ELSEVIER

Biophysical Chemistry 59 (1996) 247–257

Biophysical Chemistry

Birth of the macromolecule

Henryk Eisenberg

Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

Received 9 June 1995; accepted 25 July 1995

Abstract

The science of chemistry has made considerable advances over the last few hundred years in the characterization of "small" molecules which can be purified and studied by melting, distillation, crystallization and solubility in various liquids. When the study of "large" natural and biological molecules, limited in these properties, rose in significance at the turn of the century, it was first attempted to explain their properties by the concepts of colloid chemistry of aggregation and complex formation. The struggle for the acceptance of the concept of the natural or biological covalently bonded macromolecule, as recalled by Herman Mark, is one of the interesting chapters in recent science history. A specific phase in the establishment of the macromolecular concept centered around the development by The Svedberg of the analytical ultracentrifuge, a versatile tool of highly practical and profound thermodynamic significance.

Keywords: Macromolecules, biological; Ultracentrifugation; X-ray crystallography

1. Introduction

Bill Harrington was born in 1920 and, as we shall see below, the concept of the macromolecule was still subject to strong negating arguments well into the 1930s. During his doctoral studies in Berkeley (1948–1952), Bill was strongly involved in developing the analytical ultracentrifuge as a tool for modern protein research, and his first publications with Howard Schachman and colleagues were all linked to the ultracentrifuge as a powerful analytical structural tool. He continued his education in colloid chemistry in Cambridge, UK, and in the secrets of protein biochemistry in the Carlsberg Laboratory, Copenhagen, under the inspiring guidance of Kai Linderstrøm-Lang. There he also strongly interacted with Chris Anfinsen, John Schellman and many other active visitors at the then Mecca of protein science. Bill's work quickly veered into the study of collagen and myosin structure and the understanding of muscular contractility, a topic still actively pursued in our present days. I first met Bill at a meeting on contractility organized by Paul Flory in Pittsburgh in 1959. I also remember clearly that upon inviting Bill to lecture at the Mellon Institute, Inga called to inform him that the twins had been born in his absence. In Bethesda, in Baltimore and in Rehovot we deeply enjoyed our continued interactions with the Harringtons. Bill's untimely death created a deep hole in our lives and we must continue to probe the mysteries which kept him so cheerful and busy. I will, in the following, aim at presenting some chosen aspects of research, relating to the contributions of Herman Mark and The Svedberg to the concept of

Elsevier Science B.V.
SSDI 0301-4622(95)00132-8

the "Birth of the Macromolecule", which eventually led to the explosive eruption of the biological sciences in the second half of the 20th century.

2. Herman Mark: the macromolecule

Lawrence Bragg, outstanding early contributor, jointly with his father, to the initial stages of X-ray crystallography, which influenced chemistry and physics as well as in due course modern biology, is quoted [1] as having stated that "unless a (scientific) paper has an immediate effect, it will almost certainly play no part at all in the progress of science and might as well never have been written. Papers of the last generation are only of interest to science historians. Here science is unlike the arts, where the value of original thought is often enhanced by time. Science is like a coral reef, alive only on the growing surface. The work of the past is the foundation on which further advance has been made, but it is dead, it has been replaced by a more complete understanding". I would call these sentences equivalent to stating that we should have no more interest in the human drama of the voyage of Columbus to what is now called America, considering that this trajectory is now much more efficiently covered by four-jet engined airplanes. Closer to my own life in research, I have experienced the tendency by some young striving scientists to negate the significance of the achievements of even their own teachers. However, in my view, science is moving on a continuous organic trajectory, and history is not for the historians only, but for all of us to dwell on.

A more amiable statement to serve as introduction to this compendium on the history of macromolecular biophysical chemistry and structural biology – a happy mixture of biology, chemistry and physics, led by outstanding personalities many of whom I was fortunate to know – is due to Herman Mark, a pioneer in macromolecular chemistry. The "Geheimrat", as we all used to call him, said [2] that "All life on our earth evolves in systems which mostly consist of organic polymers and water. These organic polymers have played a surprising role in the development of mankind and were the basis for the development of all civilizations. For tens of thousands of years wood, straw, palm leaves and tanned skins served for the construction of homes; all clothing consisted and still consists today essentially of fibers of organic polymers: cotton, wool, silk, and now also synthetic fibers. With the exception of fats and salts our nourishment consists of two kinds of large classes of organic polymers: of proteins and of carbohydrates. We would know nothing of the Bible or of the songs of Homer, if they would not have been inscribed on tanned keratin or pressed cellulose fibers. All expeditions in human history into the unknown were undertaken with wooden boats, made impermeable with resins and whose sails consisted of cellulose fibers. Without wood, resins and strings we would have no bowed stringed musical instruments, and without wood, linen and hardening oils we would not know about the magnificent achievements of the great painters". There is of course much more to what has been said in these very human sentences with respect to the molecules of life. However, the early main interest of Mark was not in proteins and in nucleic acids, the study of which had not yet assumed major significance in the period between World Wars I and II, but rather in the structure of the natural macromolecules mentioned above, and in the design and production of synthetic polymers to improve and replace the natural products. We know now that the basic properties of the macromolecular "state" are essential for an understanding of the properties and performance of the molecules of "life". The term "macromolecule" was coined by Hermann Staudinger [3]. Mark, Werner Kuhn and Paul Flory were focal contributors to our understanding of macromolecular behavior. In the early days of the 20th century and into the early thirties there raged a big argument, to be discussed below, whether macromolecules, meaning large molecules, were composed of chemically precisely defined and bonded macromolecular chains, or whether they were colloidal aggregates, small molecular entities held together by weak intermolecular colloidal forces. In the words of Ostwald, colloid chemistry constituted the *world of neglected dimensions*. The fact that we now accept the concept of the large covalently bonded macromolecule, does not diminish the importance of colloid science in many aspects of the chemical and the natural life sciences.

