

## Research on Macromolecules at the ETH-Zürich. A Survey\*

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### 1. Introduction\*\*

The 100th anniversary of the birthday of *H. Staudinger* (23.3.1881–8.9.1965) will be celebrated at the Eidgenössische Technische Hochschule Zürich (ETH-Z) on May 25th, 1981 with a Symposium on "Macromolecules Today" in which the present significance of macromolecules in different areas of science will be discussed.

Professor Staudinger was professor of chemistry at the ETH-Zürich from 1912 to 1926. During that time he conceived the idea that there are no theoretical limits for the size of molecules, and that the existence of long macromolecular chains could account for the extraordinary physical properties of some compounds like natural rubber and cellulose, as well as for some organic compounds like polystyrene which had been synthesized, but not fully characterized. This concept, which needed more than ten years to be universally

accepted, has had an enormous impact on research in many different fields. In fact, the rapid development of biochemistry in the last fifty years and the advent of a new field such as molecular biology would not have occurred without a clear conception of the existence of macromolecules. Furthermore, the development of synthetic polymers was based on the realization that macromolecules with  $10^6$  or more atoms could be synthesized, and that molecular structure controls the properties of the polymeric materials thus obtained. The present state of research in the field of macromolecules at the ETH Zürich is briefly summarized in the following text. Research in this field is being carried out mainly at the Institutes of Biochemistry, of Molecular Biology, and at the Departments of Physics and of Industrial and Engineering Chemistry.

### 2. Main Research Projects in Macromolecular Science at the Institutes of the ETH-Z

#### a) Institute of Biochemistry\*

In this very year, 1981, the laboratory of biochemistry of the ETH-Zürich celebrates its 25th anniversary. For

\* This survey has been collected and edited by Prof. P. Pino, Chemisch-Technisches Laboratorium, ETH-Z. The names of the coauthors are given in footnotes to the corresponding chapters.

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the first chair in this discipline *Carl Martius* from Germany was appointed.

In the beginning biochemistry was considered in many places to be a subspecialty in chemistry. But in the past quarter of a century biochemistry has developed into an independent field of enormous dimensions. Modern biochemistry has its ramifications equally in Biology, Pharmacology, Chemistry and Medicine. As no biochemical institute has the means to cover the entire vast subject, the staff of our laboratory follows, in mutual agreement, the concept to focus on membrane biochemistry. The four sections of the institute have the following specific research interests:

*Biochemistry I* (Prof. *K.H. Winterhalter*, since 1977, successor of Professor *Carl Martius* emerited in 1976): Oxygen in Biological Systems and Cytoskeleton.

*Biochemistry II* (Prof. *G. Semenza*, since 1969): Resorption of Mono- and Oligosaccharides across the gut wall.

*Biochemistry III* (Prof. *E. Carafoli*, since 1974): Regulation of Calcium metabolism in the cell and its organelles.

*Biochemistry IV* (PD Dr. *R.J. Cherry*): Mobility of proteins in natural and artificial membranes.

(Oberassistent Dr. *A. Cogoli*): Mechanism of activation in lymphocytes and effect of conditions in outer space on biological systems (the latter in cooperation with ESA and NASA).

(PD Dr. *H. Hauser*): Structure determination on a molecular level in both artificial and biological membranes.

Biochemistry elicits vivid interest in undergraduate and PhD students and the laboratory profits from the interactions with these young scientists a great deal. Only two of the above mentioned teachers are Swiss citizens. This implies that the Federal Institute of Technology is still, true to its tradition, attractive for scientists from abroad. Alternately the laboratory for biochemistry has excellent working relationships with numerous biochemistry institutes both in Switzerland and in foreign countries.

Biochemistry is presently in a phase of rapid expansion. Therefore the coming decades are apt to bring many new developments and continuing challenges.

*b) Institute of Molecular Biology and Biophysics\**

In the institute of molecular biology and biophysics (founded 1970) research is concentrated on the structure-function relationship of peptides, polypeptides and proteins. In the various research projects of the institute three groups are involved: two in the field of molecular biology (Prof. *R. Schwyzer*, Prof. *H. Zuber*) and one in biophysics (Prof. *K. Wüthrich*).

