Chemistry of Peptides and Proteins

Editors
Voelter · Wünsch
Ovchinnikov · Ivanov

W DE G
de Gruyter

Chemistry of Peptides and Proteins

Volume 1

Proceedings of the Third USSR–FRG Symposium Makhachkala (USSR), October 2–6, 1980

Editors
Wolfgang Voelter · Erich Wünsch
Yuri Ovchinnikov · Vadim Ivanov

Walter de Gruyter · Berlin · New York 1982

Editors:

Wolfgang Voelter, Prof. Dr. rer. nat.
Director
Department of Physical Biochemistry
Institute for Physiological Chemistry
Hoppe-Seyler-Strasse 1
D-7400 Tübingen · Germany

Erich Wünsch, Prof. Dr. rer. nat., Dr. med. h.c.
Director
Department of Peptide Chemistry
Max-Planck-Institute for Biochemistry
D-8033 Martinsried · Germany

Yuri Ovchinnikov, Prof. Dr.
Vadim Ivanov, Prof. Dr.
Director
Shemyakin Institute for Bioorganic Chemistry
USSR Academy of Sciences
Moscow · USSR

CIP-Kurztitelaufnahme der Deutschen Bibliothek

Chemistry of peptides and proteins : proceedings of the ... USSR–FRG symposium. – Berlin ; New York : de Gruyter

Vol. 1. Proceedings of the third USSR–FRG symposium : Makhachkala (USSR), October 2–6, 1980. – 1982.
ISBN 3-11-008604-2

Library of Congress Cataloging in Publication Data

USSR-FRG Symposium (3rd : 1980 : Makhachkala, R.S.F.S.R.)
Chemistry of peptides and proteins.
Bibliography: p.
Includes index.
1. Peptides–Congresses. 2. Proteins–Congresses. I. Voelter, W. II. Title.
QD431.A1U88 1980 547.7'5 82-14937
ISBN 3-11-008604-2

Printing: Karl Gerike, Berlin. – Binding: Dieter Mikolai, Berlin.
Printed in Germany.

PREFACE

The USSR-FRG Symposium on the Chemistry of Peptides and Proteins was conducted to reinforce between scientists of both countries the hitherto existent contact and exchange of information at the level of the European Peptide Symposium.

Based on the scientific agreement between the Union of the Soviet Socialist Republics and the Federal Republic of Germany, represented on the one hand by the Soviet Academy of Sciences and on the other by the Deutsche Forschungsgemeinschaft, the bilateral symposium series was initiated at the 1st Meeting in Dushanbe-Tadjikistan in April, 1976.

As the scientific basis of these meetings, we have attempted to combine the three main research areas in the field of chemistry of peptides and proteins, i.e. a) isolation, b) structure elucidation and c) synthesis as a discussion platform in order to reestablish the working community previously existing in chemistry of natural products. The first meeting has already confirmed the usefulness of such a programme .

Additionally, our idea was to incorporate, if required, discussions on special, closely-related topics of particular scientific interest. Consequently, at the 2nd Meeting in Grainau-Eibsee in May, 1978, membrane chemistry was added to the programme. The positive aspects of this choice were clearly confirmed at the 3rd meeting in Makhachkala in October, 1980, where a first, tentative discussion on immunological problems in connection with the chemistry of peptides and proteins took place. We believe that this widening of the scientific programme may, in future meetings, lead to a fruitful exchange of experience and knowledge in the individual sectors of this field of chemistry. The strong resonance of the scientific communications and related discussions among

the participants of both countries is clearly shown by the increasing interest in these meetings and by the continuous demand for publication of the reports. Following a simple abstract booklet on the occasion of the 1st Meeting, a proceedings volume with shortened versions of the lectures was printed for the 2nd Meeting. In the present proceedings the detailed reports of the lectures and communications of the 3rd Symposium on the Chemistry of Peptides and Proteins are published in full.

The organizers of these series of symposia on Chemistry of Peptides and Proteins would like to take the opportunity of expressing their gratitude to the Soviet Academy of Sciences as well as to the Deutsche Forschungsgemeinschaft for their generous support and sponsorship.

For the Editors
E. Wünsch

CONTENTS

I. Isolation and Structure Elucidation of Peptides and Proteins

I Isolation and Structure Elucidation of Peptides and Proteins

THE PRIMARY STRUCTURE OF DNA-DEPENDENT RNA POLYMERASE FROM *E. coli*. NUCLEOTIDE SEQUENCE OF rpoB GENE AND AMINO ACID SEQUENCE OF THE β-SUBUNIT

Valery Lipkin, Galina Monastyrskaya, Valentin Gubanov, Sergey Guryev, Oleg Chertov, Vladimir Grinkevich, Irina Makarova, Tatjana Marchenko, Irina Polovnikova, Eugene Sverdlov, Yuri Ovchinnikov

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

Introduction

Elucidation of the transcription mechanism requires detailed knowledge of the active centers organization of RNA polymerase at the various stages of the RNA synthesis. This, in turn, can be obtained only after determining the primary and spatial structure of the enzyme.

