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Mass spectrometry (MS) and nuclear magnetic resonance (NMR) applied to biological macromolecules

The Nobel Prize in Chemistry for 2002 is to be shared between scientists working on two very important methods of chemical analysis applied to biological macromolecules: mass spectrometry (MS) and nuclear magnetic resonance (NMR). Laureates John B. Fenn, Koichi Tanaka (MS) and Kurt Wüthrich (NMR) have pioneered the successful application of their techniques to biological macromolecules.
Biological macromolecules are the main actors in the makeup of life whether expressed in prospering diversity or in threatening disease. To understand biology and medicine at molecular level where the identity, functional characteristics, structural architecture and specific interactions of biomolecules are the basis of life, we need to visualize the activity and interplay of large macromolecules such as proteins. To study, or analyse, the protein molecules, principles for their separation and determination of their individual characteristics had to be developed. Two of the most important chemical techniques used today for the analysis of biomolecules are mass spectrometry (MS) and nuclear magnetic resonance (NMR), the subjects of this year's Nobel Prize award.

MASS SPECTROMETRY (MS)

The ability to separate molecules based on different size and charge was first described in 1912 by J.J. Thompson (Nobel Prize laureate in 1906 for investigations of the conduction of electricity by gases) and expressed as the mass/charge ratio with the unit Thompson (Th). Despite years of intense MS development, the goal of analysing large macromolecules remained elusive for over 70 years. It was soon acknowledged, however, that the process would comprise a chemical preparation of charged molecules in the gas phase followed by physical separation of ions in vacuum. For biomolecules the challenge was to find a viable procedure for the chemical preparation of the sample. The processes that were finally able to make the biomolecules leave the aqueous phase and hover in the gas phase made use of an ingeneous combination of choices of material, energy, dimension, structure and chemical surroundings.

The drive to equip bioscience with novel tools to study the identity and structure, as well as the functional characteristics, of large biomolecules strongly permeated the development of mass spectrometric techniques and methods. History played a role here, as in most cross-disciplinary ambitions, and scientists were hampered by the idea that one always had to volatilise the molecules first and then ionise them. In addition, the energy needed to release the molecules was commonly supplied as heat, and molecules containing "polar groups" needed to be chemically modified to withstand and enable the heat-volatilisation. The developments superseded each other, but each step was marginal in relation to the giant step needed to be able to analyse intact large biomolecules.

One early major breakthrough, described by M.S.B. Munson and F.H. Field in 1966 [1], was the use of chemical ionisation (CI), which for the first time made it possible to ionise thermo-labile biomolecules. In CI, abundant reagent gas ions are first formed by electric discharge of a reagent gas, and the reagent ions then in turn ionise volatilised molecules of interest. Plasma desorption (PD), introduced in 1976 [2], uses high-energy ions to desorb and ionise molecules. The technique achieved some success but was never shown to be reliable for molecular masses greater than 10 kiloDalton (kDa). This can be compared to the molecular weight of common proteins, ranging from a few thousand Dalton (Da) for the hormone insulin (5,734 Da), to one hundred thousand Da for common proteins, and up to at least 5 million Da for large enzyme complexes.

It was known that ion bombardment would destroy small biomolecules. A milestone was passed when M. Barber et al. [3] described the successful use of a non-volatile chemical protection environment next to the molecules to enable polar and thermally labile compounds to survive the ionisation process. This work showed that accelerated atoms (and later also ions) of e.g. argon, caesium or xenon could be used for mass determination of small biomolecules (i.e. mol. wt. <10 kDa) combined with on-line fragmentation for structure determination. This technique, termed fast atom bombardment (FAB), and the closely related method liquid matrix secondary ion mass spectrometry (LSIMS) did not solve the problem of reaching higher masses but had a major impact on expectations of future success.

The challenge in the 1980s was how to find a way to analyse high-molecular-weight compounds by mass spectrometry and to make mass spectrometry into a powerful detector for liquid separation techniques. The introduction of the electrospray (ES) and soft laser desorption (SLD) methods could meet both needs. The resulting explosion of applications can be examplified by the demonstration that the ES technique is so mild that viral material can remain viable after an electrospray ionisation (ESI) process [4]. The SLD method allowed easy access to singly charged ionisation of intact biomolecules in complex matrices. Due to significantly improved sensitivity and ease of use compared to conventional plasma desorption, a rapid gain in popularity awaited this method.

Electrospray ionisation

Initial experiments by the physicist John Zeleny in 1917 preceded the first description by Malcolm Dole in 1968 [5] of the electrospray principle, including the charge residue model (CRM) that has survived as a main explanation for the enigmatic ESI process. According to this model, the charged droplets evaporate to a point where the number of repulsive electrostatic charges on the surface becomes so large relative to the droplet size that an explosion ("Rayleigh explosion") occurs. This produces a number of smaller droplets that also have a surface containing electrostatic charges. In his early experiments Dole used an inert gas to facilitate the desolvation in the mass spectrometric analysis of relatively high molecular weight polystyrene.

