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Journal of Chromatography B, 787 (2003) 29–41
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Analysis of complex protein–polypeptide systems for proteomic studies

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Abstract

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), followed by protein extraction and characterization with chemical sequencing or mass spectrometry (MS), is the most commonly used method to analyze complex protein systems such as cells and organelles. However, it is claimed that 2-D PAGE is a slow and labor-intensive technique and also needs subsequent efforts for one-by-one identification of proteins. Recently, the combined methods of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, with preceding separation techniques such as capillary isoelectric focusing (CIEF) or liquid chromatography, have been demonstrated as high-throughput techniques suitable for proteomic analysis of protein systems. The studies which employ FTICR MS, aimed at the analysis of complex protein systems, have been reviewed, comparing their performance with that of 2-D PAGE. Also, the possibilities of combining 2-D PAGE and the FTICR MS method to analyze and reconstruct the structures and functions of complex systems are discussed.

Keywords: Proteomics; Protein–polypeptide systems

Contents

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1570-0232/02/$ – see front matter
doi:10.1016/S1570-0232(02)00337-9

1. Introduction

Since the 1970s, when the techniques of polyacrylamide gel electrophoresis became commonly available, the total analysis of the proteins present in various complex protein systems has been attempted. However, it has become clear that the processes of protein expression from genes must be studied in order to understand the time-dependent changes in the expressed protein species and their levels, and in the last 20 years much emphasis has been placed on genome analysis (total DNA sequencing) of biological species, including humans. Several genome databases are now available and we can compare the amino acid sequence of proteins predicted from the DNA sequence with those which are actually present and function in cells or organs. These comparisons have clearly shown that very little can be determined about a protein's structure and physiological function from the DNA sequence alone, although improvements in the techniques to deduce them from the homology of the amino acid sequence can be expected.

The ultimate aim of the analysis of a complex protein system, such as an organelle, a cell, or an organ, is to reconstruct the structures and functions of the proteins or protein complexes within the system as a whole. The limitations of genomic studies in reconstructing protein systems can be realized by illustrating the structural levels present in these systems, as shown in Fig. 1. There are multiple structural levels in the system, from the DNA sequence shown as a plane positioned at the bottom of the figure, to the complex three-dimensional

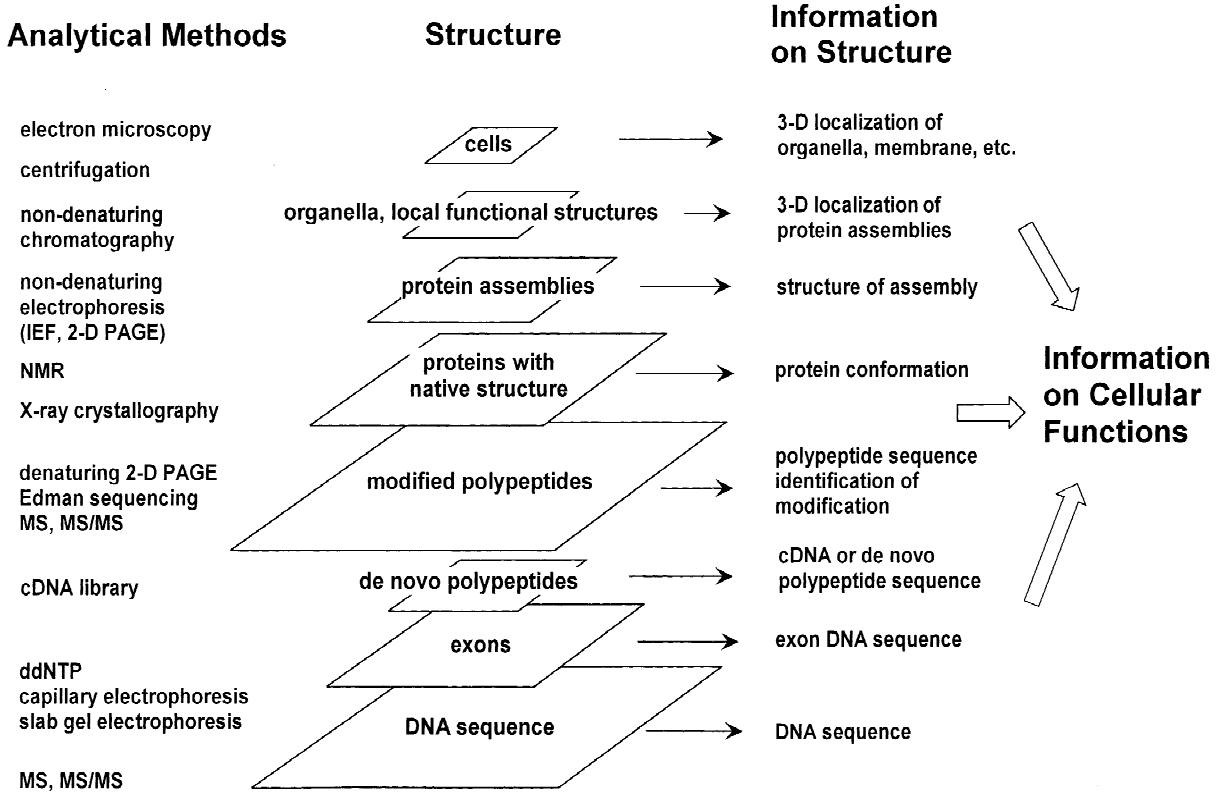


Fig. 1. Structural levels in complex protein systems. The planes show structural levels in complex protein systems, each plane containing information on all the individual molecules or structural units. To obtain complete structural information on one level does not mean the elucidation of the structures on the other levels. Information on protein functions will be obtained from each structural level, although the structures at higher levels are more directly related to the functions of the protein system.