Earlier we had established the amino acid sequence of the α-subunit of *E. coli* DNA-dependent RNA polymerase resorting solely to the ordinary methods of protein chemistry (1). In the case of the β-and β'-subunits with their much higher molecular weights (~155.000 and ~165.000, respectively), such an approach could no longer suffice.

Results

One of the approaches to the structure determination of large protein molecules is their initial cleavage into a small number of fragments, which then can be analyzed by conventional methods. The search for the conditions of limited proteolysis of the β- and β'-subunits was undertaken. Considerable obstacles were encountered in the course of these studies owing

Chemistry of Peptides and Proteins, Vol. 1

to the fact that the RNA polymerase subunits are not the native proteins. However, the conditions for limited tryptic proteolysis of the β-subunit were found. These are an enzyme/substrate ratio of 1:500, temperature 0°C, 4 hr (2). Herein there seems, what we believe, to be an optimal set of large fragments (mol. wt. 62.000, 52.000, 37.000, 24.000 and 10.000). Initial separation of the resultant hydrolysate was carried out by chromatography on Sephadex G-100 in 6 M guanidine hydrochloride. This yielded 10 fractions. Their analysis by polyacrylamide gel electrophoresis showed that all large fragments mentioned above are in the first three fractions, while the rest contain about 95 smaller peptides. 53 low molecular weight peptides were isolated from the hydrolysate. They consist of approximately 400 amino acid residues. Their sequencing was very useful for a further structure investigation (3). Isolation of the high molecular peptides proved difficult because of both the little hydrolytic specificity and the low yield of most products. So we could not use limited proteolysis as the main procedure for β sequencing.

The progress in DNA sequencing methods allowed to realize the possibility of using the genetic code to obtain information on the primary protein structure from the nucleotide sequences. However, here there are many pitfalls in the way, requiring considerable caution to avoid possible sources of error.

In the first place the mRNA can undergo processing, leading to erroneous deduction of the protein structure. This holds particularly for eukaryotic cells, wherein "splicing" has been noted. Secondly, the protein itself can be processed. Thirdly, it is often difficult to recognize in the overall DNA structure the beginning of a structural gene. Moreover, one has to bear in mind that a single error (deletion or insertion) in the DNA sequence could lead to a completely erroneous amino acid sequence of the protein.

Thus, primary structure determination of DNA cannot serve as a substitute for the direct sequencing of the protein. In view of this, we decided to utilize the methods of both protein

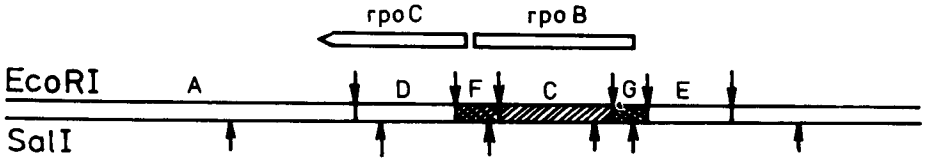

Fig. 1. EcoRI and SalI restriction cleavage map of the *E.coli* DNA region including the structural genes (rpoB and rpoC) of the β-and β'- RNA polymerase subunits.

and nucleotide chemistries, performing the parallel sequencing of the structural genes rpoB (β-subunit) and rpoC (β'-subunit) and of the corresponding proteins. Knowledge of the nucleotide sequence of the pertinent DNA segments would permit aligning of the peptide fragments from the protein analysis into an uninterrupted polypeptide chain. Such an approach provides the key to the most complicated problem in the primary structure analysis of high molecular proteins.

In Fig. 1 restriction endonucleases cleavage map of *E. coli* DNA region containing the structural genes of the β-and β'- subunits of the RNA polymerase (rpoB and rpoC correspondingly) is given. We determined the total sequence of the EcoRI-C (4), EcoRI-F (5) and EcoRI-A - HindIII fragments and partial sequence of the EcoRI-G fragment carrying the beginning of the rpoB gene (6). These fragments were obtained from DNA of $\lambda_{rif}^{d}47$ and $\lambda_{rif}^{d}18$ transducing phages, containing the *E. coli* rpoBC operon (7, 8), or corresponding plasmids by EcoRI restriction endonuclease digestion. In the case of EcoRI-A - HindIII fragment EcoRI and HindIII digestions were used.

The fragments were consecutively digested with one of the restriction endonucleases (Sau 3AI, Hinf I, Hpa II and Taq I) cleaving the DNA into relatively small blocks. The resulting subfragments were phosphorylated by means of $|\gamma\text{-}^{32}P|$-ATP and phage T4 polynucleotide kinase and the mixture was separated by electrophoresis on polyacrylamide gel. As a rule both