The well-defined breakthrough of ESI came in 1988 at a symposium in San Francisco, when John Fenn presented an identification of polypeptides and proteins of molecular weight 40 kDa [6]. Fenn showed that a molecular-weight accuracy of 0.01% could be obtained by applying a signal-averaging method to the multiple ions formed in the ESI process. The findings were based on developments that had started in 1984 [7] in Fenn's laboratory at Yale, when electrospray and mass spectrometry were successfully combined for the first time. Fenn used his knowledge of free-jet expansion to improve Dole's method with a counterflow of gas for desolvation, eliminating re-solvation of formed macromolecular ions. This discovery was closely followed by results from a Russian research group (Aleksandrov et al.) [8].

The electrospray process is governed by a large number of chemical and physical parameters that together determine the quality of the process. Its start and end can be defined by an electrical circuit that drives the spray of liquid-charged droplets (Figure 1). In this process the biomolecule starts out as an entity or complex, usually charged and dissolved in a water-rich environment. At the end of the process the same biomolecule is represented and harvested through the orifice of a mass analyser as a series of 'naked' multicharged ions. In a vacuum, the biomolecular ions then are selectively analysed according to their mass/charge ratio.
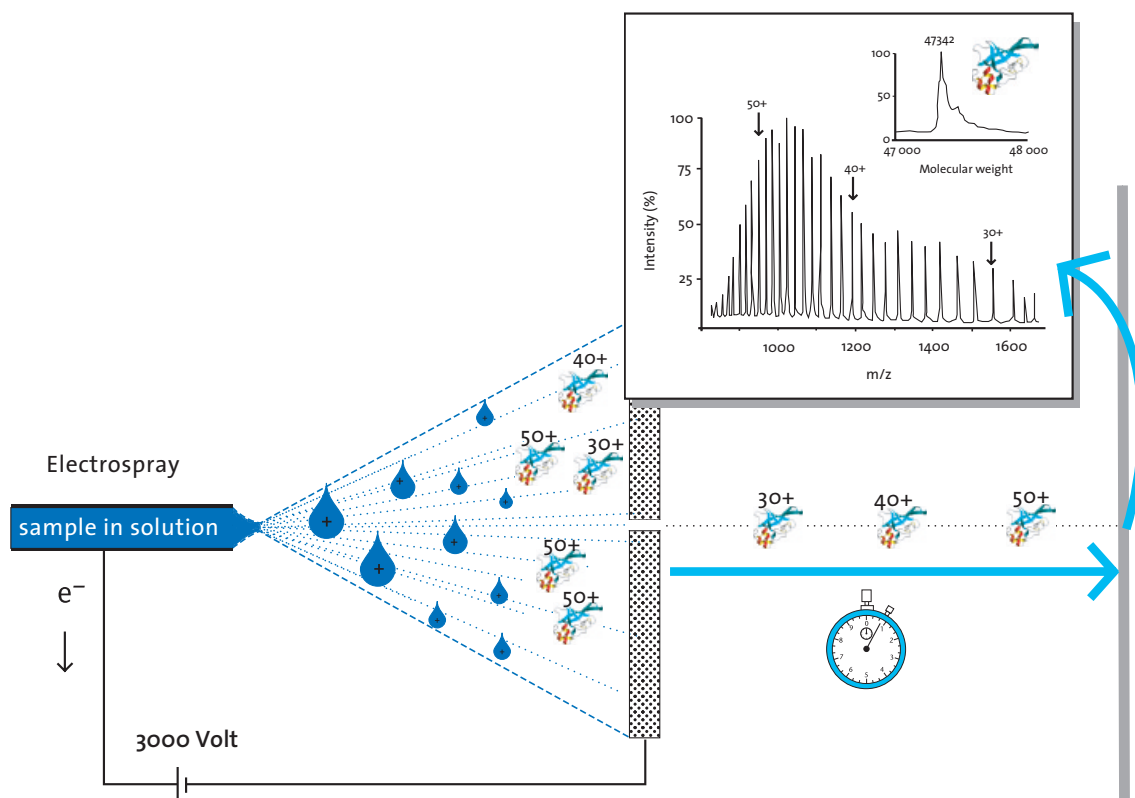
Figure 1. The electrospray process.

The initial droplets usually have positive charges. Protonated or deprotonated macromolecules are set free as the water-rich content of the droplets evaporates. In the naked macromolecule ions entering the mass analyser this charge is typically manifested as variable states of protonation at proton-accessible sites. The process results in ions with varying charges in the range +2 up to +40 or even higher. In the analysis one observes a regular series of peaks reflecting the mass/charge ratio. This added complexity to the interpretation of the mass spectra and initially confused scientists when the first results were presented. Fenn realized, however, that this very complexity adds to the information and can be used to an advantage to improve the accuracy of the molecular-weight determination. The secret was revealed in the multiple charge theory described by Fenn in 1987 [9]. The theory showed that different charge states could be interpreted as independent measurements of molecular weight and that an averaging method based on the solution of simultaneous equations could provide accurate molecular-weight estimations for large molecules.

