phoresis (in 0.5 N acetic acid or in pyridine-acetic acid buffer pH 4.9, 3 h, 350 V, it moved as a single band towards the cathode). A semi-quantitative thin-layer two-dimensional chromatography[12] of an acid hydrolysate of either peptides VII or D revealed the presence of the expected amino-acids in equimolar ratios.

The α-aminobutyryl peptide D, when tested as a catalyst for the hydrolysis of nitrophenyl acetate, showed a catalytic activity of about $^1/_3$ ($k_2 = 11$) of that of the cysteine (or cystine) peptide B and half of that of imidazole. First order kinetics were observed from about 10% to about 70% of completion of the reaction. The concentration of *p*-nitrophenyl acetate was $3.58 \times 10^{-5} M$ and the peptide D concentration ranged from $1.48 \times 10^{-4} M$ to $4.43 \times 10^{-4} M$ (in phosphate buffer 0.2 M, pH 7.7 containing 5% dioxan (V/V) at 22–24°). The formation and decomposition of an N^{im}-acetyl peptide intermediate (measured by the optical density at 245 nm) is probable as was also shown for the cysteine peptide B[6b].

Although the peptide analogues A–D are poor enzyme models, the role of the neighbouring amino-acid side chain to the catalytic imidazole group of histidine is obvious. A comparison of the catalytic coefficients of peptide B in the SH- or –S–S-form, and of peptides B and D suggests that the polarity rather than the reactivity or the geometry of the side chain group next to the histidine residue plays a more important role in the exhibition of the catalytic activity. A better peptide model showing a much higher catalytic activity is sought to study the effect of changing the functional side chain groups on the catalytic properties[13].

Zusammenfassung. Es wurde das Pentapeptid L-Thr-L-Ala-L-Abu-L-His-L-Asp synthetisiert, seine katalytische-hydrolytische Wirkung auf Essigsäure-*p*-nitrophenylester geprüft und die katalytische Aktivität ca. $^1/_3$ derjenigen seines isosteren L-Thr-L-Ala-L-Cys-L-His-L-Asp festgestellt.

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[13] This investigation was supported by Grant No. 814 to I.P. from the Royal Hellenic Research Foundation. Microanalyses were performed by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation.

[14] Work done on a leave of absence from the Research Council of Alberta, Canada.

Isolation and Characterization of a Cystine-Containing Octapeptide from Silk

The controversy as to whether silk fibroin (*Bombyx mori*) contains a small amount of cystine or whether this amino acid is entirely absent was finally resolved by the isolation of the 2:4-dinitrophenyl derivative of cysteic acid from a silk hydrolysate which had been treated with 2:4-dinitrofluorobenzene and then oxidized[1]. It is now generally accepted that the cystine content of silk is approximately 0.2%[2].

The isolation and characterization of a cystine-containing octapeptide from *Bombyx mori* silk fibroin is now reported and it is estimated that approximately $^1/_3$ of the cystine residues present in silk form part of the structure given. The experimental procedures used were as follows: Acid-degummed[3] *Bombyx mori* fibroin (1.0 g) was dissolved in 60% (w/v) lithium thiocyanate solution (5 ml) and after diluting to 50 ml with distilled water the solution was dialysed against dilute ammonia solution of pH 8. The solution of fibroin was digested with chymotrypsin (2.5 mg) for 24 h at 40 °C, the solution being maintained at pH 8 by the intermittent addition of 0.05 N ammonia. The precipitate formed was removed by centrifuging and the supernatant solution evaporated down to 20 ml and freeze dried.

The residue (0.35 g) was dissolved in 5% acetic acid (1.5 ml) and applied to a 175×2.0 cm column of Sephadex G 15 and eluted with 5% acetic acid and 5 ml fractions were collected. An aliquot of each fraction was treated with ninhydrin-hydrazine sulphate reagent (pH 5.5) and another, after acid hydrolysis, with acid ninhydrin reagent[4] to determine the distribution of the cystine residues. The cystine peptides, which were eluted before the bulk of the other peptides, gave rise to 2 main peaks, the first of which corresponded to high molecular weight material and was rejected, the second corresponding to peptides with a mean chain-length of 10 residues being retained for further examination.

The latter was subjected to diagonal electrophoresis and by this means a homogeneous cystine (oxidized)-containing peptide was isolated which contained $(CySO_3H)_2$, Asp, Pro, Ala, Val, Leu, Arg. From a partial acid hydrolysate (6 N-HCl for 45 min at 100 °C) of this material 11 peptides were isolated. The amino acid composition and N-terminal amino acid residues of these was determined, the latter by the DNS method. In addition, complete sequences of 2 of these peptides were established by the DNS-Edman method and they were shown to be Arg. Ala, and $CySO_3H$. Asp. Val. $CySO_3H$.

It was possible for these peptides to arise only from the unique structure, Arg. Ala. Leu. Pro. $CySO_3H$. Asp. Val. $CySO_3H$. Since the unoxidized peptide is basic it is most probable that the aspartic acid is present as asparagine residue and further, since it is unlikely that the half-cystine residue are in the reduced (SH) state, they must form a four-membered ring to give the structure: Arg. Ala. Leu. Pro. CyS. Asp(NH_2), Val. CyS.

Although this structure is the smallest known cystine-containing ring found in proteins, the octapeptide may be constructed without difficulty from space-filling atomic (Courtauld's) Models, the half-cystine residues being in suitable positions for linking.

Zusammenfassung. Isolierung und Strukturaufklärung eines Oktapeptides aus Seidenfibroin aus *Bombyx mori*.

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[5] We wish to thank the Wool Textile Research Council for financial assistance towards this investigation, a fuller account of which will be given in a later publication.