The group 'Informational Biopolymers and Polypeptide Hormones' (Prof. *Schwyzer*) is concerned with the

application of synthetic methods in the field of polypeptides and polynucleotides to biological problems. Since our early work on the conformation of cyclic peptides (1958) and the organization of information in linear peptide agonists (1963), we have been studying the causal relationships between primary structure, conformation, receptor interaction, and biological activity of peptides of the endocrine and nervous system. New concepts, like the deployment in space of groups of individual agonist molecules, and the use of artificial spacefilling and hydrophobic amino acids, are leading to new insights into the mechanism of peptide-receptor interactions and the localization of receptors in cells and tissues. In addition, physical methods developed in this group are being used to study the direct interaction between peptides and lipid bilayer membranes. We hope that the combination of these methods and concepts, developed in our laboratory, will further increase our knowledge in the fields of molecular pharmacology and physiology.

In the present research projects of the group "protein structure and enzymology" (Prof. *H. Zuber*) two main topics are in the center of interest. On the basis of primary structure analysis, protein chemistry and enzyme kinetics the structural and functional particularities of thermophilic enzymes are studied. In this context comparative studies on thermophilic and mesophilic enzymes are primarily focussed on the relationship between thermostability and enzyme activity. These investigations should indicate how biologically active proteins and whole organisms adapt to different environmental temperatures. The second project is concerned with the structure of pigment-protein-complexes involved in the primary processes of photosynthesis. The knowledge of these structures is a fundamental condition for full understanding of the process of photosynthesis. In this context, the primary structures of the light-harvesting pigment-protein complexes from Cyanobacteria (phycobiliproteins) and from photosynthetic bacteria are determined. In addition the amino acid sequences of the polypeptides of the reaction centers are established. The general object of these investigations is to understand the molecular structure of these energy harvesting, transducing and converting pigment-protein complexes.

The research in the field of molecular biophysics (Prof. *K. Wüthrich*) is focussed primarily on various aspects of biopolymer conformation. The current projects include studies of static and dynamic features of the three-dimensional structures of polypeptide chains in solution and bound to lipid matrices. Experimental techniques available for these investigations are the analytical ultracentrifuge, optical absorbance and fluorescence spectroscopy, circular dichroism spectroscopy, electron paramagnetic resonance and nuclear magnetic resonance spectroscopy. High field high resolution NMR has a particularly important role in our experimental investigations, and we are also engaged

\* By Prof. *H. Zuber*

in the development of new NMR methods for macromolecular studies. For the structural analysis the spectroscopic data are complemented by theoretical studies, in particular with the use of the "Zentrum für Interaktives Rechnen" (ZIR). The biological systems under investigation include the polypeptide hormones glucagon and melittin in solution and bound to lipid-water interphases, protease inhibitors from mammalian species and from snake venom, neurotoxins and cardiotoxins from the venoms of snakes, scorpions and sea anemones and a variety of c-type cytochromes from different, evolutionarily related species. A common interest in all the different projects is to obtain new data on correlations between internal mobility and functional properties of the proteins.

c) *Department of Physics* \*

A small research group in the laboratory of solid state physics (Professor *W. Känzig* and two coworkers) investigates the aggregation of biomacromolecules using the technique of quasielastic light scattering. Extensive experiments on the transition of fibrinogen to fibrin have been undertaken in close cooperation with the department of internal medicine of the University of Berne (Professor *P.W. Straub*). The hydrodynamic parameters of fibrinogen and of the fibrin monomer turned out to be the same. The polymerization of fibrin prior to gelation was studied. In the early stage the rod like molecules aggregate end-to-end, whereas later staggered overlap takes over. A significant difference between thrombin-induced polymerization and Reptilase induced polymerization occurs only beyond the sol-gel-transition. A mathematical theory of coagulation has been worked out in the group of Professor *W. Baltensperger* at the Institute of Theoretical Physics.

d) *Department of Industrial and Engineering Chemistry* \*\*

Research on synthetic macromolecules at the "Technisch-Chemisches Laboratorium" began in 1952 when Professor *H. Hopff*, who had made important contributions in the field of vinylpolymers and of polyethylene at BASF, was appointed professor of industrial organic chemistry. He enlarged the polymer group in 1962 by appointing Professor *H.-G. Elias*—now Director of the Midland Macromolecular Institute, Michigan (USA)—who integrated Professor Hopff's research in the field of the synthesis of high polymers with his research on the physicochemical properties of macromolecular compounds in solution. Furthermore, a group working in the field of natural and synthetic fibers was created under Professor *H. Zollinger*.

