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PROTEIN NANOTECHNOLOGY - A POWERFUL FUTURISTIC DIAGNOSTIC TECHNIQUE

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ABSTRACT

Healthcare can be maintained well, when diagnosis is quick, accurate, cost -effective and painless. DNA and RNA based diagnosis may not reveal the right information for certain diseases. Identification and quantification of proteins and their folding mechanism are very important in diagnosis of diseases. Small quantities of proteins, which generally escape from detection and are responsible for the diseases, now can be quantified by protein nanotechniques which aids in the diagnosis. In this review, we have summarized the recent developments in nanotechnologies such as protein microarrays, biosensors etc. and their application in diagnosis of diseases at proteomics level have also been discussed.

KEY WORDS

Biosensors, Diagnosis, Folding, microarrays, Protein estimation.

INTRODUCTION

Healthcare can be more effective if the diagnosis is rapid, accurate and sensitive. More than 1,200 genetic disorders have been identified. Most of us carry a few defective genes with no signs of disease and many of these can only contribute to susceptibility. Molecular basis of a vast majority of these diseases is not yet clear (5, 19). Since 1989, when first successful attempt was made to diagnose genetic defects before embryo implantation in human, this diagnostic technique gained lot of importance (24). After the discovery of complete genome of Mycoplasma genitalium, 8% error rate in the annotation for 340 genes was found (3). If such error rates are extrapolated to the human genome, the outcome and consequences can easily be imagined. In order to avoid such errors, verification of the gene products with protein-based methods is very essential. Generally, genetic defects are tested for by PCR, allows amplification of a selected DNA sequence of interest (25) and chromosomal abnormalities by FISH techniques (9, 10).

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Nevertheless, certain diseases are not developed due to defect in genes, for example, prion diseases. Thus it is clear that DNA based diagnosis does not always give accurate information, though it is very sensitive. RNA based diagnosis has also been tried for analysis of certain diseases to check the defective gene expression status of mRNA and compared with normal sample using a powerful technique, reverse transcriptase polymerase chain reaction (RT-PCR), when defect is not at gene level. However, it has also some limitations that RNA can not be amplified directly, but converted to cDNA using reverse transcriptase enzyme. More so, mRNA analysis will not be possible in areas where samples do not contain mRNA such as some body fluids (end products).

Beadle and Tatum in 1940 gave one gene- one polypeptide hypothesis which was disproved later; as shown that certain genes may result in dozens of proteins. These may be produced either in very minute quantity with a very short half-life, fragmented, chemically modified or the fragments of different genes are rearranged. Such modifications are going to be key elements to understand functional activity of proteins. Furthermore, protein-protein interaction, protein cross-linking and post-translation modification of proteins can not be studied through genomics. Therefore, in such conditions gene analysis is not useful in clinical diagnosis. Moreover, proteins lack DNA's copying ability. Thus separation and fractionation of proteins become more difficult,

especially when present in small quantities. It explains that, there is no PCR for proteins for those trying to unravel the proteome of human and in such conditions, one of the most crucial steps in protein study is protein study is obtaining and handling the protein sample.

Though protein based diagnoses are in vogue, the estimation of proteins are not yet sensitive enough to detect minute quantities present in tissues and/ or biological fluids. Therefore, improvements in protein detection techniques would help in diagnosing diseases with accuracy and sensitivity. Hence, developments in protein nanotechnologies, which have been carried out in recent years, are reviewed here. With the aid of nanotechnology this problem can be overcome and will be of a great promise for the human health and medicine in near future. The complexity of disease from both the diagnosis and treatment point of view will be easier even if the protein is present in zeptogram quantity with the advanced protein based nanotechnology methods.