Herman Mark was born in Vienna on May 3, 1895 and died in Austin, TX on April 6, 1992. He

studied chemistry in Vienna with Wilhelm Schlenk and moved with him as his assistant to Berlin in 1921. He became a member of the Kaiser Wilhelm, now the Max Planck, Institute, and stayed there for five years in illustrious company. There he also initiated his studies on X-ray crystallography of metals, low-molecular-weight compounds, and the structure of graphite which sent him on the path towards an understanding of the relation between structure and physical properties, which are an essential feature in our understanding of macromolecular compounds. In particular he became aware of the fact, not accepted by X-ray crystallographers on the European continent at that time, that the size of the crystallographic unit cell does not determine the size of the covalently bonded molecular structure. Additionally, he undertook studies on the basic physical properties of X-rays, a still very young field of study, and Mark capillaries are used in X-ray crystallography to the present day. In 1926 Mark was asked by Kurt Meyer of the I.G. Farbenindustrie, Germany's major chemical concern, to become head of a laboratory devoted to the study of natural macromolecules such as starch, cellulose, silk and rubber and dedicated to the creation of synthetic replacement materials. In 1928 Mark and Meyer succeeded in the first crystallographic characterization of silk fibroin, cellulose and chitin, clearly establishing the covalent macromolecular nature of these structures. These findings were summarized in 1930 in a book, co-authored by Meyer and Mark and later republished in English [4], on *Der Aufbau der hochpolymeren organischen Naturstoffe*. In 1932, following the rise of Hitler in Germany, Mark moved to the University of Vienna where, incidentally, Max Perutz was one of his young students. Mark advised Perutz to join Bernal's laboratory in Cambridge. Mark and Perutz later joined forces in World War II in participating in a top-secret project aiming to construct huge aircraft carriers out of reinforced ice, to turn the tide of the war [5]. Mark's father was Jewish, which had made his position in Germany increasingly difficult. In Vienna, Mark developed with Eugene Guth the first statistical theory of rubber elasticity. Following the *Anschluss* in 1938 of Austria by Nazi Germany, Mark left Austria and moved to head the research laboratory of a paper company in Canada for two years. This gave little scope to his scientific and educational abilities, until in 1941 he reached the Polytechnic Institute of Brooklyn, "Brooklyn Poly", where he founded the Institute of Polymer Research, the first of its kind in the US. The depth of Mark's engagement with the macromolecular state can be gauged from the fact, known to readers of the *New Yorker* magazine [6], that when Mark left Vienna, freshly occupied by a hostile regime, in a great hurry, he converted his assets into platinum which was drawn into wires, in the form of a set of cloth hangers. The unusual personality of the "Geheimrat", an appellation he cherished all his life, was a mixture of viennese *bon vivant*, a perfect sense of humor, a devotion to his friends and to all he could be of assistance to, a sharp intellectual mind, and the most relaxed way of acting in business and in pleasure. The Brooklyn Institute quickly became a pioneering worldwide known center for the study of synthetic and biological macromolecules and a focal contact point for industry and academe in the United States. Mark travelled broadly in the States and worldwide and continuously brought together ideas, concepts, people, financial resources and goodwill which led to a continued development of an important field of science. Weekly lectures and prolonged discussions in the New York suburb of Brooklyn attracted the best minds and led to continuous progress, partly reported in the *Journal of Polymer Science* founded by Mark, among the first in a long series of journals in macromolecular science. So well was Mark organized in his extended activities that when at one time his departure from Israel following one of his many visits to the Weizmann Institute was delayed for a few days by a strike at the Tel Aviv airport, the usual personal "thank you" note signed by him (or by his secretary?), arrived before he actually left. I will never forget how he explained to us, in one of his many outstanding lectures, a brandnew hydrodynamic theory of John Kirkwood, by drawing a single equation on the blackboard, and discussing it extensively from many points of view, rather than the normal procedure of the travelling lecturer confounding his audience by submerging it with an unending sequence of mathematical expressions [7].

According to Mark [2] the science of metallurgy developed for the last 400 years because analytical and synthetic chemistry could provide precisely the

composition of natural minerals and artificial alloys. However, although organic chemistry was already well developed 150 years ago, organic polymers were inaccessible to study. This is due to the fact that a small organic molecule is purified by distillation and crystallization and could then be well characterized by a variety of analytical means. However, cotton and silk could not be subjected to the same procedure, beyond solvent extraction, drying, and subsequent elementary analysis. Interestingly, it was found by a number of researchers that the cell wall of a variety of plants manifested identical chemical composition, namely $C_6H_{10}O_5$. This substance, essential for the life of plants, was called cellulose. It was an amazing observation that this chemical composition was almost identical to that of water-soluble sugars, and hydrolysis products of celluloses of various origin yielded the same hydrolysis product, a simple sugar, glucose. It thus appears that the chemical *composition* is not sufficient to characterize the chemical and physical properties of a given material, and the decisive difference is determined by the then still unknown *structure* of the molecules.