A chair of macromolecular chemistry was established after Professor Hopff's retirement. Professor *P. Pino* was appointed and charged with the task to further the development of this field at the school. According to

his concept, approved by the school in 1969, three main directions were to be developed in teaching and research: Macromolecular Chemistry (including synthesis and characterization of macromolecular compounds), Polymer Physics (of particular significance for material science and the physical fundamentals of polymer technology), and Biopolymers. The latter was necessary to provide basic education for the undergraduate and graduate students of the faculty of chemistry, emphasizing the relationship between structure and properties of natural polymers.

Professor *J. Meissner* was appointed to the chair of polymer physics in 1974; he was formerly associated with the BASF Research Laboratory.

Professor *P.L. Luisi* who, after a period spent with Professor *M. Volkenstein* in Leningrad working on conformational equilibria in synthetic polymers, had completed his education by working with Professor *S. Bernhard* at the University of Oregon in the USA, was appointed to the second chair of macromolecular chemistry to take care of teaching and research in the field of biopolymers.

d.1) *Section of Polymer Science*

I. *Macromolecular Chemistry* \*

– *Structural Isomerism Phenomena in Condensation Polymers* (*P. Pino, G.P. Lorenzi, U.W. Suter*) (In collaboration with the group of Polymer Physics)

This project concerns the investigation of the effect of structural isomerism on the physical properties of condensation polymers. The theory of the polycondensation of non-symmetric monomers with symmetric monomers has been developed, and structurally ordered and non-ordered polyamides have been synthesized. The structural order not only influences crystallinity of the polymers, as expected, but ordered and non-ordered polyamides show different properties in the glassy state (e.g. mechanical properties, solubility, water adsorption). The research continues on different classes of condensation polymers, such as polyesters and polyureas.

– *Conformational Equilibria of Polymers and Oligomers in Solution* (*P. Pino, G.P. Lorenzi, U.W. Suter*). (In collaboration with the group of Biopolymers.) The conformation of macromolecules in solution substantially determines the chemical reactivity and the physical properties of the polymer-solvent systems. The investigations are particularly difficult because of the great number of atoms forming the chains and of the large number of possible conformers. Both experimental and theoretical approaches have been used. As experimental tools, optical activity, depolarized Rayleigh scattering, Kerr-effect, and epimerization equilibrium constants have been used. As polymeric systems, optically active poly- $\alpha$ -olefins

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and polyvinylketones, as well as polymers of non-chiral monomers as polypropylene, polystyrene and poly-*t*-butyl vinylketones are presently investigated. Also under study are different series of stereo-copolymers with alternating  $\alpha$ -amino acid residues of opposite configuration.

– *Mechanism of Stereoregulation in Stereospecific Polymerization of  $\alpha$ -olefins (P. Pino)*

The significance of this research is not only connected with the attempts to obtain more active and more stereospecific catalysts for the polymerization of  $\alpha$ -olefins, but also with the attempts to understand the factors which allow a stereospecificity of the polymerization reaction of over 99% and which might be applied to other stereospecific syntheses in organic chemistry. The discovery of supported catalysts ( $\text{MgCl}_2/\text{TiCl}_4/\text{AlR}_3$ ) has opened new perspectives to approach the problem of the structure of active catalytic centers producing polymer chains with different stereoregularities. By using propylene and racemic  $\alpha$ -olefins as monomers and optically active Lewis bases as agents which control polymer stereoregularity, it has been experimentally demonstrated that the catalysts consist of catalytic centers having different structures. Furthermore, it has been shown that stereoregulation occurs with at least two different mechanisms, the first one involving an activation of non-stereospecific catalytic centers by addition of the Lewis base, the second one involving a modification of catalytic centers by complexing a Lewis base.

## II. Polymer Physics \*

The research area of the polymer physics group is polymer melt rheology which, at least for thermoplastics, connects the polymer structure with fluid dynamics and the processing behaviour of polymers and, by means of molecular orientations due to the rubber elasticity of the melts, with the anisotropy of the physical properties of the resulting solids and with the technological end-use characteristics.

