文章编号：1004-0374(2015)06-0717-02

FIRST SYNTHESIS OF A PROTEIN

by Professor H. N. Rydon
Department of Chemistry, University of Exeter

Chinese chemists have synthesized crystalline insulin, thereby achieving what has long been attempted with only partial success—the artificial production and purification of a complete active natural protein. It is a tour de force in fundamental chemical research, with no obvious technological application

A great deal of interest has been aroused by the publication, in a recent number of *Scientia Sinica*, of the details of a total synthesis of crystalline insulin carried out by a group of 21 Chinese chemists and biochemists, working in the laboratories of the Academia Sinica in Shanghai and of Peking University. Readers of *New Scientist* will be aware of similar work which has been in progress for some years in Germany and the United States and the purpose of this article is to summarize the position.

The structure of insulin, which is a small protein, was established, by degradative methods, by Sanger and his colleagues in Cambridge in 1955. Bovine insulin has the structure shown in Figure 1; in the Figure the three-letter symbols represent amino-acid residues (–NH.CHR.CO–, in which the Rs represent any of 20 different chemical groupings), which are linked together end to end to form the chains known as peptides (...NH.CHR.CO.NH.CHR.CO...). The molecule contains two such peptide chains, A and B, containing respectively 21 and 30 amino-acid residues. Each chain is terminated at the left-hand end by an amino (NH_2) group and at the right-hand end by a carboxyl (CO_2H) group. The two chains are joined together by two disulphide (–S–S–) linkages and a similar linkage, between residues 6 and 11, forms a "loop" in the A chain. Insulins from different mammalian species differ in the nature of some of the amino-acid residues; in sheep insulin residue A9 is glycine (Gly) instead of serine (Ser), while in human insulin residues A8 and B30 are threonine (Thr) and residue A10 isoleucine (Ile).

Figure 1. *The structure of bovine insulin, showing the amino acid sequences of the A and B chains, the two disulphide linkages between them, and the "loop" in the A chain*

The synthesis of such a compound from its component amino acids is a formidable task. Of the various possible strategies, all three research groups have adopted the simplest, which involves the separate synthesis of the A and B chains followed by their combination to give insulin.

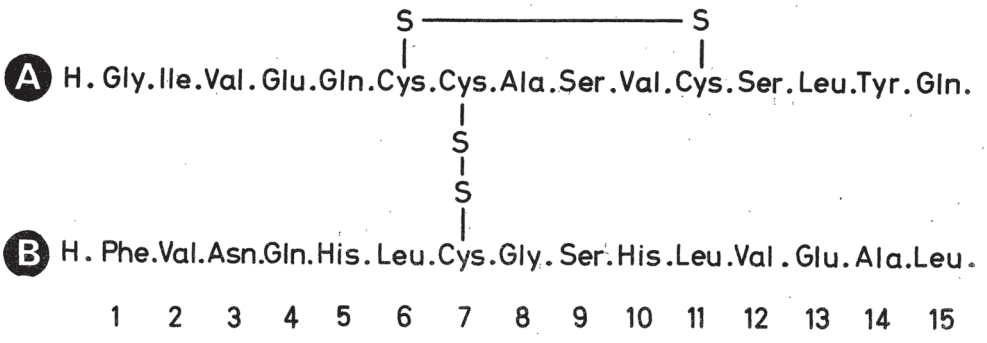
Thanks to the great improvements in methods for synthesizing peptides which have been made in recent years, the synthesis of the two individual peptide chains presents no difficulties of principle. It does, however, require hard work and great experimental skill owing to the large number of difficult chemical operations involved. The synthesis of a dipeptide from its component amino acid involves between three and six distinct chemical operations (Figure 2).