Similar observations, as described by Mark, could be made with respect to rubber, and it was already shown by Faraday that by dry distillation of this material a volatile combustible fluid was obtained, later called isoprene, of chemical composition C_5H_8. Here again there is no direct connection between composition and structure, between a flexible, sticky macromolecule, as we know it now to be, and a volatile fluid. Additional examples are other polysaccharides, proteins and proteinaceous materials and, in more recent years, nucleic acids. Already in a lecture in 1877 August von Kékulé had expressed the idea that the molecules of life, such as cellulose, starch and egg-white are likely to be constituted by long, chain-like molecules, and that their special properties derive from their unusual structures. Whereas these structures were unknown, it was known that with increasing molecular weight, organic molecules, be they aliphatic or aromatic, display increasing melting points and decreasing solubility. Between 1900 and 1920 three proposals by different authors for the "structure formula" of cellulose preferred a chain structure, whereas three other studies suggested closed low-molecular-weight rings. For starch and rubber, which could be investigated by physico-chemical methods then available, because of their solubility in selected solvents, molecular weights between 20000 and 30000, and 6500, respectively, were estimated in 1888 and 1889. Samuel Pickles, critically engaged in the study of the structure of rubber, suggested a chain structure in 1910, constituted of isoprene residues connected by normal chemical bonds. This concept was taken over by Staudinger in his path-breaking work in 1920, and has maintained its validity including the establishment of the *cis* form of the isoprene residues.

Mark described alternate views on macromolecular structure developing in the early decades of the 20th century. Alfred Werner and his school refined the valency concept and came to the conclusion that, in addition to "normal" chemical valency, other strong forces of attraction between small molecules could lead to the formation of complexes with high melting points. Such strong associative forces, also leading to high viscosities in solution, were postulated as well by the science of colloids. Colloid chemistry, in the hands of Donnan, Ostwald, Perrin, Rideal, Svedberg and Zsigmondy, was a very active field in the early days of the 20th century, historically bound to biological and physiological systems. Arguments therefore developed for the characterization of high-molecular-weight organic natural products as formed by strongly associating small-sized building blocks. The world of chemistry was not inclined to accept the basic concept that natural high-molecular weight natural products are covalently linked chain macromolecules (often folding, as we now know, into higher order structures), and the concept of association of low-molecular-weight building blocks was more naturally acceptable. I clearly remember the *tetranucleotide hypothesis* model of DNA under discussion until about 1940, claiming that nucleic acids consist of strongly associating cyclic tetrameric structures, comprising the four deoxynucleotides [8,9]. However, at that time it was already known that the DNA, isolated by Einar Hammarsten and analyzed by Rudolf Signer [10] by birefringence of flow, had a molar mass approaching 10^6 Da, larger than most known proteins, and with an elongated shape. However, the leading organic chemists of those days still believed that it was within the realm of small-molecule chemistry to describe all observations with respect to the natural

high-molecular-weight molecules. Physico-chemical experiments in the difficult field of macromolecular science were far from the advanced level achieved today and controversial results were often obtained. I will discuss below in greater detail the realization by Svedberg of the analytical ultracentrifuge which provided concrete support for the macromolecular concept. Theoretical aspects of macromolecular behavior had also not been developed before 1930 and Staudinger, for instance, believed from an analysis of viscosity data, that macromolecules are stretched rod-like structures, a concept dispelled by the work of Werner Kuhn (Reviewed in Ref. [11] and by Paul Flory in Ref. [12], valuable also for its historical introduction). These authors correctly introduced the concept of the random coil for linear macromolecular structures, deriving from rotation around covalent chemical bonds, the rules of Brownian motion forming the basis of the theoretical calculations. The equilibrium conformation of a linear randomly coiled macromolecule fluctuates because of thermal motion. Rubber elasticity is the consequence of stress deforming the equilibrium conformation and the system returning to the equilibrium conformation when the stress is removed, striving to an increase in entropy. The macromolecular chains may be crosslinked into three-dimensional, 3D, structures, akin to the well known process of rubber vulcanization. I mention now the consequences of attaching electrical charges to the macromolecular chains, forming polyelectrolytes, which are ideally suited for the study of the transformation of chemical energy into mechanical work [13]. A typical biological polyelectrolyte macromolecule is deoxyribonucleic acid, DNA. B-DNA is formed by two intertwining helices which provide mechanical stiffness, in addition to the stiffness due to electrostatic repulsion. These molecules are best represented in solution by the Kratky–Porod worm-like coil [14], with dimensions defined by the persistence length, a measure of directional persistence, or molecular chain stiffness. Short worm-like coils may be approximated by rigid rods, and long worm-like coils by random coils. Both flexible, synthetic and stiff, natural polyelectrolytes can be examined in a universal scheme [15]. Proteins are different because the basic polypeptide chains are folded into well defined nearly rigid three-dimensional structures – sometimes stabilized by SS intramolecular crosslinks – which may associate into non-covalently bonded well defined complex structures. Additional structures of major relevance are nucleic acid–protein complexes of variable stability and dynamics forming the basis of biological function.

