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Utilization of the Carboxyl Carbon of L-Phenylalanine for the Synthesis of the Amino-acids of Silk by *Bombyx mori*

PREVIOUS observations in this laboratory concerning the content of free amino-acids in the silk gland of *Bombyx mori* seemed to indicate that silk alanine might be derived from phenylalanine. In order to check this possibility, it was decided to inject silkworms with L-phenylalanine labelled with carbon-14 in the carboxyl group, before they spin their cocoons.

The L-phenylalanine was prepared from glycine labelled with carbon-14 in the carboxyl group, following the method of Bergmann *et al.*[1]. Acetylglycine was allowed to react with benzaldehyde and the azlactone obtained was coupled with L-glutamic acid; the product of the reaction was hydrogenated and the acetyl-L-phenylalanyl-L-glutamic acid was separated by crystallization from the acetyl-D-phenylalanyl-L-glutamic acid. After hydrolysis of the acetyl-L-phenylalanyl-L-glutamic acid, the two amino-acids were separated on a starch column following Stein and Moore's procedure[2]. The radioactive L-phenylalanine was crystallized in water/ethanol and the purity checked by paper chromatography (phenol/water; butanol/*N* hydrochloric acid); Kjeldahl nitrogen analyses (8·61 per cent; theor., 8·48 per cent), and optical activity determination ($[\alpha]_D^{20} = -34·5°$, litt. $-35·1°$). The scanning of the paper chromatograms showed that the radioactivity was exclusively localized at the phenylalanine spots. That the radioactivity was indeed in the carboxyl group of the radioactive phenylalanine was shown by ninhydrin decarboxylation following the method of van Slyke *et al.*[3] (see Table 1).

0·2 mgm. of this carboxyl-labelled L-phenylalanine was injected into each of ten silkworms after they had stopped eating and were ready to spin their cocoons. It must be emphasized that, at this time, the silk glands are already swollen with secretion.

Fibroin was isolated from the ten cocoons, following the procedure described by Dunn *et al.*[4]. The fibroin was then hydrolysed by refluxing in 32 per cent hydrochloric acid for eight hours. The tyrosine, glycine and alanine were isolated following Stein and Moore's method[5].

The glycine was isolated as the *p*-azobenzene

sulphonate and the alanine as the nitronaphthalene sulphonate; these sulphonates were recrystallized in water. The amino-acids were obtained by passing the sulphonates on an 'Amberlite' *IR*-120(H) resin and then eluting with 3 *N* ammonia; the amino-acids were crystallized in water/ethanol/ethyl ether and found to be chemically pure by paper chromatography (phenol/water; butanol/*N* hydrochloric acid) and Kjeldahl nitrogen analyses (18·75 per cent for the glycine and 15·75 per cent for the alanine; theor. 18·66 and 15·72 per cent respectively). Both glycine and alanine were radioactively inactive (Table 1).

The tyrosine was purified by starch chromatography following Stein and Moore's procedure[2]. The radioactivity of this purified tyrosine was determined, and the compound was found to be active. It was felt that it could not be precluded that the radioactivity of the tyrosine might be due to traces of the very radioactive phenylalanine that had been injected. To examine this possibility, ordinary phenylalanine was added to the purified tyrosine and the mixture separated by chromatography on a starch column. The eluted tyrosine was dissolved in sodium hydroxide solution and crystallized by addition of acetic acid to *p*H 5 and ethanol; the radioactivity was again measured and found to be slightly higher than previously; there was, moreover, no detectable radioactivity in the eluted phenylalanine. Both results show that the radioactivity of the tyrosine sample was not due to the contamination by carbon-14-phenylalanine and was most probably within tyrosine molecules. The chemical purity of the purified tyrosine was proved by paper chromatography (phenol/water; butanol/*N* hydrochloric acid) and Kjeldahl nitrogen analyses (7·75 per cent; theor. 7·73 per cent). The radioactivity of the isolated tyrosine was localized exclusively in the carboxyl

Table 1

	Specific activities*	
	Amino-acid (c./min./m.mole)	Carboxyl group of amino-acid (c./min./m.equiv.)
Injected L-phenylalanine	$13{\cdot}6 \times 10^6$ $\sigma = 1$ per cent	$13{\cdot}5 \times 10^6$ $\sigma = 1$ per cent
Silk glycine	0	
Silk alanine	0	
Silk tyrosine	$23{\cdot}9 \times 10^3$ $\sigma = 1$ per cent	$23{\cdot}8 \times 10^3$ $\sigma = 1$ per cent

* All determinations were performed on barium carbonate deposits.

group as it was entirely in the carbon dioxide evolved by ninhydrin decarboxylation (Table 1).

From these results, it is concluded that phenylalanine is converted to tyrosine by the silkworm and that the conversion is a fairly direct one, since the radioactivity was exclusively localized in the carboxyl group of the injected phenylalanine and the isolated tyrosine. About 15 per cent of the injected phenylalanine radioactivity was found in the tyrosine of the silk fibroin.

On the other hand, the carboxyl carbon of phenylalanine is not utilized for the synthesis of alanine or glycine of the silk by *Bombyx mori*; the tyrosine results make it very unlikely that the phenylalanine could not reach the site where such transformation occurs.

This work was nearly completed when Fukuda published results[6] showing that he had isolated radioactive tyrosine from silk after injection of 2-^{14}C-phenylalanine into silkworms; he found only a very small activity in glycine and alanine.

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