structures of cells and organelles shown as the planes at the top. The planes represent structural levels actually present in the system and they are positioned very close to, but separated from, each other. This indicates that even after we have analyzed all the structures of the constituents in one structural level, we cannot deduce them in another structural level. For example, we already know the total DNA sequence of several biological species, but the structures of exons cannot be predicted from the sequence data alone. Similarly, we might predict the exon regions, then the possible polypeptide amino acid sequences. However, from the information on polypeptide sequences alone we cannot predict how they will be subjected to co- and post-translational modifications, such as phosphorylation, glycosylation, limited proteolysis, etc. Further, the tertiary structures of native proteins, including the location of intra-molecular disulfide bonds between cysteine residues, cannot be predicted from a detailed analysis of the amino acid sequences and the modifications of expressed polypeptides. Even after all the tertiary structures of native proteins have been determined, this information alone will not be enough to deduce the complete structures of protein assemblies, such as those of ribosomes [1].

In this paper, I define proteomic studies as those which focus on the total analysis of protein structures, including all the structural levels shown in Fig. 1, and aim to reconstruct complex protein systems. For the analysis of these levels of structures, various analytical methods are now employed, as shown in the left column of Fig. 1: electron microscopy, ultracentrifugation, NMR spectrometry, X-ray crystallography, non-denaturing chromatographic methods, non-denaturing or denaturing electrophoretic methods, protein sequencers, mass spectrometric methods, etc. Each method has its own advantages in the analysis of one or a few structural levels. Chromatographic and electrophoretic methods under non-denaturing conditions are suitable for the analysis of proteins and protein assemblies. Denaturing 2-D PAGE is suitable for the total analysis of expressed polypeptides and their modification states. Mass spectrometric methods have recently been demonstrated to be quite effective in the identification of polypeptides separated by 2-D PAGE.

At present, the major emphasis in proteomic studies is on the structural analysis of polypeptides, namely the determination of amino acid sequences and the sites of co- and post-translational modifications of all the polypeptides present in a specific protein system. Therefore, in this review article I deal with developments in the methods which aim at a total analysis of polypeptides or proteins. I use the word ‘‘polypeptides’’ to mean linear polymers of amino acids which do not have, or have lost, tertiary structure, and the word ‘‘proteins’’ to mean those which have functional tertiary structures. Most of the current methods for total polypeptide analysis are based on the use of 2-D PAGE, run under denaturing conditions, and I will discuss the advantages and disadvantages of these methods in relation to the aim of proteomic studies. Then I will review in detail methods that have appeared recently which are based on the use of high-resolution (Fourier transform ion cyclotron resonance, FTICR) mass spectrometry. The advantages of FTICR MS-dependent methods over the preceding methods and their possible limitations in obtaining information on functional proteins will be discussed. Finally, I will introduce a combined method of non-denaturing and denaturing 2-D PAGE, which we have proposed to integrate the information on polypeptides with that on functional proteins or on protein assemblies.

2. 2-D PAGE and related methods for proteomic studies

2.1. Characteristics of proteins/polypeptides available for their separation

Proteins are linear polymers of amino acids and the following four structural features distinguish them from each other and can be used to separate them. (1) Charge state; the charge state of a protein is mainly determined by the amino acid composition of charged side-chains. Side-chains of aspartic acid, glutamic acid, lysine, arginine, histidine, and tyrosine, together with the N-terminal amino group and C-terminal carboxyl group, mainly contribute to the charge state. When charged or hydrophilic side-chains or terminal groups are subjected to various modifications, including N-terminal blocking, phosphorylation at serine, threonine, or tyrosine and

glycosylation at aspartic acid, serine, or threonine, changes in the charge state of the protein will occur. (2) Protein size and shape; the size and shape of a protein are uniquely determined by the amino acid sequence, including the partial 3-D structure of the active site or binding site of a protein, although we cannot predict them from sequence data alone. (3) Polypeptide chain length; the polypeptide chain length of a protein can be considered as a structural feature only when the protein is treated with denaturing agents such as a high concentration (8 *M*) of urea in the presence of reducing agents to cleave disulfide bonds. With these treatments, proteins lose their tertiary and secondary structures and the resulting polypeptide chains, without a stable structure, are called random coils. (4) Polypeptide hydrophobicity; it is sometimes convenient to calculate the hydrophobicity of a polypeptide as the sum of partial hydrophobicities of the constituent amino acids. This value can be considered to be the degree of repellency of a polypeptide chain to water or accessibility to organic solvents and increases as the chain length increases.