Returning once more to the profound controversy of our earlier discussion, the dilemma between the high-molecular and the low-molecular associated structures dramatically peaked in 1926 when Staudinger gave his *Abschieds Vorlesung* at the ETH in Zürich, before moving to Freiburg im Breisgau, Germany. I now return to the description by Mark [2] of the events which finally led to the acceptance of the current correct views on macromolecular structure.

It so happened that Mark was in Zurich at the time of the Staudinger lecture and able to attend the presentation of his scientific credo, jointly with a galaxy of current eminent researchers and teachers. Molecular weights in excess of 100000 were believed to be overestimated and unacceptable to Staudinger's predecessor in Freiburg, Heinrich Wieland, and Paul Niggli, the "crystallographic Pope" of those days, concluded that "the unit cell of these materials is small, and as the molecule cannot exceed the unit cell in size, it must be small". The majority of chemists were not concerned with this problem. Fritz Haber and Richard Willstätter strove for a clarification and arranged a special meeting of the Chemistry Section at the congress of German Natural Scientists and Medical Doctors in Düsseldorf on October 23, 1926, to achieve, if possible, a solution of the controversy of "small building blocks against large molecules". Mark was invited by Haber to present his views on the crystallographic aspects of the problem, a study which he carefully prepared. Willstätter opened the proceedings and Max Bergmann and Hans Pringsheim, supported by Paul Karrer and Kurt Hess in the discussion, emphasized the lack of necessity of the large molecule concept to explain all known facts. Mark followed by describing the achievements of X-ray crystallography and emphasized that under given circumstances a molecule can exceed the size of the crystallographic unit cell, a fact well known and accepted today. K. Weissenberg, who attended the meeting, supported this view. Staudinger then presented a summary of

his experiments on chemical modifications, hydration and dehydration, of organic polymers such as rubber, polyvinylbromide, polystyrene and others, which clearly justified the macromolecular concept. It was emphasized though that the experiments of Staudinger were restricted to hydrocarbon polymers and extrapolation to polar polysaccharide and protein chemistry was not justified without further experiments. The possible role of strong forces of association was therefore not excluded. However, the stage was now set for constructive investigation of a vital topic, liberated from the restrictive prejudices of the scientific establishment. In the following years the elementary structures of cellulose, silk, rubber and chitin could be established. W.H. Carothers and his collaborators in the DuPont laboratories demonstrated the preparation of high-molecular-weight polyesters and polyamides of which the Nylon polyamide fiber and Neoprene synthetic rubber are the best known representatives. An excellent summary of the history and achievements of polymer science has been given by Morawetz [16].

As usual this is only part of the whole story. For a description of early work on fibrous (and crystalline) protein structure in the UK and in the USA, and an elementary intellectual description of the nature of X-ray diffraction, the reader is referred to Crick and Kendrew [17]. They claim, among other didactic and entertaining comments, "that X-ray diffraction is not a difficult branch of physics: on the contrary, it is easy to the point of tediousness". In another, more recent contribution [18], Linus Pauling describes how his interest in proteins developed, leading to the discovery of the peptide α-helix in 1951. Of interest in this connection is Pauling's comment that he was working at the California Institute of Technology in the only place in the world, at that time, where X-ray crystallography was practised in a chemistry department. On the other hand "Bragg, Kendrew, and Perutz were physicists working in a physics department, the Cavendish Laboratory. None of them, I judge, knew very much about structural chemistry". Apparently, according to Pauling, Bragg did not understand when Lord Todd, head of the chemistry department at Cambridge, told him "that the amine group in the polypeptide was planar". The DNA double helix though was discovered by Watson and Crick in Cambridge in 1954 [19] and not by Pauling, who had claimed the importance in having a good understanding of the two fields, structural chemistry and X-ray diffraction. However, this is another story. I quote Frederic Richards [20] saying that "chance and fad play a strong role in both the research and the career of any scientist". I would add that scientists, even the greatest, are human beings, hardly exempt from the weaknesses of this clever species. The stage was now certainly set for an understanding of the structure, also relating to functional aspects, of biological macromolecules, a field of study which has assumed enormous proportions, bringing in theoretical and practical rewards which were hardly envisaged in the not so far away pioneering days.

3. Theodor (The) Svedberg: the analytical ultracentrifuge

Quite independently and roughly at the same time as the events reported above, studies generated by The Svedberg in Sweden, led to the realization that proteins are large, uniformly sized macromolecules, ranging in molecular weight from roughly 10000 Da, to a few million. The Svedberg was born near Gävle, Sweden on August 30, 1884, and specialized at the University of Uppsala in various aspects of colloid chemistry [21]. The work he became famous for was initially all devoted to the study of the size and the distribution of sizes of organosols of various metals, and their stability under various conditions. He already early felt convinced that the study of colloidal systems would ultimately lead to an understanding of the processes of life, at the time when the existence of large molecules as particles was still doubted by well known scientists, such as Wilhelm Ostwald and others. Physical chemistry was a new departure in Uppsala and it was not easy for Svedberg and his colleagues to construct the Zsigmondy–Siedentopf ultramicroscope at a time when "the city of Uppsala had not yet been electrified, the electric current was provided from a d.c. generator driven by a hot air engine with a heavy balance wheel which had to be started by hand, not without risk". With the ultramicroscope working they could test the Brownian motion of the particles in the metal sols and determine the influence of solvent, temperature,

viscosity and so forth. The work of Svedberg confirmed the recent theories of Einstein and Von Smoluchowski on basic aspects of Brownian motion of colloids. Svedberg was interested as well in the properties of radioactive substances which interested him throughout his life. In 1913 he received the official title of Professor of Physical Chemistry at Uppsala University, enabling him to continue his studies on the physico-chemical properties of colloidal particles and systems, their size distribution, absorption of light, diffusion and Brownian motion.