In Figure 1, a protein of molecular weight 47,342 Da is analysed with ESI. The process results in over 50 peaks of the corresponding charge pattern. The resulting mass/charge ratio equals 1-2000 Th and can thus be easily analysed in any mass analyser. The charge pattern can simply be deconvoluted and the mass of the uncharged protein determined to dramatically higher accuracy than if the interpretation of data was based on a single ion.

It was early understood that the sensitivity of the process was not increased by high mass flow. From microlitre/minute flows, the sensitivity enhancement has been visualized by lowering the flow to nanolitre/minute flows [10-11]. Sensitivities of attomole levels are commonly achieved in these low-flow applications. A further advantage is that with a proper selection of conditions, ESI can be the least invasive of all ionisation methods. It even allows studies of molecular complexes that only have weak non-covalent interactions, such as protein-protein, enzyme-substrate or protein-ligand complexes.

Soft laser desorption (SLD)

During the 1980s several groups tried to solve the volatilisation/ionisation problem of mass spectrometry using laser light as an energy source. By focusing a light beam onto a small spot of a liquid or solid sample, one hoped to be able to vaporise a small part of the sample and still avoid chemical degradation. V.S. Letokhov in Moscow demonstrated that the method could work for small but polar molecules, like amino acids. This approach was further developed by M. Karas and F. Hillenkamp in Münster. In 1985, these scientists showed that an absorbing matrix could be used to volatilise small analyte molecules, but were without initial success for large molecules.

A breakthrough for the laser desorption method in its application to large biomolecules was reported at a symposium in Osaka in 1987, when Koichi Tanaka at the Shimadzu Corp. in Kyoto presented results of a mass spectrometric analysis of an intact protein. In two publications and lectures in 1987-1988, Tanaka presented ionisation of proteins such as chymotrypsinogen (25,717 Da), carboxypeptidase-A (34,472 Da) and cytochrome c (12,384 Da) [12-14].
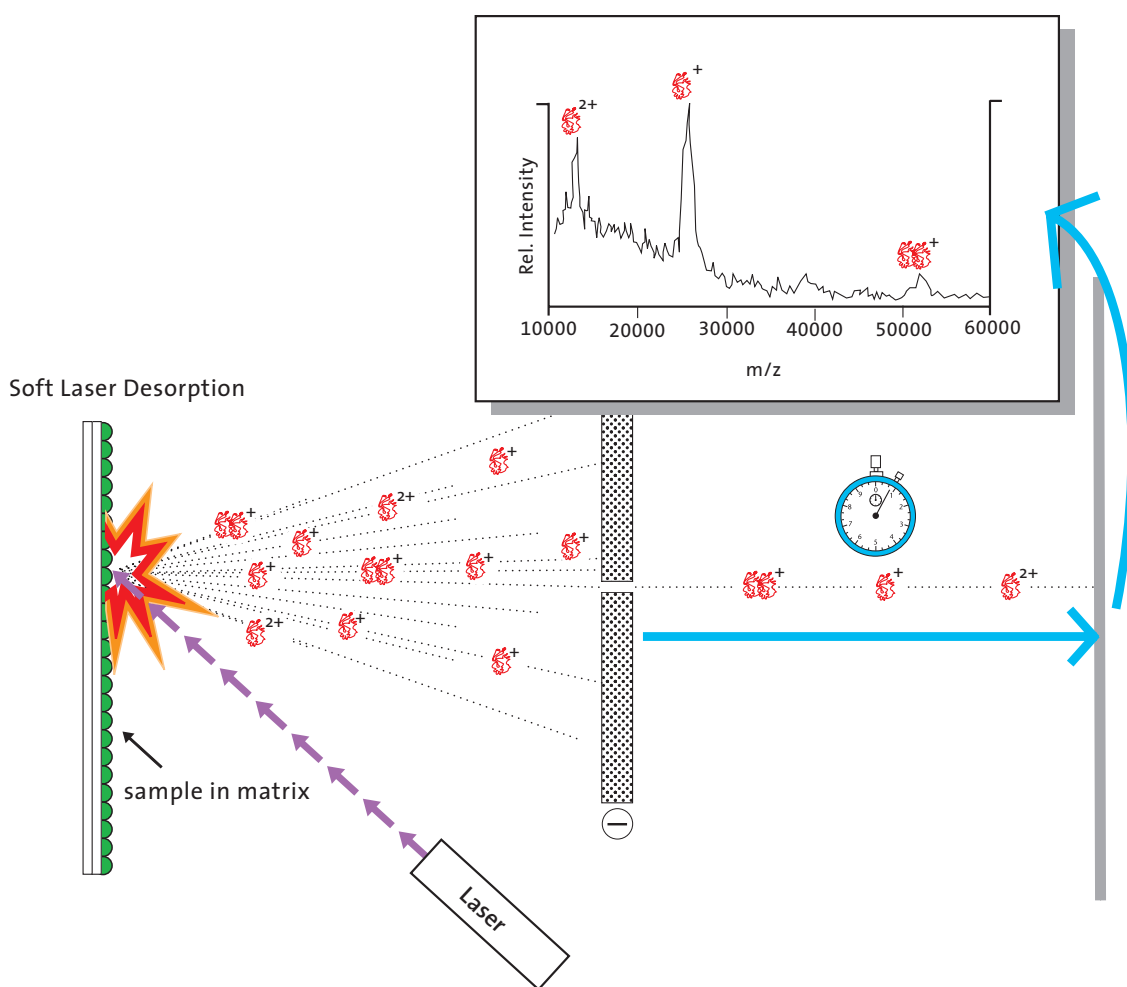