The central problem in rheology is the formulation of the "correct" constitutive equation which expresses the stress at some instant as a function of the deformation history up to this instant. Non-linear continuum mechanics has derived a general formalism for this equation, and molecular theories, based on first principles, are in the early stage of development. Due to the non-linear, rubberlike, viscoelastic behaviour of polymeric liquids conceptionally new experimental investigations are necessary, also for further progress in the different aspects of theory. In addition the more conventional methods have to be improved in accuracy and sensitivity in order to apply them increasingly for the structure analysis in polymer chemistry.

Consequently, the present research projects of the

polymer physics group are closely connected with new experimental developments:

- *For the uniaxial extension of polymer melts*, a new rheometer was built with exceptional properties: at 150°C, a branched polyethylene melt (LDPE) was extended at constant strain rate  $\dot{\epsilon}_0$  up to total strains in the HENCKY measure of  $\dot{\epsilon} = 7$  which corresponds to a stretch  $\lambda = 1100$ . Quality parameters for the test performance had to be defined and were determined experimentally for each test because at large total strains only tests with excellent performance give reliable results. Except for very small strain rates  $\dot{\epsilon}_0$  where the melt response is linearly viscoelastic, no equilibrium stress could be found in the whole range of  $\epsilon$  investigated, instead there was a maximum followed by a decrease. This result indicates that, even up to large strains, a rheologically steady state of flow does not exist. This finding was confirmed by measuring the corresponding recoverable portion of the total strain.
- Another rheometer was built for the *extension of small samples* (total mass approximately 1 g) in order to investigate correlations between the chemical structure and the rheological properties of the melt. A series of polystyrene specimens of narrow molecular weight distribution but different molecular weight was prepared (total batch mass 70 g), and one of the first intentions is to find the mixing rule for the linear viscoelastic and the onset of the non-linear behaviour of welldefined mixtures prepared from these specimens.
- The behaviour of polymer melts under *biaxial extension* is of theoretical as well as of technological interest. In order to replace the conventional "bubble-inflation-technique", a new rheometer was developed, and homogeneous equibiaxial extensions of a polyisobutylene sample could be performed up to very large total strains: The sample thickness of originally 5 mm was reduced by the equibiaxial extension down to 12  $\mu\text{m}$ . The resulting stresses allow one to compare the biaxial behaviour with the behaviour in shear and uniaxial extensional flows. Due to the modular design of this new rheometer, other modes of extensional deformation can be performed. With a slight modification of the "rotary clamp" design the total state of stress, e.g. in planar extension ("pure shear"), can be measured. From the stress components in different directions conclusions follow concerning the structure of the constitutive equation and the different material functions involved.
- *For polymer melt fluid dynamics* studies the Laser Doppler Velocimetry was applied for the first time to flows above room temperature (150°C). The velocity vector field at the entrance of a slit die and within the die was measured (measuring volume  $30 \times 30 \times 100 \mu\text{m}$ ) for a polyethylene melt in the laminar flow region. For the melt entering the die,

\* By Prof. J. Meissner

conclusions from the space derivatives of the velocity field follow for the deformation history which is largely extensional. At present, modifications to the flow system are made in order to study the entrance flow in capillaries of circular cross-section.

### III. Biopolymers\*

Two main directions of research activity are pursued by the group (*P.L. Luisi, V. Rizzo, H. Jäckle, R. Wolf*)

a) The search for new applications of enzyme catalysis and

b) The modification (by synthetic or enzymatic means) of biopolymers in order to achieve new chemical properties and reactivity.

a) With respect to enzyme applicability, there exist two main interests: The first is the study of enzymes which are solubilized in apolar solvents by means of reversed micelles (enzymes retain their activity in a medium which is composed of up to 99% isooctane). Presently, the applicability of such systems to the catalytic transformation of water insoluble substrates (as for example lipids, steroid and long-chain olefins) is under investigation. The structure of the protein-containing reverse micelles is also under study.