For the study of size distribution in colloidal systems, Svedberg decided to study the variation with height of the concentration in a sedimenting system by optical means. As the field of gravity was not strong enough for smaller colloidal particles, the idea was born to study sedimentation in a centrifugal field. Briefly, particles of large size, heavier than the medium in which they were suspended, would sink to the bottom of the vessel were it not for the disordering molecular motion discovered by the English botanist Robert Brown in 1827. Depending on the buoyant weight of the particles (and the rotational velocity of the centrifuge rotor), they would now settle towards the bottom of the cell with a velocity measurable by an ingenious optical system. Alternatively, conditions could be such that an equilibrium might be established, distributing particles in the field, somewhat like the decreasing concentration of air in the atmosphere with increasing height. Svedberg developed suitable equations to derive the correct sedimentation velocity or molecular mass, either way. This is how in 1923 the idea of the construction of an analytical ultracentrifuge was born and was stated in the context of a paper by Svedberg and Rinde on the distribution of size of particles in disperse systems, appearing that year [22]. In the same year Svedberg was invited to the University of Wisconsin in Madison to organize research in colloid chemistry, and spent eight months there, considering, among other things, plans for the construction of machinery for sedimentation in centrifugal fields, electrophoresis and diffusion. His students there, who later achieved considerable success, were E.O. Kraemer, J.W. Williams and J.B. Nichols. A major experimental difficulty, later resolved by the design of sector-shaped cells, was due to the fact that particles were carried down the centrifuge cell by convection along the walls in addition to the normal sedimentation process. Convection-free sedimentation later became possible by the construction of sector-shaped cells. In 1924 Svedberg and Rinde published the first paper on an analytical ultracentrifuge [23] in which the cell was placed in a heavy rotor resting on the axis of a modified milk separator centrifuge, spinning in a hydrogen atmosphere. According to the authors "The new centrifuge constructed by us allows the determination of particles that cannot be made visible in the ultramicroscope. In analogy with the naming of the ultra-microscope and ultra-filtration apparatus we propose the name ultra-centrifuge for this apparatus". The first centrifuge generated centrifugal fields up to about 5000 g and the rate of sedimentation of gold particles could be measured by optical absorption to a diameter of about 5 nm.

Svedberg's ideas then veered to the belief that the dispersity of proteins could be determined by the construction of improved ultracentrifuges. Emil Fischer had shown that they contained various amino acids combined by peptide linkages. They yielded complex colloidal solutions, depending on the way of dissolution, similarly to other colloidal sols. S.P.L. Sørenson, at the Carlsberg Laboratory in Copenhagen had in 1917 determined the molecular weight of egg albumin by osmotic pressure as about 34000, later (1945) recalculated by Güntelberg and Linderstrøm-Lang to be 45000. Nichols and Svedberg did not succeed in sedimenting egg albumin. However, Fåhraeus and Svedberg successfully sedimented haemoglobin to equilibrium, determining in 1925 [24] a molecular weight of 67000, which was four times the value of the peptide subunit, 16700, expected from chemical analysis of the iron content. Moreover, they reached the surprising conclusion that the protein was uniformly sized, in contra-distinction to the man-made gold colloids. They stated "The lack of a reliable method for the determination of the molecular weight of substances possessing very complicated structure has been a serious obstacle in the progress of our knowledge of the chemistry of the proteins. In the present paper such a method will be proposed, and its use will be illustrated by a few preliminary measurements on haemoglobin", and later "These measurements should be regarded more as an illustration of the method than as a precision determination of the

molecular weight of haemoglobin. A more refined technique of measurements will, we hope, enable us to communicate such determinations later on''. The road was open now for further refinement and advances in our understanding of the structure of biological macromolecules. For a full understanding of mono- or polydispersity or complex interactions between species or with solvents, additional theoretical insight and considerable experimental upgrading were required.