Figure 2. The soft laser desorption process.

The missing link to make laser desorption work for large macromolecules was a proper combination of laser energy and wavelength with the absorbance and heat transfer properties of a chemical/physical matrix plus the molecular structure of the analytes in this matrix. Tanaka showed that gaseous macromolecular ions could be formed using a low-energy (nitrogen) laser,

a fact that was not expected at the time but quickly thereafter inspired the laser desorption scientists. The principle of SLD is illustrated in Figure 2, showing the signals from singly- and doubly charged molecular ions and a protein cluster-ion with a single charge.

A nitrogen laser beam has a wavelength of 330 nm, which is not absorbed by the aromatic amino acids in proteins and peptides. This is important for avoiding fragmentation. Tanaka's physical/chemical matrix (glycerol containing colloidal particles) has not been further developed, but various types of physical/chemical matrices have been, and are continuously presented. One such matrix is used in the desorption ionisation on silicon (DIOS) method, where a physical matrix with extreme surface area and high laser radiation absorptivity is of growing interest [15].

A fast-growing version of the SLD technique, currently predominant, incorporates the macromolecules of interest in a low-molecular-weight crystalline matrix with absorption maximum matched to the wavelength of the laser pulse. This matrix-assisted, laser-desorption ionisation (MALDI) technique applied to proteins [16] appeared shortly after Tanaka's initial breakthrough. The MALDI technique presented by M. Karas and F. Hillenkamp used a YAG laser at 266 nm and a chemical matrix of nicotinic acid. Different physical/chemical matrices and wavelengths were subsequently presented, affording the SLD technique very many useful niches, including the analysis of proteins and oligonucleotides.

One advantage of the approach, early recognized, was to permit intact low-charge macromolecular transfer to the gas phase. That it also gives good results with contaminated samples has helped acceptance of the technique for large molecules, although this same contamination and chemical matrix usually hamper its usefulness for medium-size-molecular-weight compounds and low concentrations of the macromolecules.

The combination of SLD in the form of MALDI with time-of-flight mass spectrometric detection has been most important for molecular weight determinations of biological macromolecules. It is today a cornerstone of proteomics.

Recent developments and applications of MS

Some five years ago, mass spectrometry definitively crossed the border to biochemistry. The general ways that it provides structural determination, identification and trace level analysis have many applications in the biochemical field. It has become an attractive alternative to Edman sequencing, earlier dominant, and has an unsurpassed ability to identify posttranscriptional modifications and non-covalent interactions in for example antigen-antibody binding studies for identifying ligands to orphan receptors. An important development was the demonstrated usufulness of the MS technique for protein identification after 2-D gel electrophoretic separation and after liquid separation. With the recent interest in microfabricated devices for sample preparation, low-flow (nano-flow) techniques are being developed for optimised utilization of time and sensitivity.

The application to assist a high flow of sample by a high-velocity gas in "pneumatically assisted" ESI has, on the other hand, initially become more popular. This makes it possible to extend the solvent flow rates and choice of electrolyte compositions, concentrations and electric potentials needed for a well-adapted ESI to conventional liquid-separation MS applications. As already mentioned, combining low-flow liquid separation and mass spectrometric analysis still remains the ideal match in biomolecular analysis. Coupling separation in series before ESI and SLD is at present an attractive approach to medical early warning chemical diagnostic tools. Simpler on-line separation before ESI is also of interest for enhancing robustness and data information from the ESI process. In this way residual salts and electroactive compounds added for solubility or to enable separation can be removed in time from the ESI

process. A comparative advantage of an SLD approach is that the method is less affected by sample impurities.