The synthesis of a peptide containing n-amino-acid residues therefore requires between $2n + 2$ and $4n + 3$ stages; the total synthesis of the A-chain of insulin thus involves between 44 and 87 stages and that of the B-chain between 62 and 123 stages. Such syntheses have been carried out by all three groups. The A-chain peptide of sheep insulin was synthesized in 1963 by Zahn and his colleagues at Aachen and by Katsoyannis and his colleagues in Pittsburgh, who also synthesized the A-chain peptide of human insulin in 1966; the A-chain peptide of bovine insulin was synthesized by the Chinese group in 1964. The B-chain peptide of sheep and bovine insulin was synthesized by all three groups in 1963-64 and that of human insulin by the Pittsburgh group in 1966. These syntheses are all in themselves very considerable achievements.

The combination of the synthetic chain-polypeptides to give insulin is quite a different matter. It may, at first sight, seem easy to bring together the chains, bearing –SH groups where –S–S– linkages are required in the insulin, and oxidize the mixture to give insulin, a process which can be represented diagrammatically as in Figure 3.

Unfortunately this is only one of 12 ways in which the two chains can come together and Kauzmann in 1959 calculated that the probability of insulin being produced, rather than one of the 11 other possible isomers, was only 0.04; this calculation neglects combinations of the A and B chains with themselves, and the formation of products containing more than two chains, and it is clear that only very small amounts of insulin can be expected from oxidative combination reactions of this kind. Nevertheless, this is the method used by all three groups.

The first clearly successful recombina-



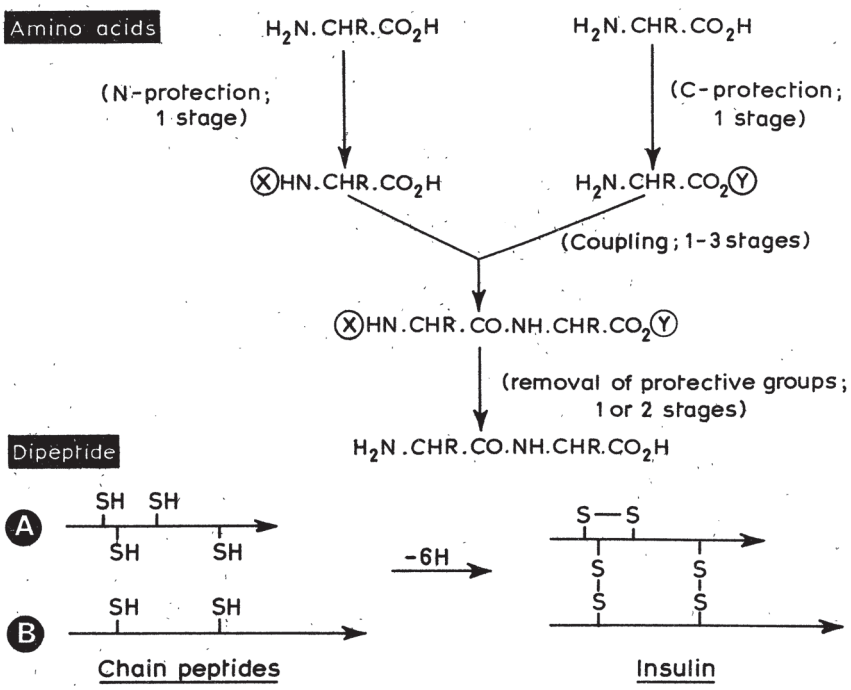


Figure 2 *Combining two amino acids together to form a dipeptide may involve as many as six separate chemical stages. Making larger peptides calls for a correspondingly larger number of operations*

