Utilizing Protein Purification Techniques to Characterize Protein Structure and Function

N Varma, A Arbab

Citation

Abstract

Protein characterization is crucial for understanding the role played by proteins in different cellular processes. In addition, protein characterization is vital in biopharmaceutical industry, wherein proteins are produced in different extraneous hosts such as bacteria, yeast and mammalian cells. Recently, wide range of protein purification techniques are made available that can be utilized to solve protein structure and function. In this short review we like to highlight some of recent developments in utilizing protein purification techniques to characterize structures and functions.

INTRODUCTION

Proteins play an important role in living systems due to their unique structures that are large, flexible and complex (Schellekens 2004, Kumar et. al, 2002). Proteins can perform important functions such as transporting oxygen, perform enzymatic activities and in general act as work-horse of the cells. Therefore, characterizing proteins by understanding the relationship between protein structure and its function is crucial for determining the key pathways of cell signal processes that can lead to understand the disease mechanisms. In addition, protein characterization techniques are also in great demand in the biopharmaceutical industry because of the advent of biogenerics.

The advances in DNA sequencing technology have generated a rapid genome sequence for a wide verity of organisms. However, understanding proteome is still at a nascent stage. Unlike genome, proteome consists of complex dynamic interaction of proteins and therefore it is essential to understand how proteins function. It has been well demonstrated that protein structure plays an important role in its function. Although there has been great progress in predicting protein structures by using in vitro and in silico models, the next logical step is to develop models that can predict functions. However, understanding protein structure-function relationship has been difficult due to the lack of protein characterization tools.

Until now it is believed that 'process is product' (Rader, 2005, Herrera 2003) and if the process is different then it is considered that the product is also different. If existing or future techniques can quantify minor structural changes, analytic methods can be developed to determine the extent of similarity between two products. However, the existing techniques that are being used to determine the structural changes are expensive and time consuming. With the rising health care costs (Bodenhelmer, 2005), there is great push to develop biogenerics.

Currently, analytical techniques such as NMR (nuclear magnetic resonance), CD (circular dichroism), gel electrophoresis and mass spectrometry are used to characterize proteins (Chirino et al., 2004). However, protein purification methods such as IMAC (Immobilized metal affinity chromatography) and IEC (Ion exchange chromatography) have been used to decipher the relationship between protein structure and its function. This article is a short review of these emerging purification techniques that complement the existing analytical tools to characterize protein structure and also evaluate its effects on protein functions.

OVER VIEW OF PROTEIN PURIFICATION TECHNIQUES

Depending on the type of protein-resin interactions, there are four types of chromatographic techniques. These techniques are ion exchange, affinity, size exclusion and hydrophobic chromatography. Though all four chromatographic techniques are used in the purification process, affinity and ion exchange chromatography are by far the widely used techniques in the biopharmaceutical industry and in

academia.

Affinity chromatogrphy is based on the principle of protein recognition. The specialized groups/moieties on resin such as antibodies and metal ions recognize the moieties/epitopes on protein. In biopharmaceutical industry, antigen antibody based affinity chromatography (e.g. protein A columns) is widely used due to their specificity. On the other hand metal-based affinity chromatography is widely used in academia due to its versatility of recognizing proteins with surface histidine/cysteiene residues.

Immobilized metal affinity chromatography (IMAC): Porath and co-workers (Porath et al. 1975) developed a metal-based affinity chromatographic technique. This technique is based on the principle of coordinate covalent bonding chemistry. Certain transition metals such as Ni^{2+}, Cu^{2+}, Zn^{2+}, and Co^{2+} can form stable complexes with certain amino acids that are electron donors (e.g. histidine, cysteines). In an IMAC column the metal is chelated to a chelating agent such as IDA (iminodiacetic acid), which is in turn immobilized to the back-bone of a chromatographic resin. When the protein is loaded on the IMAC column with a chelated metal, the surface amino acids form a coordinate covalent bond with the amino acids, thus bind to the column. The bound protein can be eluted by lowering the pH or with a buffer containing a competitor such as imidazole for histidine residues.

Ion exchange chromatography (IEC): IEC is based on the principle of charge-charge interaction, wherein similar charges repel and opposite charges attract. The protein migration in an IEC column is dependent on the interaction between the protein net charge and the charge of resin. The net charge of the protein is primarily dependent on the protein's isoelectric point (pI). In IEC, the whole protein surface with its charge moieties modulates the protein binding process. However, there are certain regions that primarily act as the contact regions and play an important role in protein binding mechanism in IEC.

IMAC IN CHARACTERIZING PROTEIN STRUCTURE

IMAC has been used to characterize not only natural proteins but also recombinant and modified proteins. IMAC was used to characterize the metal binding properties of engineered histidine sites in bovine somatotropin (Suh et al., 1991). Lectin-based chromatography has been used to determine the extent of glycosylation in glycoproteins (Qiu and Reigner, 2005). IMAC and immobilized metal-ion affinity capillary electrophoresis (IMACE) has been used as

a tool to assess the changes in histidine surface accessibility due glycosylation in R-chymotrypsin (Jiang et al., 1999). Characterization of normal cellular prions (PrP^C) and its PrP^{Sc} isoforms was performed using IMAC (Mueller et al., 2005). By using IMAC, Boden et al. (1998) were able to characterize subtle structural variations in the serine protease family. Pathange et. al. (2006) used IMAC as a tool to establish a direct correlation between the binding strength of lysozyme variants with the relative surface area (rSA) of the lone surface histidine residues. They also demonstrated that IMAC could be used to detect intramolecular hydrogen bond between surface histidines and adjacent amino acids.