The other interest lies in the use of proteolytic enzymes as catalysts for the synthesis of structurally ordered polypeptides. The advantage of this method is that the reaction proceeds under very mild conditions, and that even with racemic starting material, only residues with L-configuration are present in the products. This technique can be of special value to the pharmaceutical and food industry.

b) Also in the study of chemical properties of modified proteins there are two main lines. The first one involves thioredoxin. This protein is composed of 108 amino acids, and possesses a reactive disulfide bond between residues 32–35. The protein can be chemically cleaved into fragments 1–37 and 38–108 or enzymatically into fragments 1–73 and 74–108. Currently, attempts are being made to shorten or lengthen, via chemical and enzymatic techniques, fragments 1–37 and 1–73. This is being carried out on the one hand, to determine the influence of the length and conformation of the polypeptide chain upon the reactivity of the reactive disulfide bond, and, on the other hand, to determine the minimal length which an open polypeptide chain may have in order to acquire a globular folding. Small model co-oligopeptides are also used in this research in order to establish some of the main factors responsible for the conformational equilibrium in solution. The other interest of research involves proteins which are modified *via* the linkage to synthetic polymer chains. By this way, properties such as transport, solubility, proteolytic stability, as well as thermal and pH-stability of enzymes and proteins can be improved.

Presently, we are utilizing insulin as a model compound

for globular proteins, using polyethylenglycol as the polymeric linking agent.

### d.2) Chair of Dyestuff and Textile Chemistry\*

Before 1960 all professors of industrial organic chemistry at the ETH (a professorship created in 1890) worked partly or exclusively on dyestuffs and on their application to textile fibres. Textile fibres consist of macromolecules; in the application of dyes and other low molecular weight chemical compounds in textile finishing operations, fibrous macromolecules interact, chemically and physically, with these compounds. Up until 1960, very little work on such interactions was carried out at the ETH and at almost all other research institutions around the world. Since 1960, however, research in this area is the centre of interest in textile chemistry at the ETH. For example, it could be demonstrated in the Sixties that hydroxyl groups of the cellulose chains of cotton reactive dyes and crosslinking reagents form covalent bonds, and the particular hydroxyl groups were identified. Further, the stability of the bonds towards hydrolysis as a function of the pH values of aqueous buffer solutions was determined (*H. Zollinger*). Previously, chemists had not taken covalent bond formation into consideration at all.

At present, research on textile fibres at the ETH concentrates on the following subjects:

#### – Mechanism of Diffusion of Dyes in Fibres and Films

There are two theories concerning the mechanism of diffusion of dye molecules, ions, and other small chemical compounds from an aqueous solution into solid polymers: the pore diffusion model and the segmental motion (of polymer chains) model. The first is more probable for hydrophilic, swellable polymers with fibrillar structure such as cellulose and proteins, the second more important for hydrophobic fibres with relatively low glass transition and low melting temperatures such as polyethyleneterephthalate and (perhaps) polyamides and polyacrylonitrile. At present, diffusion measurements of cationic dyes on polyacrylonitrile fibres containing well defined volume fractions of pores in the dry and wet state allow the applicability of the pore diffusion model to be tested (*H. Zollinger, U. Meyer*).

In another project, the influence of anionic charges of cellulose containing carboxylate groups on the rate of diffusion of anionic dyes is being studied (*P. Rys*).

#### – Thermodynamics of Dyeing Equilibria

Dyeing of fibres which contain ionic charges on the macromolecules is basically a gegenion exchange process if ionic dyes are used. Superimposed on this process are sorption processes of small additional ions, e.g. ions of buffers used to keep the pH-values constant. Furthermore, many types of macromolecules used for fibres contain various types of acidic

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or basic groups, e.g. polyacrylonitrile contains  $-\text{SO}_3\text{H}$ ,  $-\text{OSO}_3\text{H}$  and  $-\text{COOH}$  groups, i.e. acidic groups with different acidities ( $\text{pK}_a$ ). The conditions (e.g. pH) under which cationic dyes interact with these groups of the macromolecules, and the extent of this interaction are under investigation (*H. Zollinger, U. Meyer*).

– *Oxidation Degradation of Cellulosic Fibres in Bleaching Operations*

Cotton can be damaged seriously by oxidative degradation when bleached with  $\text{H}_2\text{O}_2$  in the presence of certain metals and metal ions. The active oxidation reagent under such conditions and the position of attack at the cellulose chain is being studied (*U. Meyer*). In the context of this report it is interesting to note that no basic research on this problem has been carried out since *Staudinger's* original work in the Twenties!