Protein identification at nanomolar concentration

Reading the genome is the easiest part however, understanding the molecular organization of proteins require new tools and concepts which may be possible with the study of proteomics only. The complete sequence of genome is not sufficient to interpret the biological function of proteins. Proteins. the active agents of a cell are gene products and directly contribute to the drug development as almost all drugs are directed against proteins, except a few which interfere in DNA replication in cancer cells and RNA in AIDS virus multiplication.

a) Spectroscopy

It is very important to know the protein concentration in a biological sample before studying its functional activity. The accurate quantification of low abundance protein is a biggest challenge, which has been overcome by nanotechnology. Various methods exist, which measure the protein concentration up to nanogram quantity and even

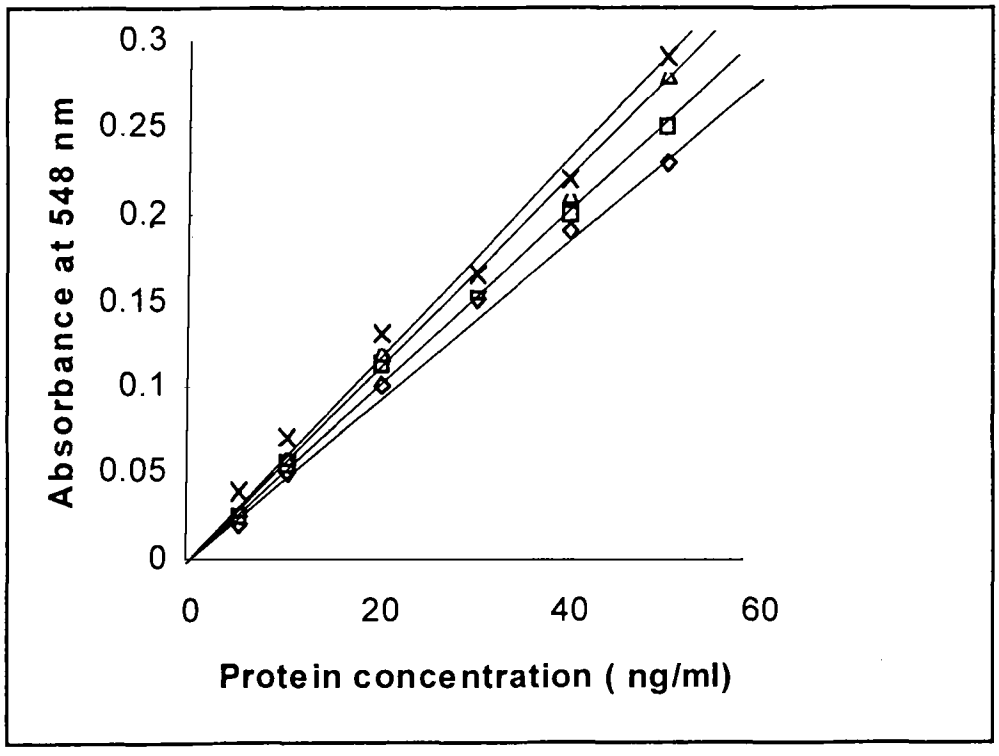

Fig. 1. Standard curve for 5-50 ng/ml of BSA (x),lysozyme ( ), creatine kinase (à) and catalase(D)

Different aliquots of BSA sample were taken in the concentration range of 5-50 ng/ml and made 0.1 ml with 0.15 M NaCl. To this 0.1ml of 0.025%citric acid and 0.01% eosin Y dye reagent were added .The absorbance was recorded at 548 nm by incubating at room temperature for 15 min against reagent blank, which contain 0.1ml of 0.15 M NaCl instead of protein. Similar experiments were followed for lysozyme , creatine kinase and catalase proteins also. These curves give straight curve up to 50 ng/ml concentration.

less. NanoOrange reagent method (22), binding of silver particles to gluteraldehyde treated proteins (17), ELISA, radioimmunoassay, immunofluorescence detection methods, flurometric assay are the methods by which proteins can be quantified in nanogram quantity, however they are multi-step, complicated and time-consuming methods. Waheed and Gupta (27, 28), developed a single-step method for estimating nanogram quantity of protein by spectrophotometer using eosin Y dye which is simple, accurate, sensitive (measures the protein concentration up to 2 ng), stable and less time-consuming (Fig. 1).

