Chemistry of Peptides and Proteins

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de Gruyter

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PREFACE

The 4th USSR-FRG Symposium on Chemistry of Peptides and Proteins took place in June 1982 in Tübingen.

The growing interest in all aspects of biologically active peptides is very well reflected in the topics discussed during the symposium. The molecular and structural basis for the biological activity of polypeptides has attracted the increasing interest of peptide chemistry. Many contributions are devoted especially to the fields of neurochemistry, neurotoxicology, immunology and membrane-active peptides and proteins. The isolation and structure analysis of new, biologically active peptides has entered a new era with the increasing sensitivity of the methods now available. Structure elucidation with a minimal amount of substance is no longer a dream. This miniaturization of methods is also important for gene technology and it seems as if the problems and progress in gene technology will give new impulses also to peptide chemistry.

The synthesis of polypeptides still poses problems despite the progress in this field, and the improved arsenal of methods for purity control steadily reveals new insights. For the first time the semisynthesis of peptides may reach production scale, as the papers on the semisynthesis of insulin show.

This symposium gives an excellent overview of the status of peptide chemistry. It should be mentioned that lively and open discussions both in and outside the lecture halls contributed to the success of the symposium. At this meeting many young scientists from the USSR and the FRG had the opportunity of participation for the first time. This certainly is important for the promotion of better understanding between the countries.

The organizers of the symposium gratefully acknowledge the generous support and sponsorship given by the Deutsche Forschungsgemeinschaft and the Soviet Academy of Sciences and they hope that this important and fruitful series of symposia can be continued for the benefit of the scientific community working in this field.

For the Editors
E. Bayer

Tübingen, February 1984

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ENZYME-CATALYZED SEMISYNTHESES WITH PORCINE INSULIN

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Introduction

Semisyntheses became the method of choice for chemical variations of large peptides or proteins from natural sources (1). Since ten years various semisynthetic procedures (2-8) have been reported which allow transformation of porcine into human insulin. Both species of insulin differ in the only position B30, where alanine is the C-terminal end of porcine insulin - and threonine of human insulin-B-chain (fig. 1).

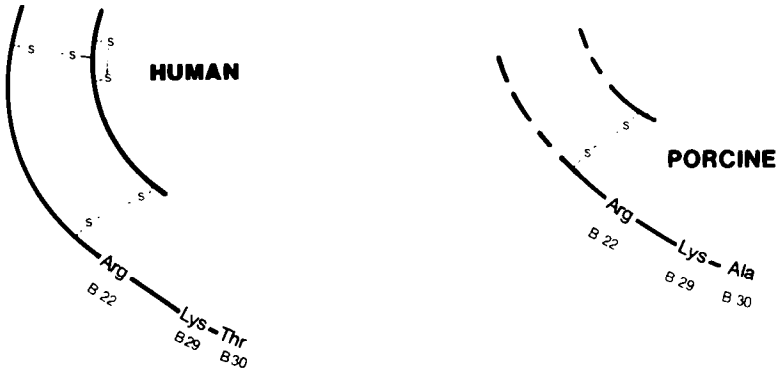

Figure 1. Schematic formula of human and porcine insulin B-chain C-terminus.

As most insulins contain only one Arg(B22) and one Lys(B29), the C-terminal region of the B-chain can be split off selectively by trypsin, which cleaves peptide bonds specifically at the carboxyl site of basic amino acids. During the last years a highly specific lysylendopeptidase (9) has been made commer-

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cially available, which even allows selective cuts only between -Lys(B29)-Ala(B30). The resulting des-Ala-B30-insulin (DAI) can be prepared alternatively by a very critical digest with carboxypeptidase A (10), however, degradation of the C-terminal Asn-A21 occurs to some extent. Thus, two readily available starting materials can be used for the semisynthetic exchange of the C-terminal sequence of porcine insulin B-chain namely desoctapeptide-B23-30-insulin (DOI) or DAI. Starting from DOI, chemical coupling methods (2, 3) with protected octapeptide corresponding to the human insulin sequence B23-30 resulted in poor yields and therefore extensive puri-