In the next step Svedberg aimed to achieve a centrifugal field of 70 000–100 000 times the gravitational field and in January 1926 the first oil-turbine driven ultracentrifuge yielded 19 000 r.p.m. instead of the 40 000–42 000 aimed for. In March this aim was achieved after turbine redesign, improved bearings, elimination of vibration and of heat-convection currents. The machine was run at 10–20 mmHg in a hydrogen atmosphere. Difficulties occurred with leaking cells and cracked cell windows and had to be overcome. Word got around fast and many new foreign students were now attracted to Uppsala to work with the ultracentrifuge. Of the many interesting projects one stood out in particular, namely the study of haemocyanin from the vineyard snail *Helix pomatia* which, according to its copper content, should have had a minimum particle weight of 15 000–17 000. Amazingly, it sedimented quickly with knife-sharp boundary, disclosing an absolutely uniformly sized particle with molecular weight in the millions [25]. Interestingly, when dynamic light scattering came into use much later [26], haemocyanin was used as a first demonstration because of its huge and uniform size. From an examination of the sizes of many proteins studied Svedberg derived certain rules of regularity which are currently receiving renewed interest [27]. This, no doubt, is part of the long and strenuous path to the discovery of the secrets of nature. Svedberg was fortunate that, unlike the unhomogeneously sized fibrous polymers discussed above, he choose to work with uniformly sized proteins, allowing obtainment of basic reliable information. Svedberg had a deep interest in nature and botany and from many "...trips with his assistants to the woods and the lakes in the surroundings of Uppsala, all kinds of snails, worms, snakes, crayfishes and tiny creatures were collected.." [21], also in other places in Sweden and abroad, and many seed proteins, chromoproteins and respiratory proteins were studied with four ultracentrifuges put into operation. Closely similar proteins were mixed and centrifuged together to observe tiny differences between related species. By March 1931 new high-speed centrifuges with speeds up to 56 000 r.p.m. came into operation and new problems arose with rotor explosions at these high speeds. Rotors were redesigned, metals were changed and improved and centrifugal fields up to 400 000 *g* could be achieved in normal operations. "A perfect rotor design was finally achieved with Rotor XXI early in 1939" [21], "Two oil-turbine ultracentrifuges with Rotors XXI and XXIV of the final design from 1939 are still (at the time of writing of Ref. [21], in 1972) in daily use in Uppsala", and "Motor-driven centrifuges of later design may be used over a more extended temperature range and are in some respects easier to operate, but the Svedberg ultracentrifuge has never been surpassed in accuracy and reliability". For more details the interested reader is referred to *The Ultracentrifuge* [28], by Svedberg and Pedersen, and many other collaborators, active in the development of this path-breaking instrument.

The fact that proteins were large and well defined molecules led to renewed thinking and enterprise. James Sumner in 1926 obtained an enzyme, urease, for the first time in crystallized form [29] after many years of stubborn research, and provided convincing evidence for the protein nature of the enzyme. The crystal structure of urease from *Klebsiella aerogenes* has now finally been determined in 1995 at 2.2 Å resolution [30]. However, the great organic chemist Richard Willstätter did not believe in 1928 that an enzyme had been crystallized [31] although haemoglobin crystals had been known for quite a while [32]. At that time the protein nature of enzymes had not been established and, in the view of Willstätter and other chemists, it was believed that enzymes consisted of a low-molecular-weight organic catalytical unit colloidally connected to protein-like entities, from which it could be separated. Bitter arguments ensued. Sumner also collaborated with the laboratory of Svedberg to establish the high-molecular-weight nature of enzymes by use of the ultracentrifuge [33]. J.H. Northrop crystallized pepsin (as well as trypsin and chymotrypsin) in 1930 [34] and demonstrated unchanged composition, pro-

teolytic activity and optical activity throughout repeated cycles of crystallization, estimating that it is a protein with a molecular weight of 35 000–36 000. In 1933 John Philpot came to Uppsala to study pepsin, obtained from crystals, in the ultracentrifuge. He then took pepsin crystals in their mother liquid back to the UK, and in the same year J.D. Bernal and D. Crowfoot could publish a letter [35] on "X-ray photography of crystalline pepsin": "Not only do these measurements confirm such large molecular weights, but they also give considerable information as to the nature of protein molecules and will certainly give much more when the analysis is pushed further", and "from the intensity of the more distant X-ray diffraction spots it can be inferred that the arrangement of atoms inside the protein molecule is also of a perfectly defined kind, although without the periodicities characterizing the fibrous proteins". Emil Fischer, as early as 1907 [36], in his Faraday Lecture at the Royal Society, supported the protein-like nature of enzymes. The study of protein and nucleic acid crystal structures constitute a central theme in science today.

Svedberg extended his ultracentrifugation studies into polysaccharides from many sources, and revived his interest on radiation effects in biology and radioactive isotopes [21]. His early interest in protein electrophoresis was continued by Arne Tiselius in his laboratory. He was also instrumental in the construction of a 185 MeV synchrocyclotron in Uppsala in 1949. He showed great interest in close contacts between science and industry and in the manufacture of precision scientific instruments. Till the end of his life he was active in many enterprises closely related to the study of nature. He died on February 25, 1971.

Intense activity in analytical ultracentrifugation next moved into the USA and in 1958 John Warren Williams [37], who had been influenced by Svedberg on his visit to Madison in 1923, published a review summarizing progress in the experimental and theoretical aspects of the field with Ken van Holde, Robert (Buzz) Baldwin and Hiroshi Fujita [38]. Not much later, Howard Schachman, Howie to his many friends, published his book on Ultracentrifugation in Biochemistry [39], an opus in the same distinguished class as the more general Physical Chemistry of Macromolecules by Charles Tanford in 1961 [40]. Both volumes maintain unabated usefulness to the present day, even though lacking more recent advances. Major experimental aspects discussed by Howie include the design of electrically driven and magnetically suspended ultracentrifuges, design of rotors and cells, optical – Schlieren, Lamm scale, interference and light absorption – methods, analysis of boundaries, systems of reversibly interacting components, and discussion of experimental results. In his classical remarks in 1955 [41], G.A. Gilbert could show that even from a qualitative analysis of sedimenting boundaries of concentration gradients in mixed interacting systems, it was possible to derive conclusions with respect to the interactions. Yet the complex nature of the flow, the necessity to disregard diffusion, the imperfect knowledge of the frictional coefficients, and other simplifying assumptions, make a quantitative analysis [42] extremely difficult. Jerry Vinograd was a pioneer in the analysis of sedimentation equilibrium in a buoyant density gradient [43], an approach which convincingly led in 1958 to the establishment of the rules of semiconservative replication of DNA in *Escherichia coli* by $^{14}N/^{15}N$ isotope substitution by Meselson and Stahl [44].