In the trend following the paradigm shift in the mid-1980s, with the introduction of two complementary soft ionisation processes (ESI and SLD), the techniques have matured into viable tools for the analysis of biomolecules and many other classes of molecule. This paradigm shift is what has led mass spectrometry into fields other than physics and chemistry.

Nuclear magnetic resonance (NMR)

NMR phenomena were first detected experimentally by Felix Bloch and Edward Purcell (Nobel Prize in Physics, 1952) in 1946. In the early 1950s the chemical shift was discovered that demonstrated that the NMR signal carried information about the chemical environment of the nucleus studied. This prompted a powerful development of NMR as an analytical tool in chemistry, however with the important drawback of low sensitivity. The potential sensitivity of NMR was dramatically increased with the discovery that Fourier transform methods could be applied to NMR. Instead of slowly recording spectra in the frequency domain, radiofrequency pulses were applied to the sample and time- dependent responses were monitored. As a last step the time response was Fourier- transformed to give an NMR spectrum as a function of frequency. The Fourier transform methods led to much more rapid recording of signals, and also paved the way for two- and multidimensional NMR techniques. This very important development is based mainly on the work of Richard Ernst (Nobel Prize in Chemistry, 1991).

During the 1970s, NMR studies increasingly addressed biochemical problems. Greater sensitivity and resolution were due to methodological as well as technical developments, such as two-dimensional experiments and the availability of stable magnets with stronger fields. Scientists began to contemplate the use of NMR to study the detailed properties of biological macromolecules.

Since that time, high-resolution NMR has become a most important tool for studying the structure, dynamics and molecular interactions of biological macromolecules in aqueous solution. At present, NMR stands besides single-crystal X-ray diffraction as the major method for determining the three-dimensional structures of proteins and nucleic acids. The first protein structure studied at atomic resolution was determined by X-ray crystallography for myoglobin in 1957 by Max Perutz (Nobel Prize in Chemistry, 1962, shared with John Kendrew). Structural biology has since undergone tremendous growth. The NMR contributions started around 1985. Of the total 14,734 atomic coordinate sets deposited in the Protein Data Bank by May 2002, 2,763 or about 20% had been determined by NMR. The great majority of structures had been obtained with single-crystal X-ray diffraction, and only a few by other techniques such as electron or neutron diffraction.

Besides high-resolution NMR for chemical studies of molecules in rapid motion in solution, very significant developments have also emerged in the past few decades in the techniques of solid-state NMR and magnetic resonance imaging (MRI). Solid-state NMR permits study of large and immobile molecular assemblies, although at much lower resolutions than in solution-NMR. MRI lies behind the magnet camera used in many hospitals for diagnostic imaging. These fields will not be further described here.

NMR STRUCTURE DETERMINATION

The development of NMR as a powerful method in structural biology has involved a number of critical steps: identification of NMR parameters useful for determining molecular structure; development of methods for reliable assignment of the many resonances in an NMR spectrum to their respective nuclei in the macromolecule; development of methods to measure a suffi-

cient number of structure-related parameters to give the information needed for unique structure determination; and finally, development of computational techniques that could translate the structural parameters into the unique three-dimensional structure. Kurt Wüthrich has pioneered all these steps.

The most important parameter for structure determination based on NMR is the nuclear Overhauser enhancement (NOE) effect. This provides information about inter-atomic distances between nuclei close in space. Observation and quantification of NOE effects between protons in close proximity remains the basis of NMR-based determination of three-dimensional structure.
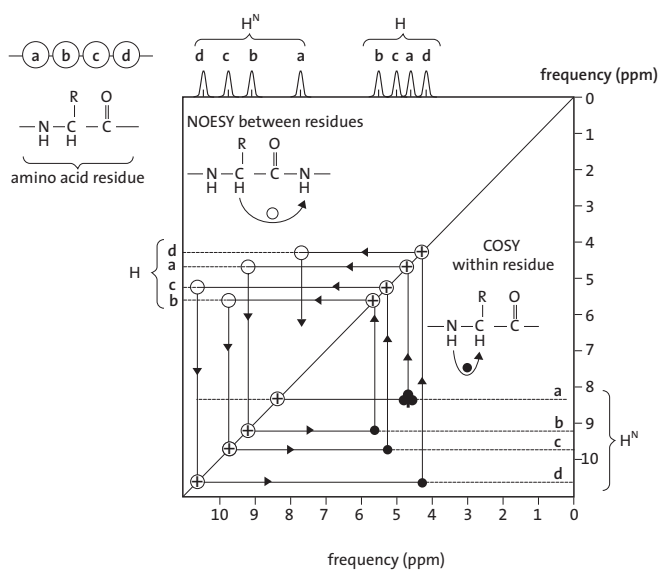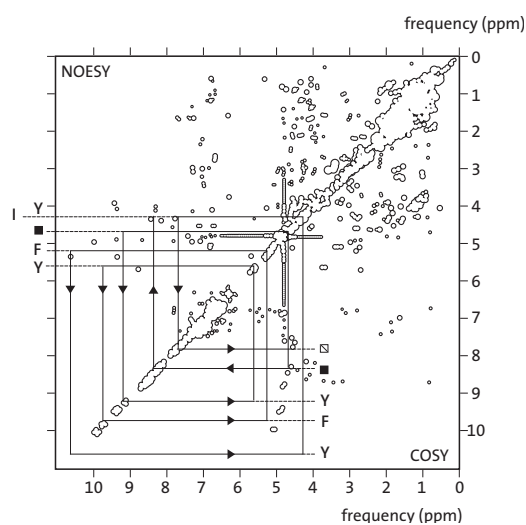

