The Modification of Silk Fibroin by Alkali

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THE formation of lysinoalanine (LyAl) by alkali treatment of proteins has been reported[1-3] and ascribed to the reaction of lysine residues with aminoacrylic residues, the latter being formed by alkali degradation of protein-bound cystine. A previous report[4] on the reaction of native and denatured wool with 0·7M-K_2CO_3 at 50° substantiated the fact that lysine residues were involved in the reaction; however, the total content of cystine, cysteine, lanthionine, and cysteic acid

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[2] K. Ziegler, *J. Biol. Chem.*, 1964, **239**, PC 2713.
[3] A. Patchornik and M. Sokolovski, *J. Amer. Chem. Soc.*, 1964, **86**, 1860.
[4] P. Mellet and O. A. Swanepoel, *Naturwiss.*, accepted for publication.

was found to remain constant throughout a reaction period of up to 32 hours, indicating that lysinoalanine may be formed in a reaction not involving the cystine and/or cysteine in the protein. This hypothesis has now been proved through the use of cystine/cysteine-free silk from *Bombyx mori*.

Degummed, purified silk giving negative tests[5] for thiol and disulphide was treated for 24 hours in 0·7M-potassium carbonate solution at 50° (liquor ratio 50 : 1) and 16·7% of the silk went into solution. The undissolved portion was filtered off, rinsed, dried, and analysed by column chromatography in 0·35M-sodium citrate buffer (pH 5·28) at 50° on a 15 cm. resin column. A peak emerged corresponding to the normal position[1] of lysinoalanine. Further characterisation was done by high-voltage paper electrophoresis (acetic acid/pyridine pH 5·2, 266 volt/cm., 5 hours) and by chromatography[6] in 0·38M-sodium citrate buffer (pH 4·26) at 30° on a 50 cm. resin column, both identifications being carried out on the desalted[7] eluant of the LyAl peak collected from the 15 cm. column. The undissolved silk contained 20 μM LyAl/g. of fibre.

The solution containing the dissolved portion of the silk was first dialysed for 24 hours against 3 litres of distilled water which could then be shown to contain traces of amino-acids (total of less than 3 μM/g. of initial silk) and also some short peptides. Dialysis of the dissolved silk fraction against fresh distilled water was continued for two more days, the protein solution freeze-dried and analysed. LyAl content = 86 μM/g. of dry protein.

In view of a total quantitative approach to the formation of LyAl, silk (3·0 g.) was denatured and dissolved by treatment for 10 minutes in 8M-lithium bromide solution (250 ml.) at 100°. The protein solution was dialysed against distilled water for 3 days and then freeze-dried. The fibroin residue tended to precipitate from 0·7M-K_2CO_3 but dissolved almost completely in 0·2M-K_2CO_3. This latter solution (0·4 mg. silk/ml.) was heated at 50°, 5 ml. aliquots removed after specified intervals, hydrolysed under nitrogen (24 hours, 5N-HCl) and analysed. (See Table.)

Significant decreases in amino-acid content are noticeable for lysine (which is duly represented in the formed lysinoalanine), arginine, threonine, and serine, of which the latter may be responsible for providing aminoacrylic residues to react with lysine in the formation of lysinoalanine.

TABLE

Amino-acid analyses (μM/100 *ml. silk solution*, i.e. *approx.* 40 *mg. protein*) *of silk treated in* 0·2 M-K_2CO_3 *at* 50°

Time (hours):	0	1	24	48	96	168
LyAl ..	—	3·1	4·8	6·8	8·1	8·2
Lys ..	25·2	22·5	21·1	18·4	17·3	17·6
Hist ..	11·1	10·9	11·2	10·5	10·4	10·0
Arg ..	32·3	31·2	31·9	31·0	29·7	26·8
Asp	153	155	154	156	153	158
Thr ..	88·7	87·4	84·9	81·0	79·6	74·0
Ser	750	750	699	703	651	648
Glu ..	62·3	62·2	56·7	63·4	61·5	59·8
Pro ..	15·5	15·5	13·1	14·8	14·8	14·8
Gly	1639	1654	1664	1684	1664	1660
Ala	1078	1092	1087	1114	1075	1133
Val	76·3	75·8	73·9	73·6	79·2	77·2
Met	1·8	1·2	1·7	1·6	1·9	1·5
Iso-Leu ..	14·5	14·0	17·4	14·4	15·1	13·7
Leu	15·3	15·3	16·5	15·2	16·1	15·4
Tyr ..	172	173	170	173	169	174
Phe	21·9	21·8	22·4	21·8	22·0	22·4

(*Received, August 2nd,* 1965; *Com.* **481.**)

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