Analytical ultracentrifugation reached a peak in activity and in usefulness in the seventies, in the determination of molecular weights, subunit structures and in the study of interacting systems. Its popularity declined when sequencing and cloning methods became available and the primary structure of both proteins and nucleic acids could be precisely characterized on a molecular level. A classical procedure, mass spectrometry, of historical but later limited scope in the analysis of isotope mixtures, recently became a powerful tool in the ultraprecise establishment of molecular weights as large and larger than 500 kDa of biological macromolecules, matching mass determination by sequencing [45–47]. Electrophoresis [48], later extended to zonal or "gel" electrophoresis [49] in a matrix of polyacrylamide or agarose, became the standard biochemical and molecular biological procedure for determining the molecular weight of minute amounts of proteins and nucleic acids, and for separating complex mixtures. However, new technologies in the construction of analytical ultracentrifuges and refined computer analysis, as well as use of precisely known molecular weights – whenever available from molecular se-

quencing or mass spectrometry – presently allow the study of high-level interactions between proteins, protein–nucleic acid complexes, and other systems of biological macromolecules. It should also be kept in mind that gel electrophoresis procedures, however useful, rely on empirical calibrations and anomalous results defying standard calibrations may be unwittingly obtained for materials of unknown structure, are sometimes reported and often accepted. Electrophoretic motion, for instance, of nucleic acids in agarose gels is a complex hydrodynamic phenomenon under difficult experimental conditions. Its relation to the reptation model of de Gennes [50], reminiscent of worm-like motion through narrow channels, has been suggested, however this model is applicable only in a given range of experimental conditions [51]. At this point it should be emphasized that equilibrium sedimentation is an absolute method from the point of view of classical Gibbs thermodynamics. It is based on the fact that at sedimentation equilibrium the chemical potentials of all components in a multi-component or, for simplicity, three-component system – containing a macromolecular and a low-molecular-weight neutral or electrolyte component in a neutral solvent – must be constant all along the sedimentation radius. This allows exact treatment of multicomponent systems in ultracentrifugation and in the evaluation of the scattering of light, X-rays and neutrons, deriving from fluctuations under strict thermodynamic constraints [52–54]. Precise evaluation of errors and approximations lead to the obtainment of interesting results in the study of hydration and complex cosolvent interactions [55], protein–DNA [56] and protein–protein [57] interactions. Much of this work is based or related to classical studies of G. Scatchard, W. Stockmayer, J. Kirkwood, R.G. Goldberg, A. Vrij, J.J. Hermans and J.Th.G. Overbeek. I believe that, in addition to the practical achievements, the intellectual rewards derived from these studies of biological macromolecules and interactions are not negligible in even our highly competitive world.

Acknowledgements

The author is grateful for the hospitality of the Dipartimento di Scienze Biochimiche, Universita di Roma, La Sapienza, where this work was initiated in September 1994, and to the Chemical Heritage Foundation in Philadelphia, PA, for the opportunity to present a brown-bag lunch lecture on the topic on March 29, 1995. It is part of a more extensive personal view in preparation on Focal Contributions to Aspects of Molecular Biophysics and Structural Biology.

References

[1] M. Perutz, Acta Crystallogr., A26 (1970) 183–185.
[2] H.F. Mark, Die Naturwissenschaften, 67 (1980) 477–483.
[3] H. Staudinger, Die Hochmolekularen Organischen Verbindungen: Kautschuk und Cellulose, Springer, Berlin, 1932.
[4] K.H. Meyer and H. Mark, Der Aufbau der Hochpolymeren Organischen Naturstoffe, Akademische Verlagsgesellschaft, Leipzig, 1930; K.H. Meyer, Natural and Synthetic High Polymers, Interscience, New York, 1942.
[5] M. Perutz, New Yorker, August 12 (1985); cf. also D. Eisenberg, Protein Science, 3 (1994) 1626–1628.
[6] M.M. Hunt, New Yorker, September 13 (1958) 48–71; September 20 (1958) 46–79.
[7] H. Eisenberg, in G. Semenza and R. Jaenicke (Eds.), Comprehensive Biochemistry, Vol. 37, Selected Topics in the History of Biochemistry: Personal Recollections III, Elsevier, Amsterdam, 1990, pp. 265–348.
[8] G.M. Blackburn and M.J. Gait, Nucleic Acids in Chemistry and Biology, IRL Press, Oxford, 1990.
[9] K.E. van Holde, Chromatin, Springer, New York, 1989.
[10] R. Signer, T. Caspersson and E. Hammarsten, Nature, 141 (1939) 122.
[11] H. Kuhn, Chimia, 38 (1984) 191–211.
[12] P. Flory, Principles of Polymer Chemistry, Cornell University Press, Ithaca, NY, 1953.
[13] H. Eisenberg, Biophys. Chem., 7 (1977) 3–13.
[14] O. Kratky and G. Porod, Recl. Trav. Chim. Pays-Bas, 68 (1949) 1106–1122.
[15] N. Borochov and H. Eisenberg, Macromolecules, 27 (1994) 1440–1445.
[16] H. Morawetz, Polymers: The Origins and the Growth of a Science, Wiley, New York, 1985.
[17] F.H.C. Crick and J.C. Kendrew, Adv. Protein Chem., 12 (1957) 133–214.
[18] L. Pauling, Protein Sci., 2 (1993) 1060–1063.
[19] J.D. Watson, The Double Helix, Weidenfeld and Nicolson, London, 1968.
[20] F.M. Richards, Protein Sci., 1 (1992) 1721–1730.
[21] S. Claesson and K.O. Pedersen, Biogr. Mem. Fellows R. Soc., 18 (1972) 594–627.
[22] T. Svedberg and H. Rinde, J. Am. Chem. Soc., 45 (1923) 943–954.

