THE IODINATION OF SILK FIBROIN

by

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In their study of the formation of thyroxine during the iodination of proteins, LUDWIG AND VON MUTZENBECHER[1] found that the amounts of thyroxine obtained under their standard conditions varied greatly with the protein used, casein and the serum proteins being outstandingly the best in this respect. On the other hand silk fibroin, with the highest tyrosine content of all the proteins investigated, only gave poor yields of thyroxine. LUDWIG AND VON MUTZENBECHER give no details of their experiment with silk fibroin, so that the result reported might be due to either or both of two factors: the great insolubility of silk fibroin at p_H 8 at which their iodinations were carried out, and the structural distribution of tyrosine residues in the protein. If the first of these factors were alone responsible for the poor yield of thyroxine, the solubilising of the protein should lead to greater yields. It was therefore thought to be of interest to determine whether silk fibroin which had been rendered soluble by the method of COLEMAN AND HOWITT[2] might be found, after iodination, to give yields of thyroxine consistent with the high tyrosine content. This has not proved to be the case, and silk which has been iodinated in solution and incubated at p_H 8 has only yielded traces of thyroxine after alkaline hydrolysis.

EXPERIMENTAL

Degummed silk was prepared for the experiments by washing with ether, alcohol, dilute acetic acid and water. The washed silk was air dried.

1. Silk fibroin (10 g) was dissolved in 100 ml of cupriethylenediamine solution containing 6% of cupric hydroxide and 8% of ethylenediamine. After 3 min the solution was made neutral to litmus by addition of 1.25 *N* acetic acid, and was dialysed against running tap-water till colourless. The solution was then made 0.5 *N* with respect to ammonia, and 10% iodine in KI solution (4.5 atoms of I per molecule of tyrosine, it being assumed that the tyrosine content of the protein was 11%) was added slowly with gentle mechanical stirring. The solution was then dialysed against running tap-water until most of the ammonia had been removed, and brought to p_H 8 by addition of 3 g of sodium bicarbonate. It was then incubated at 37° for 24 h and dialysed again to remove iodide. The final volume of the solution was 320 ml and contained 3.43 mg nondialysable iodine per ml.

Hydrolysis. 150 ml of this solution were treated with 30 ml 10 *N* NaOH and boiled under reflux for 14 h. Fractionation into butanol followed by the usual purification led to the isolation of 2 mg pure DL-thyroxine. The thyroxine content of the iodinated protein was therefore 0.042%, assuming that there had been no loss of protein during

References p. 225.

the experiment. The iodinated silk solution remained crystal clear for 3 weeks at 4°, after which it slowly set to a gel and after several months broke up into fibres.

2. The silk fibroin (28 g) was made soluble as above and was iodinated with 0.4 *N* iodine (4.5 atoms of I per molecule of tyrosine) in 5% NH_4OH after which it was kept at 4° for 18 h. The product was then precipitated by addition of dilute hydrochloric acid to p_H 4.5 and was collected, washed thoroughly with water, and dried *in vacuo* over H_2SO_4. The product weighed 22.7 g and had an iodine content of 12.7%. Alkaline hydrolysis of 20 g of the product as described above led to the isolation of 12 mg of pure thyroxine, whence the thyroxine content was 0.06%. Baryta hydrolysis of iodinated casein has been shown by REINEKE AND TURNER[3] to yield 0.4% pure thyroxine.

3. A sample of silk fibroin which was iodinated in a similar manner to (2), but with 8 atoms of I per molecule of tyrosine, had an iodine content of 11.7%. Preparations (2) and (3) may therefore be considered as practically saturated with iodine.

DISCUSSION

It appears that the absence of thyroxine formation during the iodination of silk fibroin under conditions which have been shown by ROCHE, MICHEL, AND LAFON[4] to give good yields of thyroxine with casein and thyroglobulin is not the result of insolubility and it seems that the structure of the protein must be the determining factor. It has been suggested by COLEMAN AND HOWITT[2] that all or most of the tyrosine residues in silk fibroin are situated in short parts of the protein chain. If this is so, iodination of the protein must lead to the formation of diiodotyrosine residues which are also grouped together, and for steric reasons, these might not be easily available for subsequent conversion into thyroxine. In this connexion the following observation may be pertinent: during experiments on the conversion of an acylated diiodotyrosine to the corresponding acylated thyroxine by simple aerobic incubation (PITT-RIVERS[5]) it was found that whereas N-acetyldiiodotyrosine gives good yields of N-acetylthyroxine, the N-benzoyl derivative gives no thyroxine derivative at all. It is suggested that the reaction fails with the benzoyl derivative for steric reasons, the benzoyl group preventing the approach of the second diiodotyrosine molecule required to effect the coupling for thyroxine synthesis. In a protein such as silk fibroin, in which it must be assumed that the diiodotyrosine residues are in close proximity, a similar steric effect may be expected to operate.

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SUMMARY

Silk fibroin has been made soluble by the method of COLEMAN AND HOWITT[2] and has been iodinated in solution.

Hydrolysis of the iodinated silk fibroin has led to the isolation of only minute yields of thyroxine. The relatively small yields of thyroxine per molecule of tyrosine obtained from iodinated silk fibroin compared with those obtained from other proteins, especially casein, may be explained by the difference in structure of the proteins.

References p. 225.

RÉSUMÉ

La solution de fibroïne de soie a été iodée, après avoir été rendue soluble par la méthode de COLEMAN ET HOWITT[2].

L'hydrolyse de la fibroïne de soie iodée a conduit à l'isolement de très petites quantités de thyroxine.

Les faibles rendements de thyroxine par molécule de tyrosine obtenus avec la fibroïne de soie iodée, beaucoup plus bas que ceux obtenus avec les autres protéines, spécialement avec la caséine, peuvent être expliqués par la différence de leur constitution.

ZUSAMMENFASSUNG

Die Seidenfibroinlösung wurde jodiert, nachdem sie mit Hilfe der Methode von COLEMAN UND HOWITT[2] löslich gemacht wurde.

Die Hydrolyse des jodierten Seidenfibroins führte zur Isolierung von sehr kleinen Thyroxinmengen.

Der Umstand, dass die mit dem jodierten Seidenfibroin erzielten Thyroxinmengen pro Thyrosinmolekül geringer sind als diejenigen, welche mit den anderen Proteinen, und insbesondere mit Kasein, erzielt werden, lässt sich durch die Verschiedenheit ihrer Struktur erklären.

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