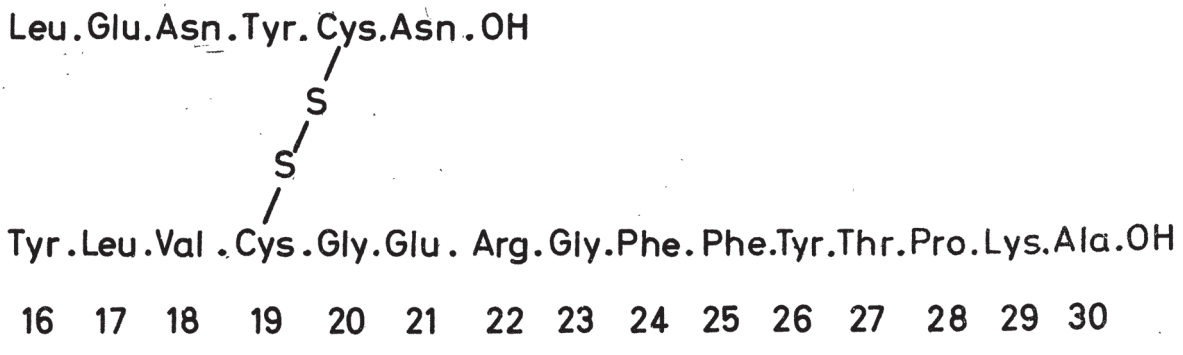
Figure 3 (bottom). *The A and B insulin chains combine together by oxidation, losing hydrogen atoms and forming disulphide linkages. Unfortunately there are 11 alternative ways in which the two chains can combine*

tion of the A and B chains of insulin was carried out in 1960 by Dixon and Wardlaw, who split natural bovine insulin into its component chains and recombined them to give a product shown by bio-assay and immunological methods to contain 1 to 2 per cent of insulin. The method was greatly improved in 1961 by Du, Zhang, Lu and Tsou who developed an experimental procedure which gave increased yields (usually 5 to 10 per cent, sometimes as much as 20 per cent); these workers also succeeded in isolating from the recombination product crystalline insulin, which was 76 per cent pure (by bio-assay) and chemically identical with natural insulin. The Chinese group later (1965) described a procedure giving reproducible yields of 50 per cent and, more recently (1966), a similar claim has been made by the Pittsburgh group.

The first combination of synthetic A and B chain peptides to give sheep insulin, in 0.5 to 1 per cent yield, was announced in December 1963 by the Aachen group. A similar combination to give an unspecified yield of insulin was reported by the Pittsburgh group in March 1964; another publication, in July 1964, seems to indicate that the yield was very low (about 0.05 per cent). Very recently (January 1966) the Pittsburgh group announced a combination of synthetic human A and B chain peptides to give a 2 per cent yield of the insulin. Although there is little real doubt that the activity of these various products was indeed due to the presence in them of insulin the formal evidence in support of this view is very slender and neither group has succeeded in isolating anything like a pure product.

This is not the case with the work of the Chinese group, who announced the combination of the synthetic bovine A and B chain peptides to give bovine insulin last November *(Scientia Sinica*, Vol. 14, p. 1710) and who published the full details of their work this April *(Scientia Sinica*, Vol. 15, p. 544). Although their yield of insulin, as assessed by biological assay, was only 1.25 to 2.5 per cent, they nevertheless succeeded in purifying their product fully, eventually isolating crystalline insulin, 87 per cent pure on the basis of the internationally accepted mouse-convulsion assay. The identity of this crystalline product with natural bovine insulin was fully confirmed by several crucial chemical and immunological methods. This synthesis must, therefore, be held to be the first fully established synthesis of a protein and, as such, must be hailed as a truly outstanding scientific achievement.

The significance of this work is likely to be scientific rather than technological. The length of the synthesis, with the inevitable loss of material at each stage, is such as to make it almost inconceivable that synthetic insulin could ever compete with the natural product, even if the yield in the final combination stage were greatly improved. Scientifically it is clear that modifications of the synthesis can lead to modified insulins which are likely to be of great value in relating chemical structure to biological activity and also by facilitating X-ray crystallographic studies on the shape of the insulin molecule. However, the greatest scientific significance of the synthesis no doubt lies in the stimulation and encouragement it will provide for work directed towards the synthesis of larger, more typical, proteins; this is a truly seminal piece of work.



本文原发表于 New Scientist, 1966: 766-7