Using this method protein concentration has been estimated at nanogram level in various tissues and biological fluids as shown in (Table 1).

Later this technique was applied with suitable modifications for the estimation of collagen in our laboratory. This technique can be used in collagen-regulated diseases for accurate quantification of collagen.

Table 1. Estimation of protein in tissue homogenate and body fluids by the eosin Y method

Sr.No.	Tissues/ body fluids	Concentration (ng/µl)
1	Lens epithelial cells	1400
2	Lens cortex	2050
3	Lens capsule	70*
4	Serum	5000
5.	Ascitic fluid	3000
6.	Pleura fluid	3500

- All tissues were homogenized in extraction buffer (containing 0.1 M Tris-HCl, 0.1 M NaCl, 1mM EDTA and 2 mM β-mercaptoethanol). 5 µl of homogenates were taken and the proteins were analysed by the standard assay of eosin Y.
- Serum samples were centrifuged at 5000 x g for 20 min to remove cells and other contaminants. 1 µl of 1:10 diluted serum was used for the estimation.
- Protein values were expressed in µg/µl present in homogenate, however the measurements were done at higher sensitivity (0.2-2 µg/ml) and therefore protein concentration was measured more accurately.
- Values represented here are the average of triplicates and were calculated using BSA standard.

b) Protein Microarrays/Chips

The central tools for protein identification and characterization between healthy and diseased samples are 2-D gel electrophoresis and highly sensitive mass-spectrometric methods (sensitivity to femtomole-attomole protein concentration). In addition to the above methods, protein microarray offer a powerful tool for screening thousands of proteins at a time, where variety of proteins such as antibodies and enzymes are immobilized in an array format on glass slide by robots. The surface of the glass slide is then probed with sample of interest that binds to relevant antibodies on chip which will be analysed by relevant detection method on array (23). Refinement in microarrays miniaturization (with the advent of nanotechnology) will further contribute to molecular diagnostics and the development of personalized medicine (14). Profiling proteins on arrays will be used in distinguishing the proteins of normal cells from early stage cancer cells and malignant metastatic cancer cells (18). It is also applicable to study the protein-protein interaction, the functional activity of proteins and in the discovery of disease markers and diagnosis (12, 13). It can also be used for profiling of the serum to differentiate patients with pancreatic cancer from those with other pancreatic diseases and from healthy control (16). Heart failure, arising from systemic disease or specific heart muscle disease, is one of the leading causes of morbidity and mortality in developing countries (4). Proteomics based approach in characterizing overall changes in protein expression in heart disdeases may provide new insights into the cellular involvement of interactions. Replacement of growing blood vessels in heart attack will be an addition to nanotechnology. The endothelial cells harvested in silicon molds shapes like capillaries; these cultivars would be delivered to the heart via microscopic machines called 'Angiochips' which will repair the damage caused by heart attack (20). Thus, protein microarrays offer the possibility of developing a rapid global analysis of the entire proteome, leading to protein-based diagnostics and therapeutics. It has been successfully applied to the study of complex trait i.e. cardiovascular diseases, cancer and type II diabetes.

Recent development in microarrays has come with technique called "Immunosensing", where patterned microarrays of antibodies to specific bacteria were used to perform a series of bacterial immunoassay and characterized using scanning probe microscopy (11).