Figure 3a

Figure 3b

Figure 3.

a) The principle for a sequential assignment of the backbone protons (H^N and H^α) in four consecutive residues (abcd) in a protein, based on two-dimensional NMR spectroscopy. The NMR spectra are depicted as contour maps with resonance frequency along both axes, and with a representation of the one-dimensional proton spectrum along the diagonal. Only the frequency region which contains the H^N and H^α resonances is shown.

Record first two different types of two-dimensional NMR spectra: a COSY spectrum, which gives crosspeaks between resonances from protons bound to adjacent carbons or nitrogens, and a NOESY spectrum, which gives crosspeaks between resonances from protons close in space. Then paste together the upper half of a NOESY spectrum with the lower half of a COSY spectrum, so that they coincide in the diagonal, providing a connectivity diagram.

Now assume that we know (or guess) that the COSY crosspeak ♣ belongs to residue a, so that frequencies for H^N and H^α are known for this residue. Now go vertically to the diagonal peak, then horizontally until meeting O in the NOESY spectrum. Go vertically towards the diagonal to find the H^N resonance frequency of residue b. Continue horizontally to ● in the COSY spectrum, vertically to the diagonal and you have determined the H^α resonance frequency for residue b. We have now assigned the two backbone proton resonances of residue b and can continue towards c and d. The resonances for the protons in the respective sidechains can easily be found in the other regions of the two-dimensional spectra, once the sequence-specific H^α for each residue is known. Finally, there will be a number of remaining NOESY crosspeaks, which are not used in the assignment procedures. Here one such crosspeak is illustrated by ♥ between H^N of residue a and H^N of residue d. It contains the information that these protons are close in space, typically less than 5Å apart. This is an example of the kind of structural information that is used to model a three-dimensional structure of the molecule. In a real study, there is a redundancy of such proximity conditions between proton pairs, and the modelled structure should fulfil all these conditions.

b) A real assignment example showing a two-dimensional combined NOESY/COSY connectivity diagram for the protein BPTI (Bovine Pancreatic Trypsin Inhibitor) in deuterated aqueous solution. The indicated residues are part of a β-sheet secondary structure, which gives rise to a characteristic spiral pattern in the assignment procedure. The strategy for the sequential assignment was outlined by Wüthrich et al. in 1981. From ref. 17 and Wüthrich, K. NMR of proteins and nucleic acids, p. 147, see list of Further reading.

In the late 1970s Wüthrich developed new ways of applying two-dimensional NMR methods to macromolecules, partly in cooperation with Richard Ernst. However, the most important hurdle was to assign the proton resonances in the macromolecule. Sequential assignment based on NOE observations along the protein backbone was developed by Wüthrich in a series of publications shortly after 1980 [17-22]. The method is based on combining the results of two-dimensional correlated spectroscopy (COSY) and two-dimensional NOE spectroscopy (NOESY), as illustrated in Figure 3.

In 1985 Wüthrich reported use of the methodology to determine the three-dimensional structure of a small protein in solution, proteinase inhibitor IIA, from bull seminal plasma, BUSI IIA[23]. Many structural constraints, mainly long-range NOE observations, were recorded and a mathematical method based on metric matrix distance geometry [24] was used to calculate the three-dimensional structure for the protein based on these constraints. Thus the last step in the development of a reliable method for NMR-based structure determination for a biological macromolecule had been accomplished. The method is robust in the sense that the resulting structure is usually over-determined by an excess of experimentally-observed structural constraints. Figure 4 illustrates the structure determined for BUSI IIA and also shows schematically how NMR-derived structures are often presented.
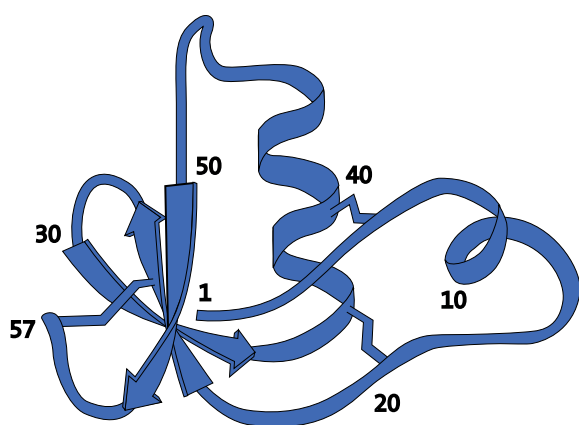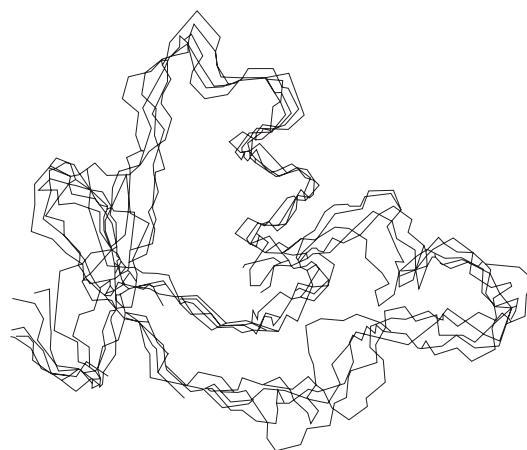

