At the end of the synthesis, after the last operation (number 21), the protected nonatetracontapeptide resin was extensively washed with CH_2Cl_2 and dried to constant weight (1.74 g) in vacuo over P_2O_5 (35 °C). To a suspension of the peptide resin in CH_2Cl_2 (10 ml), TFA (25 ml) in CH_2Cl_2 (15 ml) was added at 25 °C with vigorous stirring to cleave all the protecting groups and the peptide to resin bond. After 50 min at room temperature, the resin particles were removed by filtration, and the solvent evaporated under reduced pressure (bath temperature: 30 °C). The obtained residue was triturated with diethyl ether to give 211 mg of crude product as a white powder (19% overall yield).

Results and discussion. The crude peptide was purified by gel filtration on sephadex G-50 followed by Sephadex LH-20. Further purification was accomplished with CM-cellulose by gradient elution using pH 6.5 ammonium acetate buffer (0.001–0.3 M)[4]. The chromatographic purifications were monitored by absorbance at 205 nm due to the peptide bond. After desalting on Bio Gel P-2 of symmetrical main peak fractions in 1 N acetic acid, lyophilization gave a colorless residue of synthetic peptide, 155 mg (14% overall yield).

Thin-layer chromatography (Silica gel, Merck 60 F-254) in n-butanol-ethyl acetate-acetic acid-water (1:1:1:1) gave a single ninhydrin-positive, Sakaguchi-positive spot, R_f 0.59 (Fujino et al.[4]: 0.60). Paper electrophoresis on Whatman 3MM at pH 1.9 (formic acid-acetic acid buffer), 600 volt, 60 min, gave a single ninhydrin-positive spot, R_{Lys} 0.62 (Fujino et al.[4]: 0.59). $[\alpha]_D^{25}$ was found to be −72.4° (c = 0.3, 5% aqueous AcOH), which is in good agreement with the value (−75°) reported for the nonatetracontapeptide synthesized by conventional solution methods[4]. Serine was found to be the only amino terminal amino acid by the dansyl technique[15,16]. Arginine was shown to be the C-terminal amino acid by carboxypeptidases A and B digestion[17]. The results of amino acid analysis of acid and enzyme hydrolysates of a sample of purified peptide are reported in table 3.

From the reported data of physicochemical analyses applied to the synthetic nonatetracontapeptide corresponding to the sequence proposed for TP II, it appears that the product obtained possesses an acceptable degree of homogeneity which enables it to be used for biological studies.

To conclude, the present results suggest that the base-labile N^{α} temporary protecting group Fmoc may be a useful and favourable alternative to the acid-labile groups widely employed up to now during the solid phase synthesis of medium-sized natural peptides.

Table 3. Amino acid analyses of synthetic Thymopoietin II

Amino acid	Theoretical	Acid hydrolysate[a,c]	Enzyme digest[b,c]
Lys	5	5.11	5.24
Arg	2	1.96	1.92
Asp	2	4.20	2.02
Asn	2		
Thr	4	3.88[d]	13.12
Ser	3	2.69[d]	
Gln	4	8.17	
Glu	4		3.87
Pro	2	2.05	1.89
Gly	1	1.02	1.11
Ala	3	3.12	3.07
Val	6	5.94	6.20
Leu	8	8.15	8.14
Tyr	2	1.89	2.07
Phe	1	1.04	0.95

[a] Hydrolysis was carried out with 6 N HCl for 24 h at 110 °C in sealed evacuated tubes. [b] Digestion with acid protease for 24 h at 37 °C, followed by 24 h digestion with trypsin and chymotrypsin, followed by 48 h digestion with leucine amino-peptidase. [c] Average of 3 determinations. [d] Uncorrected for loss in hydrolysis.

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Localization of sucrase activity in the cocoon of *Bombyx mori*

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Summary. Enzymes capable of hydrolyzing sucrose, trehalose, lactose and cellobiose were detected in the cocoon of *Bombyx mori*. Sucrase activity was found to be highest and was present only in the outermost layer of the cocoon.

The occurrence of enzymes capable of hydrolyzing disaccharides has been reported in different insect species[1]. Most of these enzymes involved in digestion and metabolism of carbohydrates are found in salivary glands, gut and haemolymph[1,2]. The presence of sucrase activity in liquid silk has been shown in *Bombyx mori*[3] and in *Philosamia ricini*[4]. This

activity disappears in the liquid silk at the beginning of spinning and reappears in the cocoon[4,5]. At present, the physiological significance of this enzyme in the cocoon and the mode of its incorporation into silk proteins remain unexplained. This paper reports on the localization of the enzyme in the silk fibres and also on the presence of other disaccharidases in the cocoon of *B. mori*.

