The Formation of Lysinoalanine during the Treatment of Wool with Alkali

By M. C. CORFIELD and C. WOOD

Wool Industries Research Association, Torridon, Leeds 6

and A. ROBSON, M. J. WILLIAMS and J. M. WOODHOUSE

Department of Textile Industries, University of Leeds

(Received 2 February 1967)

Patchornik & Sokolovsky (1964) isolated a new amino acid from both the native and dinitrophenylated forms of ribonuclease after these had been treated with alkali. The amino acid was named 'lysinoalanine', since it was formed by reaction between lysine residues and residues of dehydroalanine, which were said to arise from residues of cystine, or of *S*-dinitrophenylated cystine, by β -elimination. The discovery was confirmed by Bohak (1964), who established the identity of LAL* by oxidative studies and confirmed it by synthesis. He also demonstrated that all proteins containing lysine and cystine residues did not yield LAL on treatment with alkali, whereas phosvitin, with neither cystine nor cysteine residues in its structure, suffered conversion of almost 80% of its lysine residues into LAL when so treated. In this case Bohak (1964) claimed that the dehydroalanine residues required were formed from phosphoserine residues by β -elimination. Despite these findings he tentatively suggested that LAL forms most readily when the sequence -CyS-Lys- is present in a protein, and perhaps also when half-cystine and lysine residues are in other spatial relationships.

Ziegler (1964) found LAL in alkali-treated wool, and suggested that it most probably constituted the cross-link between lysine residues and modified half-cystine residues previously postulated by Speakman (1933). On the basis of his early work Ziegler (1964) maintained that both lysine and half-cystine residues were essential for LAL formation, but later proposed that some part of the LAL found might arise from dehydroalanine residues derived from serine (Ziegler, 1965). In contrast to Ziegler's claims, Mellet & Swanepoel (1965) produced evidence to show that half-cystine residues were not involved in LAL formation when wool is treated with alkali, but the necessary dehydroalanine residues were derived entirely from some other source, such as serine residues. In support of this Mellet & Louw (1965) cited the formation of LAL in alkali-treated silk fibroin as stemming wholly from dehydroalanine residues from serine, because they maintained that silk contains no cystine. Several workers, however, have found

small, but significant, amounts of cystine in silk fibroin (Lucas, 1966).

We now present evidence to show unequivocally that most of the LAL formed during the treatment of wool with alkali stems from half-cystine residues.

Through the courtesy of Dr A. M. Downes of the CSIRO Division of Physiology, Prospect, New South Wales, Australia, we received a sample of wool clipped from a Merino sheep 13 days after it had been given an intravenous injection of 1 mc of DL- $[\beta$ - $^{14}\text{C}]$ cystine. Amino acid analyses showed that all the ^{14}C activity was present in the cystine residues of the wool. The specific activities of the cystine, lanthionine and LAL in hydrolysates of alkali-treated portions of this wool should be in the proportions 2:2:1, in accord with the formulae given below, if the whole of the dehydroalanine residues involved in the formation of LAL and lanthionine stem entirely from half-cystine residues:

Lysinoalanine

Cystine

Lanthionine

A 200mg. sample of wool was immersed in 0.1*N*- Na_2CO_3 for 18hr. at 55°, washed with water and hydrolysed with 10ml. of 5.7*N*-HCl for 24hr. under reflux. The HCl was removed by distillation *in vacuo*, and the residue dissolved in 25ml. of water. The specific activities of cystine, LAL and lanthionine in this hydrolysate were measured simultaneously in our two Laboratories.