Figure 4a

Figure 4b.

Figure 4.
One of the first three-dimensional NMR solution structures determined by Wüthrich, in 1985. From ref. 23.

a) A schematic view of the topology of the polypeptide backbone of BUSI IIA (bull seminal plasma proteinase inhibitor IIA). The structure was calculated with distance geometry, from numerous distance constraints obtained from two-dimensional NOESY spectra and additional structural constraints such as torsion angles. The structure represents an average of several computed structures that fulfil the structural constraints.

b)A set of five backbone structures of BUSI IIA, calculated with distance geometry using the NOE distance constraints. Such computed structures were used to calculate the average structure presented as Fig. 4a.

The protein backbone depicted in Figure 4b is obtained from repeated calculations (typically about 20: here 5 are presented) that all fulfil the mathematical criteria required by distance geometry. The overall agreement between the calculations is a measure of the precision of the structure determination. The parts of the molecule that seem particularly disordered are often highly mobile. Their varying mobility and the timescales and amplitudes of their motion are parameters that describe the dynamics of a molecule. The dynamics and the three-dimensional structure taken together are very important for how the molecule will be able to interact with other molecules in its environment. Using NMR, it is possible to obtain detailed experimental information on the dynamics of a molecule (see below).

NMR-based structural biology thus gained momentum in the middle of the 1980s, by which time several other researchers were employing and further developing the new NMR techniques that became available in terms of new methods and improved spectrometers. In 1985, the group of R. Kaptein in Ütrecht published an NMR-derived structure of the lac repressor headpiece [25]. At about the same time, similar NMR methods were used to determine solution structures of oligonucleotides. The first of these, in 1986, was of a DNA hairpin [26]. An important proof of the principle came in 1986 when NMR and X-ray crystallography were used independently to solve a new protein structure, that of the α-amylase inhibitor Tendamistat. Almost identical three-dimensional structures in terms of the global fold of the polypeptide chain were demonstrated in accompanying publications [27, 28].

Recent developments and applications of NMR

The 1990´s saw further rich development of NMR-based studies of biological macromolecules. Isotope labelling of the molecules with the NMR-active nuclei ^{15}N and ^{13}C has become a standard procedure, growing out of the methods for recombinant protein expression in host bacteria. The possibilities of isotope labelling have also led to the development of heteronuclear three-dimensional NMR techniques, particularly through the work of A. Bax, National Institutes of Health (NIH). The new techniques have greatly enlarged the repertoire of NMR for biomacromolecules, and have provided e.g. additional procedures for resonance assignment. Heteronuclear relaxation provides the basis for NMR studies of molecular dynamics in a macromolecule, showing that the parts of a molecule that appear disordered in a structure determination are often associated with high mobility. The dynamic behaviour of a molecule is an important characteristic, particularly for understanding molecular interactions and molecular recognition. NMR stands in the forefront as a method for providing site-specific quantitative information about this dynamic aspect of biological macromolecules. NMR has also emerged as an important tool for studies of protein folding, i.e. how an unstructured protein chain attains equilibrium three-dimensional structure in a time-dependent process.

At present, single-crystal X-ray diffraction, the grand old method of three-dimensional structure determination, can provide three-dimensional structures of very large biological macromolecules and assemblies with excellent atomic resolution. Recently reported structures represent molecular weights up to the order of megaDa in extreme cases. Compared to these large single-crystal structures, NMR solution structures generally concern smaller molecules, typically below 30 kDa, and they are often less precise. However, recent developments have advanced also the NMR frontiers. With TROSY and CRINEPT techniques [29, 30], also pioneered by Wüthrich et al., it is now possible to assign resonances and study a protein assembly as large as 900 kDa, as shown in the recent study of the molecular chaperone GroEL-GroES complex [31]. New methods to achieve even greater precision in the NMR-determined structures have also emerged, most notably by observation of residual dipolar couplings induced by partial alignment in particular alignment media, as described by N. Tjandra and A. Bax [32].