The structural nature of proteins or polypeptides in relation to the various treatments for their analyses is illustrated in Fig. 2 [2]. Proteins may form an assembly with a quaternary structure and sometimes the structure itself is a prerequisite of the physiological function. With treatments of the assembly under relatively mild conditions, the proteins or protein subunits dissociate, and they may keep their original functions since their tertiary structure or conformation is maintained. Separation under non-denaturing conditions will not destroy the protein assemblies or conformations and the difference in charge, shape, or partial three-dimensional structure is employed for their separation. The separation methods which utilize charge differences are ion-exchange chromatography and isoelectric focusing. Those utilizing size differences are gel permeation chromatography, hydrophobic interaction chromatography, and gel electrophoresis in the absence of denaturing agents. Those utilizing specific structures are affinity chromatography and batch-type methods to detect interactions with the structure. The protein conformation, including the secondary structure, is destroyed in the

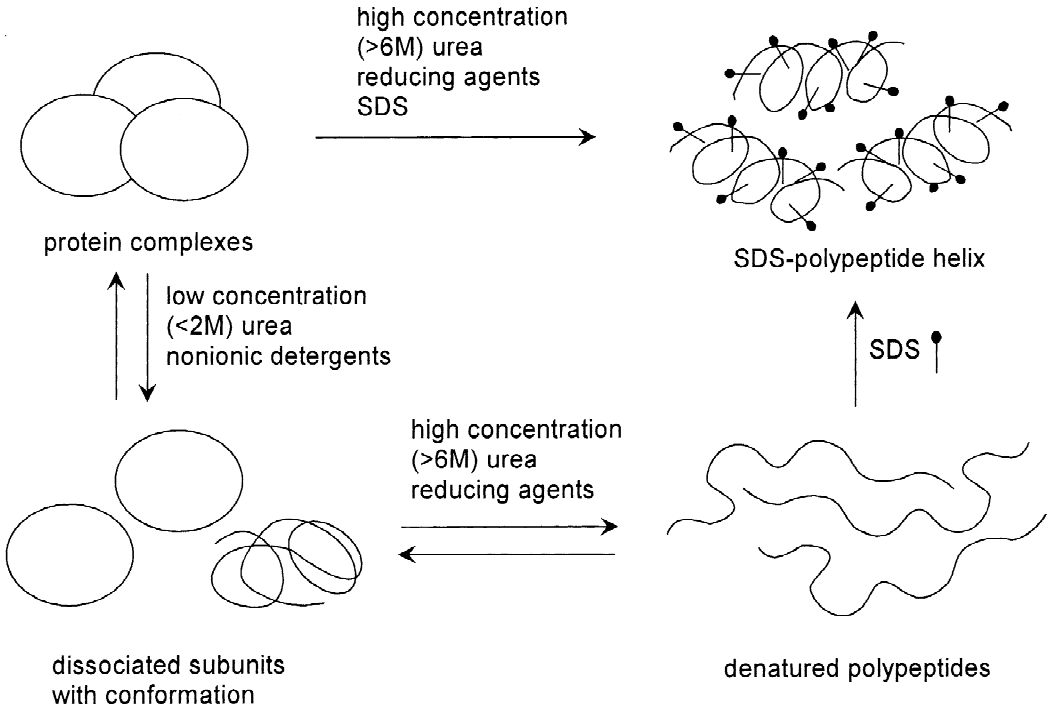


Fig. 2. Changes in protein structure during the course of various treatments.

presence of high concentrations of urea (>8 *M*) or guanidine hydrochloride (>6 *M*), and reducing agents which cleave the disulfide bonds, forming polypeptide random coils. In this state, the polypeptides have charge states which reflect the composition of amino acids with ionizing side chains and can be separated according to the differences in charge, such as by isoelectric focusing. When denatured polypeptides are treated with a short-chain anionic detergent such as SDS, they form complex micelles with the SDS molecules to form SDS–polypeptide helices. Since the shapes and the ratio of acquired negative charge per molecular mass are similar between the helices, they can be separated according to the differences in helix size, which can be correlated with the polypeptide molecular mass by a method such as SDS–gel electrophoresis. Protein assemblies can also be treated simultaneously with urea, a reducing agent, and SDS, to form SDS–polypeptide helices if single-step separation with SDS gel electrophoresis is attempted.

2.2. Denaturing 2-D PAGE and related methods

For proteomic studies, the number of protein molecular species can be above 10 000 for one eukaryotic cell and we have to employ the above structural features to obtain maximal resolution. Ion-exchange chromatography combined with gel permeation chromatography under denaturing conditions or with reversed-phase chromatography can be a candidate 2-D method for the separation of polypeptides. However, the liquid chromatographic methods may have peak capacities ranging from 20 to 40 for proteins and even 2-D separation will separate, at maximum, 1000 protein species. Electrophoresis in a polyacrylamide gel support can eliminate the causes of the deterioration of resolution in liquid chromatography, namely the diffusion of proteins in free liquids and the heterogeneous liquid flow-rate by the presence of solid support particles.

