractions using the Sanger method⁴ for amino-endgroup determination and digestion by carboxypeptidase⁵ for the carboxyl-end-group estimation. The results were as follows:

Fraction A is a peptide composed of 13 amino acids, viz. 1 cysteic acid, 1 serine, 1 glutamic acid, 1 alanine, 2 glycine, 2 valine, 1 isoleucine, 2 leucine, and 2 proline. The N-terminal amino acid is cysteic acid and the C-terminal leucine.

Fraction C is a larger peptide containing about 50 amino acids. Of the species present in chymotrypsin only histidine and arginine are missing in C. Alanine is the N-terminal amino acid and tyrosine the C-terminal.

Fraction B seems to be a long peptide chain with an N-terminal isoleucine and a C-terminal tyrosine.

It is rather surprising that all three separated peptides are attacked by carboxypeptidase inasmuch as native α -chymotrypsin only contains two C-terminal end-groups as estimated by degradation with this enzyme⁵.

The higher susceptibility of oxidized α -chymotrypsin to the action of carboxypeptidase may be due to an "unmasking" of a group which, although it conforms to the specific requirements of the enzyme, is inaccessible to it in native α -chymotrypsin under normal conditions.

The same effect would naturally be observed if a peptide bond had been broken during the oxidation procedure, but we feel justified in ruling out this possibility since no new N-terminal group seems to be formed.

The presence of a hidden C-terminal group in α -chymotrypsin suggests that of an identical group in chymotrypsinogen.

Oxidized, dialyzed chymotrypsinogen was therefore incubated with carboxypeptidase and the reaction mixture analyzed for free amino acids in the usual way.

Fig. 1. Schematic drawing of chymotrypsinogen and α -chymotrypsin. The S—S bridges which link the peptide chains together are symbolized by the dotted lines. The A peptide is arbitrarily connected to the B peptide.

Paperchromatograms revealed that tyrosine and leucine were released as the first two amino acids, followed by valine, alanine, and serine.

Also in this case oxidation produced no new N-terminal groups in stoichiometrically significant amounts and in agreement herewith only one boundary was formed in free electrophoresis of the oxidized protein.

In an attempt to unmask the C-terminal group in a milder way than by oxidation, carboxypeptidase was allowed to act on chymotrypsinogen in 6 M urea⁶. In one of the experiments 0.8 mole of tyrosine, 0.7 mole of leucine, and 0.4 mole of valine were liberated per mole of chymotrypsinogen together with smaller quantities of phenylalanine, serine, and alanine (quantitative estimation by chromatography on Moore and Stein columns⁷).

On the basis of these experiments it may be stated with considerable certainty that the third C-terminal group found in oxidized α -chymotrypsin is not formed by rupture of a peptide bond during oxidation but is a hitherto not identified terminal group in chymotrypsinogen.

This result together with Bettelheim's discovery of the N-terminal half cystine⁸ establishes chymotrypsinogen as an open peptide chain with a C-terminal tyrosine.

This fact is easily brought in relation to previous considerations concerning the structure of α -chymotrypsin and its precursors^{4,5}. This is seen in Fig. 1. It will be observed that in the model assumed here the activating splitting should take place at a distance of 15 amino acids from the N-terminal group (peptide A + ser-arg).

A more detailed report of the experiments will appear in *Comptes Rendus des Travaux du Laboratoire Carlsberg*.

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Received July 9, 1956.