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SYNTHESIS OF AN INSULIN B-CHAIN DISULFIDE POLYMER[+)]

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The insulin B-chains as synthesized and published previously contain S-benzyl-cysteine-residues in the positions 7 and 19 (1-5).

Removal of the S-benzyl protecting groups necessitates treatment with sodium in liquid ammonia according to the well established method of Sifferd and du Vigneaud (6). Unfortunately this deblocking treatment severs the B-chain and lowers the yield of pure S-sulfonate chains to 10-30 % (3-5).

One obvious way to overcome these difficulties is to synthesize symmetrical cystine peptides instead of S-protected cysteine peptides and to convert them into cysteine peptides in the final stages of the synthesis.

This idea, however, was hardly popular and used only for preparing shorter peptide fragments (f.i. du Vigneaud's first Oxytocin synthesis (7)). In the case of larger cystine peptides decomposition of SS-bonds was believed to occur if this scheme were followed. In addition cystine peptides having the double molecular weight compared to cysteine peptides should become quite insoluble.

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Two large cystine peptides, however, with the amino acid sequences found in the B-chain of bovine (sheep) insulin $(B\ 1\text{-}16)_2$ and $(B\ 17\text{-}30)_2$ could be easily prepared by condensing the fragments $(B\ 1\text{-}8)_2$ with B 9-16, and $(B\ 17\text{-}23)_2$ with B 24-30 respectively using conventional methods of peptide synthesis (see FIG. 1).

No decomposition of cystine sulfur occured during the many steps of the syntheses involving peptide couplings mainly with the nitrophenylester method, azide coupling mixed anhydride and carbodiimide method as well as the repeated selective removal of amino protecting groups.

Coupling of the large cystine peptides I and II by the azide method should give a mixture of a monofunctionally reacted product, B-chain dimer and B-chain polymers. The B-chain polymers (III) were the main product which could be isolated easily by chromatography on Sephadex G 100 or G 200 (optical rotation: $[\alpha]_D^{23} = -31^\circ$, (c = 1, in dimethylformamide)). After removal of the protecting groups (IV) the following numbers of amino acid residues per molecule were found in an acid hydrolysate (6 n HCl, 110°, 24 h): 1.25 Lys, 2.00 His, 1.09 Arg, 0.65 Asp, 1.29 Thr, 0.93 Ser, 3.06 Glu, 1.22 Pro, 3.00 Gly, 2.10 Ala, 0.56 $(Cys)_2$, 2.86 Val, 3.99 Leu, 2.09 Tyr, 3.17 Phe.

Conversion of the B-chain polymer into the 7.19-S-sulfonate (V) was achieved by oxidative sulfitolysis. S-sulfonate chain was precipitated by acidifying to pH 5.3 according to Niu et al. (3). The yield of pure S-sulfonate ranged from 20 to 44 % based on crude polymers. Amino acid analysis of the synthetic B-chain S-sulfonate gave the following molar ratios: 1.13 Lys, 2.00 His, 1.00 Arg, 0.73 Asp, 1.24 Thr, 1.18 Ser, 2.84 Glu, 0.93 Pro, 3.00 Gly, 2.34 Ala, 0.64 $(Cys)_2$, 2.73 Val, 3.98 Leu, 2.14 Tyr, 2.89 Phe.

The insulin forming potency of this preparation was distinctly higher than that of synthetic B-chains which had undergone the usual sodium treatment.

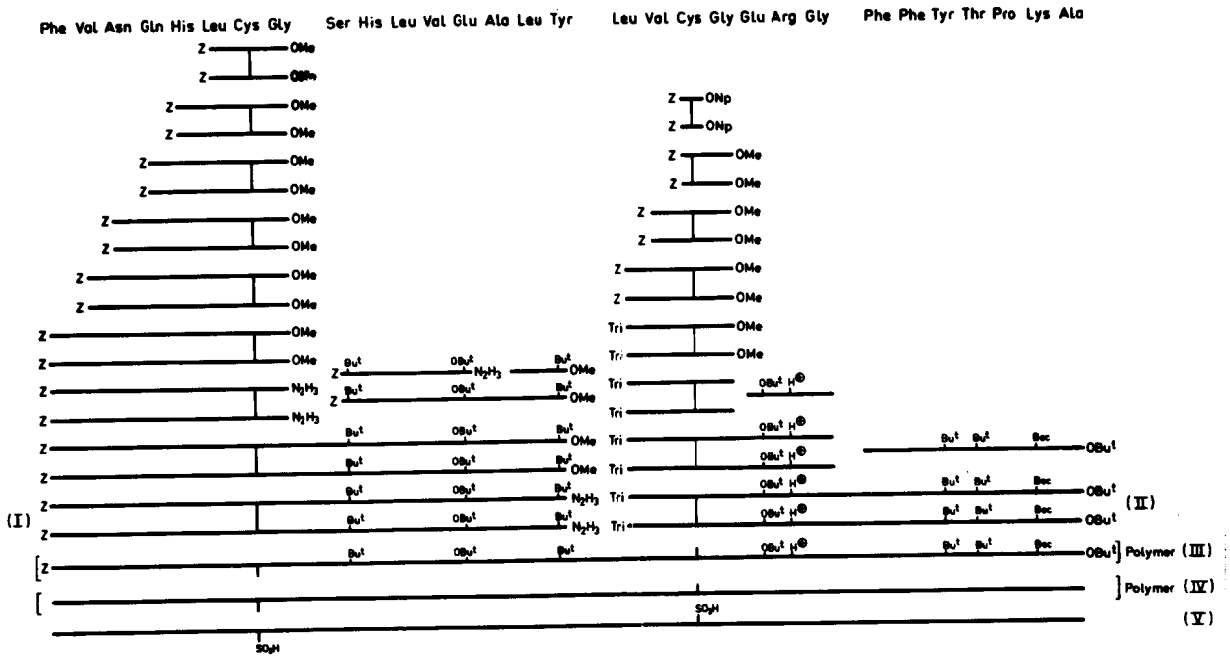

FIG. 1

Scheme of synthesis for the triacontapeptide S-sulfonate from the B-chain polymer

Combining a B-chain S-sulfonate isolated from natural insulin with an A-chain S-sulfonate from native hormone in a molar ratio of 1 : 1.5 according to Du et al. (8) at pH 10.6 yielded combination products with an insulin content of 2-3 I.U./mg[+]. Repeating this experiment with the synthetic B-chain S-sulfonate derived from our B-chain polymers gave preparations with 1 - 2.6 I.U./mg, whereas S-sulfonates obtained from S-protected synthetic B-chains after sodium treatment gave 0.5 - 0.75 I.U./mg only (5).

The new method for synthesizing insulin B-chains should be especially interesting for the synthesis of the B-chain of human insulin, which we had failed to achieve in our laboratory (5) by using the sodium deblocking technique entailing the destruction of the C-terminal threonine.

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+) Niu et al. reported 10 - 20 % activity (2.5 - 5.0 I.U./mg) [Scientia Sinica 15, 236 (1966)].