O'Farrell [3] found, using an *E. coli* lysate, that 70 bands could be separated by denaturing gel IEF, which separates random-coiled polypeptides according to the differences in charge state, and 100 bands by SDS–PAGE, which separates SDS–polypeptide helices according to the differences in their size. The combination of the two methods, denaturing 2-D PAGE, will give about 7000 peak capacity. By refinements of electrophoretic techniques in both dimensions, Klose and Kobalz [4] obtained 9551 spots on a 20 cm (IEF)×30 cm (SDS) slab gel and 10 345 spots on a 40 cm (IEF)×30 cm (SDS) gel for mouse testis soluble proteins, and this level of resolution might be the maximum expected for denaturing 2-D PAGE. We can expect the number of polypeptide species present in one stage of the eukaryotic cell cycle to be around 10 000, but since each polypeptide will be subject to modifications, the number of polypeptides to be analyzed should be multiplied by the average number of possible modification states. Therefore, the resolution of 2-D PAGE may still not be sufficient for the total analysis of complete polypeptides, especially when the protein system is eukaryotic cells or organs. Actually, the number of species at this structural level might be the largest, as illustrated by the large plane in Fig. 1.

Polypeptides separated by denaturing 2-D PAGE can be subjected to various methods for detection, quantitation, and characterization [5]. Detection methods, such as dye staining, silver staining and autoradiography, provide information on (1) the number of species, (2) isoelectric points, and (3) the apparent molecular mass as SDS complexes, for polypeptides. Quantitation methods, such as image analysis, provide information on the changes in quantity when multiple gels obtained from different sample stages are compared. Polypeptides are identified immunochemically after blotting them onto fibrous hydrophobic supports such as nitrocellulose or poly(vinylidenedifluoride) (PVDF) membranes and treating them with specific antibodies labeled with color-developing or radioactive tags. However, in principle, this method requires one specific antibody for each polypeptide. More specifically, polypeptides are characterized by determining partial amino acid sequences. In the case of chemical sequencing, polypeptides on a 2-D PAGE gel are first blotted onto a PVDF membrane and the spot areas on the membrane are cut out and applied to a sequencer one by one. The minimal quantity of polypeptides for reliable sequencing is about 0.1 μg for polypeptides with an apparent molecular mass of 50 000 (2 pmol), which means the spots must be those which are obviously stained with Coomassie

Brilliant Blue. Mass spectrometry is now commonly used for polypeptide identification after separation by 2-D PAGE. The stained spots on the gel are cut out and equilibrated in a trypsin-containing buffer. The trypsin digests are extracted from the gel and subjected to matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI–TOF-MS) for identification by peptide mass fingerprinting [6–10] and post-source decay [11,12] or electrospray ionization tandem mass spectrometry (ESI-MS–MS) for amino acid sequencing by the mass-tag method [13–15]. The quantities of polypeptides required for identification by MS methods can be smaller than those needed for chemical sequencing and the constant improvements in the resolution of MS apparatus further reduces the required quantity.

2.3. Non-denaturing 2-D PAGE and chromatographic methods

Gel isoelectric focusing followed by size-separation gel electrophoresis, both run in the absence of denaturing agents (non-denaturing 2-D PAGE), gives high resolution of proteins which retain biological activity [16,17]. Enzyme activities can be detected by immersing the gel just after electrophoresis (without fixing) in a solution containing specific substrates, a buffer to maintain the optimum pH, and ions necessary for the activity [18,19]. All the methods used for the detection, quantitation and characterization of polypeptides on denaturing 2-D gels can be used for the detection of proteins on non-denaturing gels. Immunochemical identification on the blots is effective since antibodies are more specific to native proteins than denatured polypeptides [20]. Amino acid sequencing of proteins separated on non-denaturing 2-D gels has rarely been attempted, since a chemical sequencer requires a single N-terminal amino acid, which means a single polypeptide, in a stained spot. However, mass spectrometry enables amino acid sequencing of multiple trypsin peptides without separating them. This means that a protein composed of multiple polypeptides or a mixture of such proteins can be identified to be present in one spot [21,22]. This will enhance the use of non-denaturing 2-D PAGE in studying proteins in relation to their function.

Similarly, liquid chromatographic methods, which have been assumed to be of low resolution and not suitable for purification and structural analysis of proteins, must be re-evaluated, combining them with high-resolution mass spectrometry. The chromatographic methods have the advantage that they can be automated, and tandem column (2-D) chromatography can also be automated [23,24]. In contrast, the automation of a gel electrophoretic method is quite difficult because of the use of a gel support. When one attempts to run 2-D PAGE and identify the proteins or polypeptides on the gel, at least 20 steps are needed to accompany manual handling. Although we require high-throughput methods for the total analysis of complex protein systems to obtain information on each of the structure levels shown in Fig. 1, most researchers are using 2-D PAGE and related techniques at present.

3. Use of FTICR MS for proteomic studies

Mass spectrometry has become one of the major techniques for the structural analysis of polypeptides in the last 10 years. The reasons for this are the improvements in the resolution of mass spectrometers, together with the refinements in techniques of protein ionization (MALDI and ESI). Once enzyme-digested peptides could be effectively ejected in air as floating ions, and once the mass accuracy obtained by the apparatus reached a certain level, it became possible to identify peptides by comparing the mass values of the fragment peptides with those of the predicted peptides. Further, the high mass accuracy enabled the determination of the sequence of unknown peptides. The high mass accuracy and high throughput which can be expected from FTICR mass spectrometers may similarly result in a dramatic change in the methods of analysis and characterization of polypeptides and proteins. Several attempts at the application of this method for proteomic studies are briefly summarized below.