In the first Laboratory lanthionine and cystine were isolated by chromatography according to the procedure of Spackman, Stein & Moore (1958). No

* Abbreviation: LAL, lysinoalanine.

isotopic separation of the radioactive from the non-radioactive forms of these two amino acids was observed when the effluent fractions were examined by both ^{14}C and amino acid assay techniques. The amino acid content of a portion of the lanthionine fraction was determined with the (Chinard, 1952) ninhydrin reagent. The ^{14}C content of a duplicate portion was measured to an accuracy of 2% with a Beckman liquid-scintillation spectrometer, appropriate corrections being made for loss of count by quenching. The cystine was oxidized to cysteic acid with performic acid, and pure samples of cysteic acid were isolated by high-voltage electrophoresis on paper at pH 1.85. Duplicate portions were then taken for amino acid assay by the method of Atfield & Morris (1961), and for ^{14}C content as described above. Pure specimens of LAL were isolated by high-voltage electrophoresis on paper at pH 5.2 (Atfield & Morris, 1961), and determinations of the ^{14}C and amino acid contents were made on duplicate portions. For calibration purposes pure authenticated samples of the three amino acids concerned were subjected to all the procedures described. Six analyses were carried out.

In the second Laboratory LAL was isolated from the wool hydrolysate by chromatography on a 15 cm.-wide strip of Whatman 3MM paper, eluted with butan-1-ol-acetic acid-water (4:1:5, by vol.) for 2 days. The LAL was washed from its location on the paper with water, and duplicate portions of the eluate were taken for the determination of amino acid content by the method of Atfield & Morris (1961) and ^{14}C content by the method described above. Under the paper-chromatography conditions used lanthionine and cystine run together. They were washed from the paper strip with water and separated from each other by high-voltage electrophoresis on paper at pH 1.85. The procedures used to determine their specific activities

were the same as described for LAL. Analyses were carried out in triplicate.

The specific activities obtained, expressed in the proportions cystine:lanthionine:LAL, were respectively 2.00:2.03:0.70 in the first Laboratory and 2.00:1.98:0.78 in the second. We regard the discrepancies between the two as being within the limits of experimental error.

It is therefore apparent that under the conditions of alkali treatment we have used, which are similar to those employed by Ziegler (1965) and Mellet & Swanepoel (1965), about one-quarter of the LAL produced stems from dehydroalanine residues that were not half-cystine residues, whereas all the dehydroalanine residues required for lanthionine formation come from half-cystine. There are thus at least three acid-stable cross-linkages formed in wool when it is treated with alkali: the lanthionine linkage, an LAL linkage between lysine residues and dehydroalanine residues from half-cystine, and an LAL linkage between lysine residues and dehydroalanine residues that arise by modification of other amino acid residues in wool. Some of these are probably serine residues.

- Atfield, G. N. & Morris, C. J. O. R. (1961). *Biochem. J.* **81**, 606.
 Bohak, Z. (1964). *J. biol. Chem.* **239**, 2878.
 Chinard, F. P. (1952). *J. biol. Chem.* **199**, 91.
 Lucas, F. (1966). *Nature, Lond.*, **210**, 952.
 Mellet, P. & Louw, D. F. (1965). *Chem. Commun.* **17**, 396.
 Mellet, P. & Swanepoel, O. A. (1965). *Naturwissenschaften*, **52**, 495.
 Patchornik, A. & Sokolovsky, M. (1964). *J. Amer. chem. Soc.* **86**, 1860.
 Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
 Speakman, J. B. (1933). *Nature, Lond.*, **132**, 930.
 Ziegler, K. (1964). *J. biol. Chem.* **239**, 2713.
 Ziegler, K. (1965). *3rd Congr. int. Recherche Textile Lainière*, vol. 2, p. 403.

Biochem. J. (1967) **103**, 16c

The Binding of Sterols in Cellular Membranes

By J. M. GRAHAM and C. GREEN

Department of Biochemistry, University of Liverpool

(Received 8 February 1967)

One well-established property of the unesterified cholesterol molecules of the erythrocyte membrane is the ability to exchange *in vitro* with those of soluble plasma lipoproteins (Hagerman & Gould, 1951). This has not been investigated with other cellular membranes, although many studies of

cholesterol turnover *in vivo* in the various body tissues have been made. Cellular membranes differ in morphology (Sjöstrand, 1963) and in composition (Ashworth & Green, 1966) and many types of structure have been proposed for them (e.g. Parpart & Ballentine, 1952; Vandenheuvel, 1963;