IMAC can be used as a tool to characterize protein structure because of the specific nature of the interaction between the immobilized metal ion and the protein's specific amino acids (e.g. Histidine and Cysteines) that can donate electrons. This specific interaction can be exploited, as shown above, to characterize engineered proteins and natural proteins. Thus it will provide powerful discrimination between small differences in protein sequence and structure. In this view Pathange et al. (2006) work on quantifying histidine's relative surface area was used to characterize the protein folding, while previously presentlrrently characterized using labor intensive techniques such as NMR and X-ray crystallography. The combination of analytical technologies and IMAC can be utilized to characterize protein. In the future, integrated IMAC techniques will greatly contribute to solve functional and structural properties of proteomes.

PROTEIN PURIFICATION TECHNIQUES USED IN DECIPHERING PROTEIN STRUCTURE AND ITS RELATION TO FUNCTION

Pathange et al. (2008) used IMAC to quanitify short and medium range perturbations in T4 lysozyme due to mutations. By measuring the differences in protein binding strength between the variant and the control, they were able to determine the change in the histidine's pKa (ΔpKa) due to perturbation. By calculating ΔpKa and change in free Gibbs energy due to binding ($\Delta\Delta$GB) values from experimental protein binding strengths, they were able to quantify electrostatic effects due to single point mutations. They also calculated theoretical change in Gibbs free energy due to perturbations using coulomb's law ($\Delta\Delta$GE). By correlating theoretical and experimental electrostatic effects they demonstrated that the electrostatic effects due to perturbations could be predicted. In cases, where there were microstructural contributions by the adjacent amino acid residues to the perturbations, these microstructural

contributations were identified (e.g. salt bridges and hydrogen bonds). These experimental perturbations and the ability to predict the change in Gibbs free energy help in elucidating the structure to function relation.

Determining protein functional changes due to changes in protein structure are extremely difficult. Though there are techniques such as protein kinetics that can be used to understand structural effects due to mutations, these techniques are limited to changes that impact protein's catalytic sites. On the other hand, IMAC can characterize structural changes that are independent of the catalytic sites and on any type of proteins. The underlying principle of IMAC's versatility in characterizing any type of protein is the subtle changes in the protein structure affecting the protein net charge, which in turn affects the pKa of the electron donor amino acids such as histidines. The only limitation of IMAC is that the protein should have electron donating amino acids on its surface.

IEC is already a proven tool in characterizing protein structure and is widely used in biopharmaceutical industry and academic research. IEC was used to characterize different forms of rFVIII (Pock et al., 1999). Pathange et al. (2008) demonstrated that IEC could be used as a tool to characterize subtle protein micro-structures and detect presence of intramolecular bonds or steric hindraces. They demonstrated there is a correlation between relative retention time (rRT) and protein net charge, and that microstructure of proteins can affect behavior of protein in an IEC column. Due to the presence of intermolecular bonds and steric hindrances, the interactive sites (amino acids that interact with ion exchange) were rendered less interactive, and thus affecting the protein behavior in the cation exchange chromatography quantified by rRT.

IEC works on the principle of electrostatic forces, which also plays a key role in protein structure and function. The pI of the protein, which dictates protein behavior in an IEC, is function of the sum of all the charges in the protein. When subtle changes occur in protein structure due to mutations they alter the protein net charge and the pI of the protein, and consequently its behavior in the IEC columns also alters. By performing systemic mutations, Pathange et al (2008) have demonstrated that IEC is sensitive enough to identify the subtle variation in the protein's microstructure such as intramolecular bonds and steric hindrances. Therefore, the ability of ion exchange to quantify subtle changes in protein's microstructure will aid in characterizing not only protein structure but also its function.

SUMMARY

IMAC and IEC can be exploited not only to characterize minor variations in protein structure but also to decipher protein structure function relationship. The microstructural effects such as flexibility and steric effects of the amino acids play an important role in protein functions such as protein recognition and protein ligand interactions. Thus, the above protein characterization methods (IMAC and IEC) illustrates that the protein structure, which can be obtained from protein sequence, can be used as tool to understand protein function

SIGNIFICANCE AND FUTURE DIRECTION

Protein functions are often affected due to mutations because complex electrostatic networks between amino acids are altered. Consequently, though structure remains intact, the protein function is adversely affected. By using protein purification tools such as IMAC and IEC, it is possible to characterize protein structure via its function. Furthermore, with the advent of biogenerics, these protein characterization techniques are in great demand in biopharmaceutical industry.

Due to the complex nature of the protein electrostatic effects, all site-directed mutagenesis, especially the amino acids that are either involved in catalytic sites, do not yield in desired protein functions. Therefore, successful reverse engineering of amino acid pairs (e.g. in a hydrogen bond) or catalytic sites is not trivial. However, some of the future application of the above mentioned characterization techniques can be used as a guide for developing new and reliable methods for engineering proteins to increase the efficiency (Pathange et al. 2008).

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Author Information

Nadimpalli Ravi S. Varma, PhD

Cellular and Molecular Imaging Laboratory,, Department of Radiology, Henry Ford Hospital,

Ali S. Arbab

Cellular and Molecular Imaging Laboratory,, Department of Radiology, Henry Ford Hospital,