3.1. Capillary isoelectric focusing followed by FTICR MS

Mass spectrometry is a high-resolution method for the separation of proteins and polypeptides according to their differences in molecular mass, and a candi-

date method to be combined with it for the high-throughput analysis of complex protein systems is capillary isoelectric focusing (CIEF) [25–27]. Since a gel support is not required, CIEF can be automated and has a comparable, or even higher, resolution of proteins than isoelectric focusing in column gel or slab gel format [28]. In order to combine it with CIEF, the method of protein/polypeptide ionization for mass spectrometry must be ESI, which enables on-line analysis of the charge-separated molecules [29]. FTICR MS is the MS technique of choice for combination with CIEF and ESI [30–35] because of its high resolution and high sensitivity in peptide MS analysis.

On the ion cyclotron resonance (ICR) principle, the ions produced by the various ionization methods, including electrospray, are stored in an homogeneous magnetic field where they move in circular orbits with a frequency (cyclotron frequency) determined by the product of the magnetic field strength and the mass-to-charge ratio of the ions. When ions are exposed to an oscillating electric field which has the same frequency as the specific cyclotron frequency, they are coherently accelerated to a larger radius of gyration, whereas ions having a different cyclotron frequency are not accelerated. When the ions are stored, the cyclotron motion of the ions induces an alternating current in an external circuit which has the same frequency as the cyclotron frequency, and the amplitude is proportional to the number of ions. A sinusoidal pulse of an electric field with a frequency linearly swept across the entire range of cyclotron frequencies of interest is applied, the pulse is turned off, and the induced current is stored. These steps are repeated, the data accumulated to improve the signal-to-noise ratio, and then Fourier transform of the transient signal yields the mass spectrum.

The advantages of CIEF coupled on-line with ESI-FTICR MS were fully demonstrated by the analysis of cell lysates harvested from *E. coli* cultured in normal and isotopically depleted media [31]. This method enabled the detection of about 900 proteins with unique putative protein masses plotted as scan number (can be correlated with p*I*) versus molecular mass to obtain a 2-D display pattern. The 2-D pattern must be able to be correlated with the patterns obtained by non-denaturing 2-D PAGE since both show the pattern of proteins, not polypeptides. The detected proteins were in the mass range 3–60 kDa, because a dual microdialysis technique [36] was employed for on-line sample pretreatment which utilizes two cut-off membranes of 8 and 300 kDa. Since the authors of that paper [31] compared their 2-D pattern with that of denaturing 2-D PAGE, the correlation between them was not so obvious. In the case of non-denaturing 2-D PAGE, the mass values obtained from the 2-D patterns must be apparent values since the mobility in the size-separation dimension is affected by the differences in the net charge of the proteins at the pH of the separation buffer. The mass values may deviate from about 5% (for proteins which have p*I* values at least two pH units apart from the pH of the buffer) to more than 100% (for proteins with p*I* values close to the buffer pH) [17]. In FTICR MS separation, the protein mass can be obtained at 0.001% error. Culturing the cells in isotope-depleted (~99.95% ^{12}C, ~99.99% ^{14}N, and >99.995% ^{1}H) media can also help to obtain a high resolution of the protein mass [30]. High resolution was obtained by loading ~300 ng protein in a CIEF capillary, which is about the maximum quantity that can be loaded into a capillary with a liquid volume of about 1 μL [26], whereas, in 2-D PAGE, normally 200 μg to 1 mg protein can be loaded. In spite of the advantages of this method, it should be noted that the number of proteins which can be analyzed by MS–MS to obtain partial sequence information is limited because the increased time required for the sequencing procedure may allow less frequent data acquisition and, consequently, reduce the effective resolution of CIEF.

3.2. Capillary liquid chromatography followed by FTICR MS

The CIEF–ESI-FTICR MS analysis was further extended to capillary liquid chromatography–ESI-FTICR MS [37–40]. The use of tandem FTICR MS seems to be particularly promising for high-throughput peptide identification, because partial sequence information can be obtained [40]. Fused-silica capillary columns (30–60 cm, 150 μm I.D.×360 μm O.D.) packed with 5 μm C_{18} particles were employed. Cellular soluble proteins from *D. radiodurans* cells were denatured by the addition of guanidine hydrochloride (6 *M*) and DTT (1 m*M*)