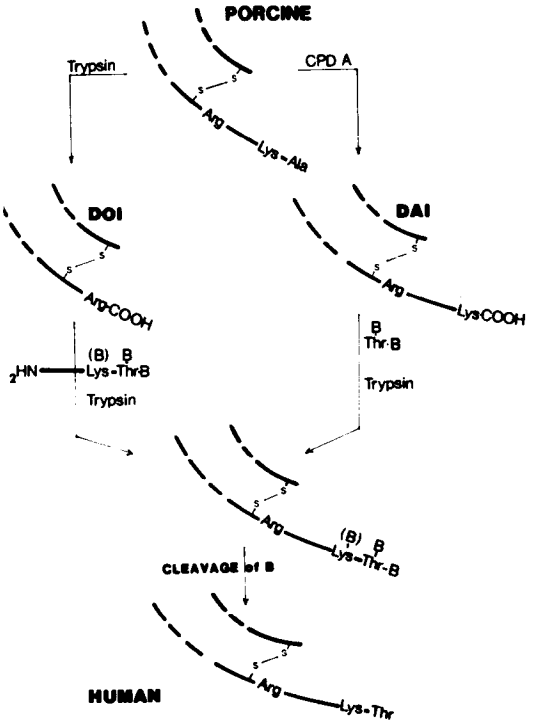

Figure 2. Multiple step enzymatic conversions of porcine into human insulin.

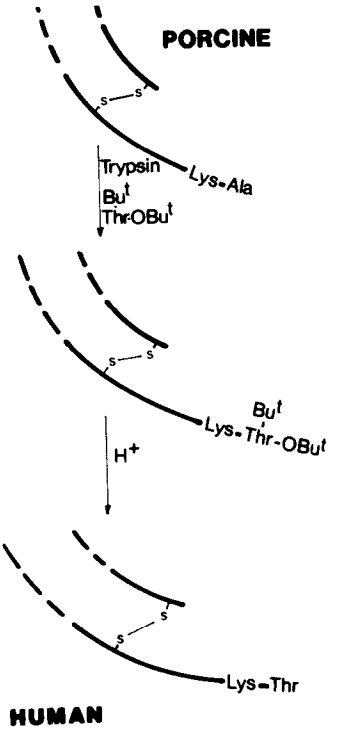

Figure 3. One step enzymatic conversions of porcine into human insulin

fication procedures. Substitution of the chemical coupling reagents by trypsin, however, raised yields up to 60-80 % (4). Enzymatic coupling of DAI with threonine esters (5) was an interesting simplification, based on the observation that under certain conditions the cleavage of the -Arg(B22)-Gly (B23) bond by trypsin is suppressed. When lysylendopeptidase became available, even that potential side reaction could be excluded (11) (fig. 2).
Very recently various groups (6, 7, 8) have independently found an essential improvement of the enzymatic semisynthesis with insulin. Under slightly variable conditions a one-step trypsin-catalyzed transamidation can be performed at the -Lys(29)-Ala(B30) peptide bond (fig. 3). In the following paper we would like to report some of our results on large-scale conversion of porcine to human insulin, insulin analogs and reaction rate and equilibria.

Results

The conversion of porcine to human insulin is exemplified by the following reaction. Porcine insulin (1 g, 0,17 mMol) is dissolved in 5 ml of 20 % acetic acid/water. Crystalline Thr-(But)OBut·Hac (6 g, 21 mMol) is added and the pH is adjusted to 4.5 by addition of acetic acid. 0,5 ml of an aqueous trypsin solution (80 mg) is stirred into the chilled reaction mixture. The reaction is kept overnight in the cold room. Conversion rates are monitored by HPLC. The reaction is finished, when about 90 % of the porcine insulin has been converted to di-tert-butylthreonine-B30-human insulin. The crude material is isolated by precipitation with methanol/ether, extensive washing with ether and drying in vacuo. HPLC of this material (fig. 4) exhibits 1-3 % DOI, 5-8 % unreacted starting material and 87 % human insulin ester. The difference to 100 % is covered mainly by desamidoinsulin respectively ester. Sepa-

ration of the human insulin ester can be achieved by various chromatographic methods e.g. partition or ion exchange chromatography (3). In each case the less acidic and more hydrophobic insulin ester is clearly separated from unreacted porcine insulin and traces of DOI (fig. 4).