Materials and methods. *B. mori* (bivoltine race L×KA) larvae were reared on fresh mulberry leaves at 27 °C. Mature 5th instar larvae were allowed to spin in paper cups and whole cocoons were used as enzyme source. In another experiment the silk fibres were collected at intervals of 24 h for 3 days and were used for assaying enzyme activity.

The enzyme activity was assayed by adding 100 mg of silk fibres to 5 ml of 0.04 M buffer solution containing 2% of a sugar (sucrose, maltose, lactose, cellobiose or trehalose, obtained from Sigma Chemical Company, USA). The buffers used included phosphate buffer, pH 6.5 for invertase and pH 6.0 for trehalase and acetate buffer, pH 4.5 for cellobiase and maltase and pH 4.0 for lactase. After incubating for 1 h at 37 °C, the reaction mixture was placed in a boiling water bath for 6 min. The amount of glucose present in the supernatant was determined by 'Glucostat Special', obtained from Worthington Biochemical Corporation, New Jersey, USA. When acetate buffer was used, the reaction mixture was brought to neutral pH using 1 N NaOH before terminating the reaction by boiling.

Results and discussion. Enzymes capable of hydrolyzing sucrose, trehalose, cellobiose and lactose were detected in the cocoon of the silkworm *B. mori*. Sucrase activity was found to be the highest as compared to the other hydrolases, which were present only in trace amounts. The cocoon was devoid of maltose hydrolyzing enzyme (table 1). The sucrase activity present in the silk fibres that were spun on different days is shown in table 2. Enzyme activity could be detected in the silk fibres spun during the first 24 h. Further studies with the silk fibres produced on the first day revealed that the sucrase was present only in the outer layer of the cocoon.

The presence of sucrose hydrolyzing enzyme in the whole cocoon of *B. mori* and *P. ricini* has been reported[4,5]. The present studies reveal that the sucrose hydrolyzing enzyme is localized in the outermost layer of the cocoon. The silk gland of *B. mori* is divided into 3 segments. The posterior segment synthesizes fibroin and exports it to the middle segment where it is stored until the larva starts spinning. The middle segment of the silk gland synthesizes a number of digestive enzymes apart from serosin during the feeding stage. Since fibroin starts accumulating at the middle segment 2–3 days before the larva starts spinning, it is likely that at least part of the enzymes secreted during this period get entrapped in the fibroin. Fibroin has been shown to be synthesized by the mature larvae for a period of 3 days after the onset of spinning[6]. Since the middle segment ceases to secrete saccharidases at the post-feeding stage, the silk fibres formed at the latter period are devoid of enzyme activity. The absence of maltase activity in the cocoon suggests that the sucrose hydrolysing enzyme of the cocoon is not α-glucosidase but probably a β-fructosidase.

Table 1. Disaccharide hydrolyzing enzymes of *B. mori* cocoon

Disaccharide	μmole glucose formed/h/g cocoon
Sucrose	80
Lactose	14
Maltose	0
Cellobiose	13
Trehalose	17

Table 2. Sucrase activity in silk spun on different days

Day	μmole glucose formed/h/g cocoon
1	212
2	0
3	0

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The role of the cysteinyl and one of the tryptophyl residues in the neurotoxic action of suberitine

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Summary. The selective photooxidation of the single cysteinyl residue and one of the 3 tryptophyl residues of suberitine has been performed by irradiation using crystal violet and proflavine respectively as photosensitizers. Crystal violet and the protein form a 1:1 complex with a consequent partial inhibition of the neurotoxic activity. The latter is completely abolished by specific photooxidation of cysteine which is probably involved in the active site of the protein. The modification of the tryptophyl residue induces a large loss of the activity as a consequence of a photoinduced extensive denaturation of suberitine.

We have previously described[1] some physico-chemical properties of suberitine, the toxic principle of the marine sponge *Suberites domuncula*[2]. Amino acid analysis and spectroscopic studies showed that this protein contains a single cysteinyl residue and one largely solvent-accessible tryptophyl side chain per protein molecule.

To shed further light on the structure-function relationships in suberitine, we investigated the role performed by the aforementioned amino acid residues in conformational stability and biological activity. The specific modification of cysteine and tryptophan was achieved by the dye-sensitized photooxidation technique which, by a proper