followed by boiling for 5 min, and digested with trypsin. Although the quantity of protein applied on the column was not reported, it can be up to 50 μg, since a sample volume of 10 μL was injected onto the RPLC column. The quantity is much larger than that applied on the CIEF capillary, and this enabled MS–MS analysis of the peptides. Capillary LC–FTICR analysis of the tryptic peptides of a *D. radiodurans* cell lysate provided a 2-D map consisting of over 13 000 peptide ''spots'', which were drawn based on their molecular mass and LC elution time, in a single run. FTICR MS–MS enabled the analysis of multiple peptides included in a single peak in the RPLC elution pattern. Therefore, the high throughput of this method is in contrast to 2-D PAGE-dependent MS analysis, in which MS–MS data acquisition is, in principle, spot by spot. However, it should be noted that the 2-D map obtained by capillary RPLC–FTICR MS is a trypsin-peptide map listing the structures of the polypeptides present in the lysate. In the list, polypeptides are arranged mainly according to the differences in their hydrophobicity. Therefore, although the purpose of this approach is similar to that of denaturing 2-D PAGE and we can expect high-throughput protein identification, it is quite difficult to correlate the 2-D map with that obtained by denaturing 2-D PAGE. The latter separates polypeptides according to differences in their isoelectric points and apparent molecular masses. Also, the 2-D map cannot be correlated with the map obtained by CIEF–FTICR MS since the latter shows the distribution of proteins, not of polypeptides.

4. Correlation of physiological functions with protein/peptide structure

As shown in Section 3, FTICR MS combined with liquid-phase separation techniques provides high-throughput and highly sensitive methods for the structural analysis of polypeptides and proteins. The total list of polypeptides and proteins which are actually present at a certain stage of the cell cycle will provide insights into the functions of proteins, like that provided by the total DNA sequencing of biological species. However, considering that our final aim is to reconstruct the structure and function of complex systems, there are various ways to correlate the functions with the various levels of structure shown in Fig. 1. As illustrated in Fig. 3, several polypeptides which have undergone post- or co-translational modifications may compose a functional protein, several functional proteins may compose a protein assembly which shows a concerted function as a whole, and several protein assemblies may compose a local structure in a cell. In studying the process to construct complex structures from relatively simple structures, we need to separate the functional units preferably according to their differences in function. This might be the reason why 2-D PAGE is so widely used in spite of the many manual procedures included in the method; it separates polypeptides and proteins according to their isoelectric point and apparent molecular size, which may be related to their functions. These values can be used to correlate the works on proteins or polypeptides carried out in various laboratories and the data can be accumulated as polypeptide/protein databases. However, there have been quite a few attempts to correlate the separation pattern of polypeptides with that of proteins for a complex protein system.

Recently, we proposed a method to correlate the information on polypeptides with that of proteins, comparing 2-D PAGE patterns obtained by stepwise changes in the separation conditions [41] using human plasma as a model protein system. As shown in Fig. 4, the dissociation or association steps can be visualized for multiple proteins on the 2-D patterns by comparing the four types of 2-D PAGE patterns. Since we have established a micro-2-D PAGE system which enables a simultaneous run of 20–30 micro-2-D gels (38 mm×38 mm×1 mm) within 5 h [42,43], comparative studies are quite feasible. In a non-denaturing 2-D pattern (a type I 2-D pattern), proteins keep their function, as their enzyme activities can be detected on the gel [19,20], and also assembled structures such as haptoglobin polymers can be observed. Employing the same conditions of non-denaturing IEF in the first dimension, when an IEF gel column was equilibrated with a SDS solution and then SDS–PAGE was run (type II 2-D), protein assemblies with non-covalent interactions dissociated, e.g. high density lipoproteins (HDL) dissociated into apolipoproteins A-I, A-II, and C. When an IEF gel was run under the same conditions and