c) Nanobiosensors

Nanobiosensor is a probe that integrates a biological component, such as a whole bacterium or a biological product (e.g., an enzyme or antibody) with an electronic component to yield a measurable signal. The nanoprobes are fabricated with optical fibers pulled down to tips with distal ends having sizes of approximately 30-50 nm. The nanoscale size of these sensors allows for measurements at single cell level. The fiber optic nanoprobes are covalently bound with antibodies that are selective to target analyte molecules. Excitation light is launched into the fiber and the resulting transient field at the tip of the fiber is used to excite target molecules bound to the antibody molecules. The florescence emission from the analyte molecule is then connected to a microscope. Aging diseases such as diabetes and Alzheimer's, and chemical warfare agents cause changes in metal ion concentrations in the body. These changes can be detected and measured by nanobiosensors, and may help in availing information about changes in disease states and exposure to toxins. A new type of biosensor has been developed, that can detect the macromolecules in nanomolar concentration (21). Application of fiber optic based nanobiosensors for *in vivo* measurement of carcinogen benzo (a) pyronine can potentially provide both quantification and identification of protein analyte at single molecule level (15).

d) Microfluidic technology

Microfluidic technology depends on the special behaviour of fluids flowing in channels with diameters equivalent to that of a human hair. This uses the inherent properties of liquid and gases at the microscale, combined with semiconductor with their compact size, disposable nature, increased utility and use of less concentration of sample have shown a real potential for efficiency in drug delivery and diagnostics (1). It has been also used for the analysis of protein. The development of microfluidics offers an alternative for conventional protein analysis technique such as SDS-PGE and colorimetric protein quantitation methods (2). Microfluidics allows active control of fluids in microfabricated channel with a few micrometers in dimension without any interference. Recently, the first commercial microfluidic system has been introduced named bioanalyser, which allows rapid and automated separation of proteins, integrating multiple experimental procedures such as, sample handling, separation, staining, destaining, detection and data analysis. The system provides protein identification by on-line protein digestion and analysis of digested proteins using electrospray ionization mass spectrometry. Thus, microfluidic system enables rapid identification of proteins in minutes instead of hours, consumes very little sample (nanogram or less) and provides on-line interface with upstream protein separation schemes for the analysis of complex mixtures such as cell lysates (8). The novel use of microfluidic technology at a cellular level includes the handling of mammalian embryo, the manipulation of embryos and oocytes in assisted reproduction and isolation of motile spermatozoa. It is also applicable for gene expression and differentiated display analysis.

Protein folding and Nanotechnology

Since proteins play such a fundamental role in biology, scientists have sequenced the human genome "a blue print for these proteins" which tells us very little about what the protein does and how it does. Before the protein become functional, it assembles in a specific manner for specific function. This self-assembly is called folding. Sometimes proteins will fold into a wrong shape, called as misfolded proteins, like unwanted proteins or degraded by ubiquitin pathway. However, if they are accumulated, they become causative factors for many diseases. Deposit of large amounts of a single, insoluble protein around the degenerative nerve cells of Alzheimer's diseases eventually provided an answer to understand the protein misfolding diseases. Familial amyloidotic polyneuropathy (FAP) and mad cow diseases also derive from the accumulation of toxic, insoluble junk due to the protein misfolding. The most common hereditary disease-cystic fibrosis is caused due to the dissociation of the transport regulator protein from one of its chaperons. Cataract is also considered as crystalline misfolding disease and there is no medical treatment available for this disease except surgical extraction. In all these aspects, key is to understand the mystery of misfolding which is possible with the help of protein nanocrystallography and to find a small molecule, drug that can either stabilize the normally folded structure or disrupt the pathway that leads to a misfolded protein.

Future directions

From nanotechnology it is only one step to nanomedicine, which may be defined as the monitoring, repair, construction and control of human biological systems at the nanolevel using nanodevices and nanostructures. Proper diagnosis and efficient delivery of drugs are very necessary in the medical fields where nanosize materials have practical implementations. Such nanotools still await construction and at present, they are in the progress. Nevertheless, they might be very helpful

and become a really diagnostic tool in the near future. The use of nanodevices such as the artificial RBCs-respirocytes (6, 7) will definitely solve the problem of thalassemia patients, which has become a curse to these people. The other important application of nanotechnology is the design of nanorobots (26), which can enter inside the body and repair the tissue. Thus, the use of nanotools and nanomachines will be possible not only for laboratory experiment but also for the health point of view in the near future.

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