Knowledge of the three-dimensional structures of biological macromolecules is fundamental for our understanding of life processes. NMR provides this knowledge of structures in aqueous solutions approximating physiological conditions. It can be applied where single crystals are difficult to obtain, typically of molecules with disordered and highly flexible parts. One example of this class of molecules is the prion proteins. The concept of prions as new infective agents was presented by Stanley Prusiner (Nobel Prize in Medicine, 1997). The major constituent of prions is a prion protein, which can exist in a benign cellular form or undergo transition to a disease-related, 'scrapie', form. The scrapie form is related to certain neurodegenerative diseases, which include bovine spongiform encephalopathy (BSE, "Mad cow disease"), scrapie

in sheep and the related Creutzfelt-Jacob disease in humans. NMR structures of the benign cellular form show that one half of the prion protein backbone has a well-ordered solution structure whereas the other half is a highly mobile, extended coil. Figure 5 shows a structure of the benign cellular form of the murine prion protein determined by Wüthrich [33].
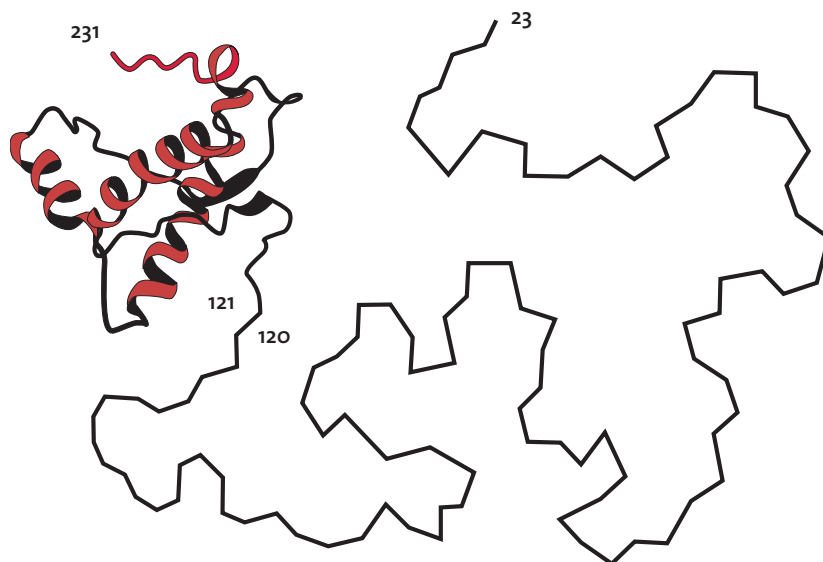

Figure 5. NMR structure of the recombinant murine prion protein, determined by Wüthrich in 1997. (From ref. 33). First the well-ordered structure of a fragment comprising the C-terminal residues 121-231 was determined. Then the intact protein 23-231 was studied and it was found that the N-terminal 23-126 segment formed an extended, highly flexible coil with high mobility. Prion proteins from other species, including bovine and human proteins, have similar structures with flexible N-terminal segments.

The combined structure and dynamic information illustrated in Figure 5 is characteristic of NMR-determined three-dimensional structures. In the case of prion proteins it may lead to understanding of the processes that bring about the transition of the benign form of the protein to the disease-related scrapie form.

Direct practical applications of biomacromolecular NMR in pharmaceutical industrial research include screening studies when a particularly important protein is being considered as a target for new drugs. The protein is exposed to a variety of small molecules, potential leads for a new drug. When a particular small molecule interacts with the macromolecule, the NMR spectrum of the macromolecule will change. If the resonances of the macromolecule have been assigned, it is also possible to determine which residues are involved in the interaction, and the potential use of the small molecule in further drug development can be assessed.

Final remarks

The last five years have seen the appearance of the "omics world" in life sciences, exemplified by new concepts such as genomics, proteomics or metabonomics. The new aspect of these concepts is the global view and the large-scale investigations, in contrast to the problem-oriented reductionistic view prevailing in earlier studies. It is now possible to describe the whole genome of an organism. Similarly, the whole set of proteins that appear at a certain stage in a living cell can at least be considered, even if not quantitatively described, and the same should in principle hold for the total flow of metabolic products. These new possibilities are in part due to the development of new methodologies, of which mass spectrometry and NMR applied to biological macromolecules are important examples. However, side by side with the

new large-scale enterprises of mapping the molecular properties of living organisms, there remains the increasing need for deeper understanding of how the biochemical processes occur at a detailed molecular level. In this world of fundamental biochemical science, mass spectrometry and NMR applied to biological macromolecules are among the important cornerstones for improved understanding of the life processes.

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