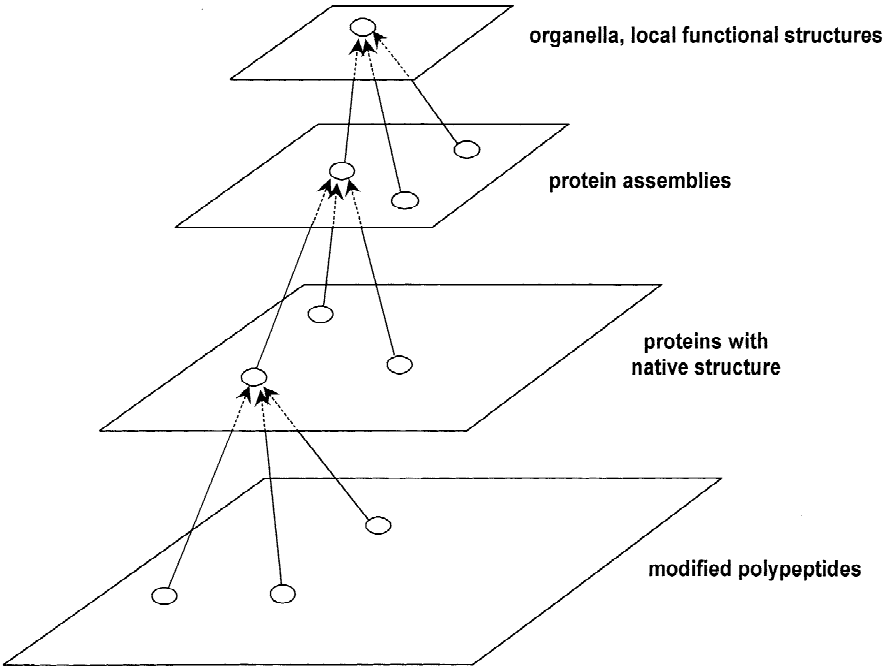
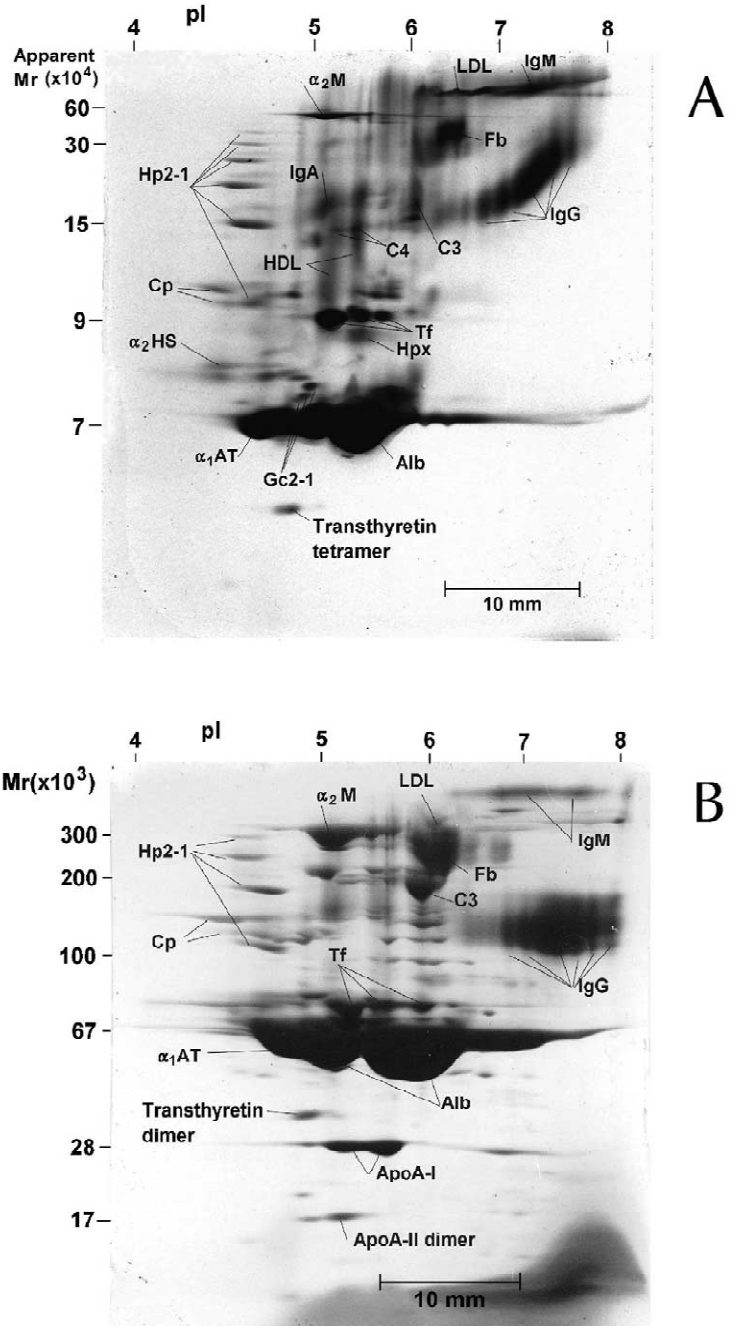


Fig. 3. Correlation of protein data between different structural levels. The data on each structural level, shown as a plane in the figure, must be correlated with those on other structural levels to analyze the functions of proteins, protein assemblies, or higher structural units.

equilibrated with urea (8 *M*)–reducing agent–SDS, then SDS–PAGE was run (type III 2-D), the proteins dissociated into their constituent polypeptides, although each polypeptide resides at the same p*I* as the original protein and this makes the analysis of the dissociation process easy. The information obtained on polypeptides by type III 2-D can be correlated with that obtained by denaturing 2-D PAGE (type IV 2-D), which is commonly used and for which various structural data on polypeptides are available in relation to their locations on the denaturing 2-D map.

The above example shows one approach to correlate the information on functional proteins with that on polypeptides and we need similar approaches to correlate the structures and functions of proteins and protein assemblies with those of organelles. The protein assemblies or organelles are better separated with their functions retained, therefore the separation methods need to be function-oriented, namely each fraction separated must exhibit a specific function. Two-dimensional separation in solution, employing differences in charge and size, appears to be promis-

Fig. 4. 2-D PAGE patterns of human plasma proteins separated using four different conditions (reprinted from Ref. [41]). (A) Non-denaturing (type I) 2-D PAGE; (B) non-denaturing/SDS (type II) 2-D PAGE; (C) non-denaturing/reduced/SDS (type III) 2-D PAGE; (D) denaturing (type IV) 2-D PAGE. A sample of normal human plasma was subjected to IEF in the absence of denaturants (types I–III). In type I 2-D, the IEF gel (35 mm long×1.3 mm I.D.) was set on a micro slab gel (38 mm×38 mm×1 mm) of polyacrylamide pore gradient (4.2–17.85% T linear gradient, 5% C), and the second dimension electrophoresis was run in the absence of denaturants. In type II, the IEF gel was equilibrated with a buffer which contained 2% SDS, then the second dimension electrophoresis was run in the presence of 0.1% SDS. The size and the acrylamide gradient of the slab gel were the same as in Fig. 1. In type III, the IEF gel was equilibrated with a buffer which contained 8 *M* urea–5% mercaptoethanol–2% SDS, then the second dimension electrophoresis was run in the presence of 0.1% SDS. In type IV, the plasma sample was treated with SDS–mercaptoethanol and subjected to IEF in the presence of 8 *M* urea and 1% NP-40, the gel was equilibrated with a buffer which contained 8 *M* urea–5% mercaptoethanol–2% SDS, then the second dimension electrophoresis was run in the presence of 0.1% SDS. For abbreviations, refer to Ref. [41].