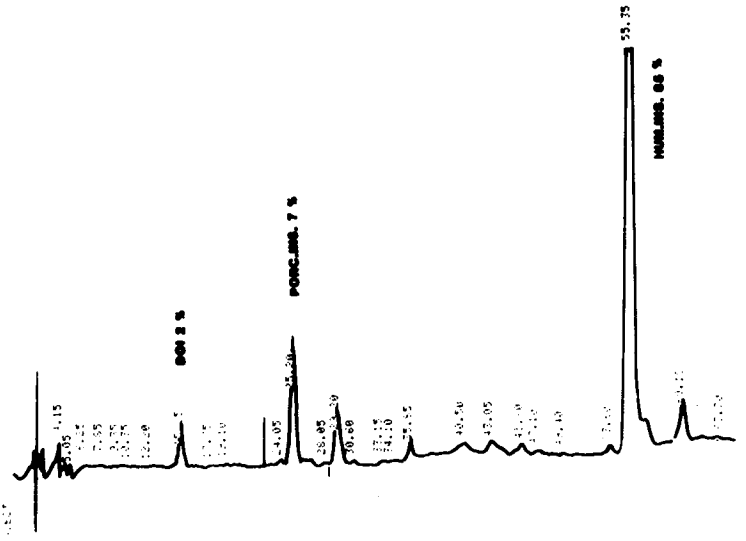

Figure 4. HPLC of crude reaction product (RP-8, Waters RCCS, 10 um, 8 x 100 mm, 0,05 M tetraethylammonium phosphate, 0,15 M $NaClO_4$, pH 3.0, gradient acetonitrile).

Cleavage of the protective groups is performed in the usual way by treatment with trifluoroacetic acid. The deblocked human insulin is isolated be precipitation with ether. The product, which already shows full biological activity, is then purified by means of ion exchange chromatography. The main fraction contains highly purified human insulin in an overall yield of 55-60 % based on the insulin content of the starting material. The insulin purity exceeds 98 %. HPLC reveals only trace amounts of desamidoinsulin. Further

impurities cannot be detected neither by HPLC nor by RIA. This material is presently produced in kilogram amounts for therapeutic application.

However, based on this transamidation type of reaction we also could easily prepare a number of B30-insulin analogs in order to investigate their immunogenic potency in pigs. These experiments are presently under way. Identical activity in blood sugar lowering tests and fat cell assay, compared to porcine and human insulin, have been determined and expected for DAI, Gly-, Ser-, Val-, Leu-, Phe- and Tyr-B30-insulin.

So far we have not been able to detect any unexpected biological properties in the series of B30-analogs. However, the synthesis of analogs showed some interesting characteristics, regarding the reactivity of -Lys-B29-X and amino acid derivate.

Conversion rates of porcine insulin and insulin esters with various amino acid esters are listed in table 1.

Insulin-Lys(B29)-X(B30)+Y $\xrightarrow[pH\ 4.5]{TRYPSIN}$ Insulin-Lys(B29)-Y(B30)+DOI			
X	Y	-Lys-Y (%)	DOI (%)
-	Thr(But)OBut	30	10
Ala	-	-	-
Thr(But)OBut	-		70
Ala	AlaOBut	25	10
Ala	Thr(But)OBut	85	3
Ala	ThrOMe	65	15
Ala	GlyOBut	25	5
Ala	Ser(But)OBut	30	15
Ala	ValOBut	85	4
Ala	LeuOBut	90	3
Ala	PheOBut	80	5
Ala	Tyr(But)OBut	75	10
Ala	D-Glu(OBut)$_2$	65	10
Ala	Leu-ProOBut	75	5
ThrOBut	Thr(But)OBut	40	4
ThrOMe	Thr(But)OBut	95	1
Thr	AlaOBut	5	1

Table 1. Conversion rates of various B30-insulin analogues.