[23] T. Svedberg and H. Rinde, J. Am. Chem. Soc., 46 (1924) 2677–2693.
[24] T. Svedberg and R. Fåhraeus, J. Am. Chem. Soc., 48 (1926) 430–438.
[25] T. Svedberg and E. Chirnoaga, J. Am. Chem. Soc., 50 (1928) 1399–1411.
[26] R. Foord, E. Jakeman, C.J. Oliver, E.R. Pike, R.J. Blagrove, E. Wood and A.R. Peacocke, Nature, 227 (1970) 242–245.
[27] A.L. Berman, E. Kolker and E. Trifonov, Proc. Natl. Acad. Sci. USA, 91 (1994) 4044–4047.
[28] T. Svedberg and K.O. Pedersen, The Ultracentrifuge, Oxford University Press, London, 1940.
[29] J.B. Sumner, J. Biol. Chem., 69 (1926) 435–441.
[30] E. Jabri, M.B. Carr, R.P. Hausinger and P.A. Karplus, Science, 268 (1995) 998–1004.
[31] A.B. Costa, Chem. Ber., 25 (1989) 788–790.
[32] E.T. Reichert and A.P. Brown, The Differentiation and Specificity of Corresponding Proteins and other Vital Substances in Relation to Biological Classification and Organic Evolution; The Crystallography of the Hemoglobins, Carnegie Institute, Washington, DC, 1909.
[33] J.B. Sumner and N. Gralén, J. Biol. Chem., 125 (1938) 33–36; J.B. Sumner and I. Eriksson-Quenset, J. Biol. Chem., 125 (1938) 37–44.
[34] J.H. Northrop, J. Gen. Physiol., 13 (1930) 739–766; 14 (1931) 713–724.
[35] J.D. Bernal and D. Crowfoot, Nature, 133 (1933) 794–795.
[36] E. Fischer, J. Chem. Soc., 91 (1907) 1749–1765.
[37] R.L. Baldwin and J.D. Ferry, Biogr. Mem. Natl. Acad. Sci. USA, 65 (1994) 375–389.
[38] J.W. Williams, K.E. van Holde, R.L. Baldwin and H. Fujita, Chem. Rev., 58 (1958) 715–806.
[39] Howard K. Schachman, Ultracentrifugation in Biochemistry, Academic Press, New York, 1959.
[40] C. Tanford, Physical Chemistry of Macromolecules, Wiley, New York, 1961.
[41] G.A. Gilbert, Discuss. Faraday Soc., 20 (1955) 68–71.
[42] L.M. Gilbert and G.A. Gilbert, Methods Enzymol., 27D (1973) 273–296.
[43] J. Vinograd, Methods Enzymol., 6 (1963) 854–870.
[44] M. Meselson and F.W. Stahl, Proc. Natl. Acad. Sci. USA, 44 (1958) 671–682.
[45] J.A. McCloskey (Ed.), Mass Spectrometry, Methods Enzymol., 193 (1990) (whole volume).
[46] T. Matsuo, R.M. Caprioli, M.L. Gross and Y. Seyama (Eds.), Biological Mass Spectrometry, Wiley, New York, 1994.
[47] W. Senko and F.W. Lafferty, Annu. Rev. Biophys. Biomol. Struct., 23 (1994) 763–785.
[48] M. Bier (Ed.), Electrophoresis, Academic Press, New York, 1959.
[49] K.E. van Holde, Physical Biochemistry, Prentice Hall, Englewood Cliffs, NJ, 2nd edn., 1985.
[50] P.-G. de Gennes, Scaling Concepts in Polymer Physics, Cornell University Press, Ithaca, NY, 1979.
[51] B.H. Zimm and S.D. Levine, Quart. Revs. Biophys., 25 (1992) 171–204.
[52] E.F. Casassa and H. Eisenberg, Adv. Protein Chem., 19 (1964) 287–395.
[53] H. Eisenberg, Biological Macromolecules and Polyelectrolytes in Solution, Clarendon Press, Oxford, 1976.
[54] H. Eisenberg, Quart. Rev. Biophys., 14 (1981) 141–172.
[55] H. Eisenberg, Biophys. Chem., 53 (1994) 57–68.
[56] S.-J. Kim, T. Tsukiyama, M.S. Lewis and C. Wu, Protein Sci., 3 (1994) 1040–1051.
[57] R. Ghirlando, M.B. Keown, G.A. Mackay, M.S. Lewis, J.C. Unkeless and H.J. Gould, Biochemistry, 24 (1995) 13320–13327.