pI
4
5
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8
Apparent
Mr (x10^4)
60
30
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9
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A
α2M
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IgA
IgG
C4
C3
HDL
Cp
Tf
Hpx
α2HS
α1AT
Gc2-1
Alb
Transthyretin
tetramer
10 mm
pI
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7
8
Mr(x10^3)
300
200
100
67
28
17
B
α2 M
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Fig. 4.

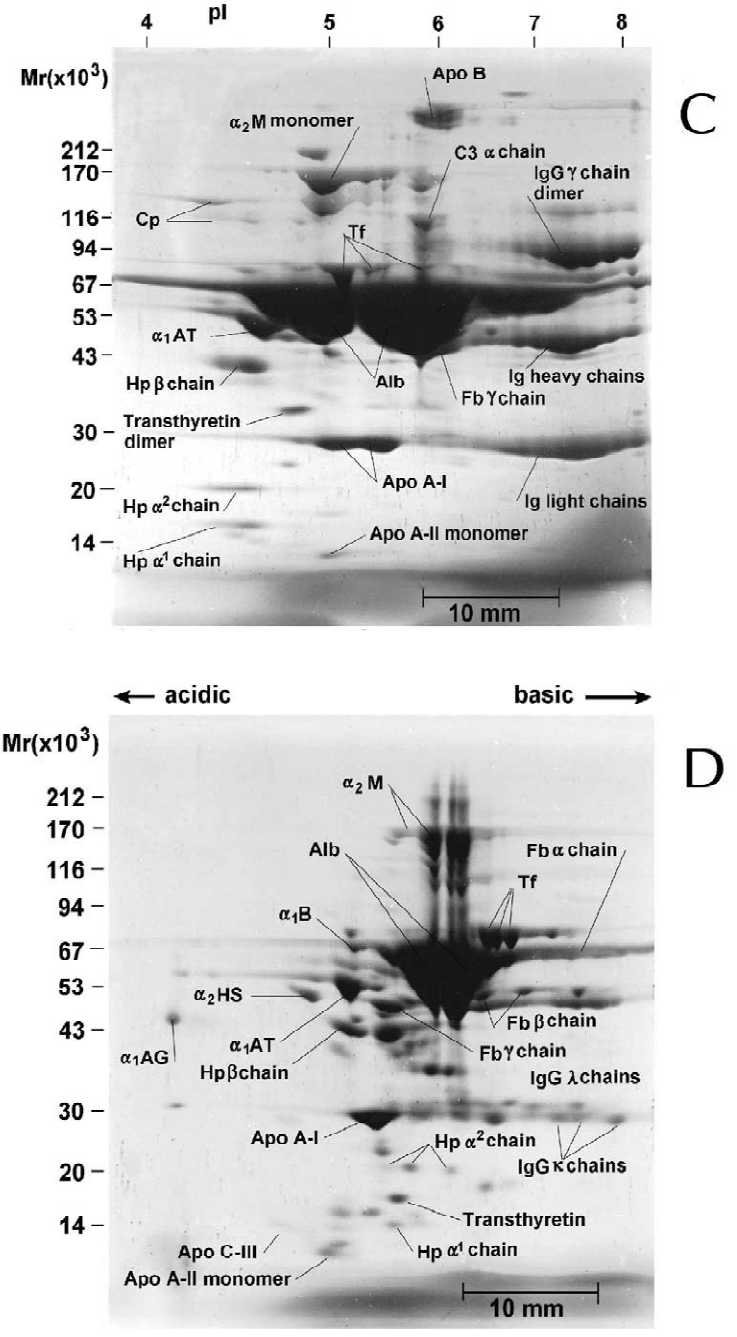

Fig. 4. (*continued*)

ing for the development of methods to separate protein assemblies and higher-order structural units of proteins.

5. Conclusions

FTICR MS combined with CIEF or capillary LC is a high-throughput and highly sensitive method for proteomic studies since a single run will give information on p*I* values and accurate masses of 1000 proteins in a cell lysate or structural information on 10 000 tryptic peptides from the lysate. However, 2-D PAGE will remain in use for proteomic studies since it can detect the functions of multiple proteins on the gel. With the aim of proteomic studies being the reconstruction of the structures and functions of complex protein systems, it is important to establish methods for the total separation of protein assemblies and higher-order structural units of proteins.

6. Nomenclature

2-D PAGE	two-dimensional polyacrylamide gel electrophoresis
CIEF	capillary isoelectric focusing
FTICR	Fourier transform ion cyclotron resonance
MS	mass spectrometry

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