PROTEIN PURIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of the filing date of Sep. 11, 2002 under 35 U.S.C. 119(e) of Provisional Application Serial No. 60/410,334.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to protein purification. In particular, the invention relates to a method for purifying a polypeptide (e.g. an antibody) from a composition comprising the polypeptide and at least one contaminant using the method of ion exchange chromatography.

2. Description of the Related Art

The large-scale, economic purification of proteins is an increasingly important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either eukaryotic or prokaryotic cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cells typically used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through".

Ion exchange chromatography is a chromatographic technique that is commonly used for the purification of proteins.

In ion exchange chromatography, charged patches on the surface of the solute are attracted by opposite charges attached to a chromatography matrix, provided the ionic strength of the surrounding buffer is low. Elution is generally achieved by increasing the ionic strength (i.e. conductivity) of the buffer to compete with the solute for the charged sites of the ion exchange matrix. Changing the pH and thereby altering the charge of the solute is another way to achieve elution of the solute. The change in conductivity or pH may be gradual (gradient elution) or stepwise (step elution). In the past, these changes have been progressive; i.e., the pH or conductivity is increased or decreased in a single direction

U.S. Pat. Nos. 6,339,142 and 6,417,355 (Basey et al.) describe ion exchange chromatography for purifying polypeptides.

U.S. Pat. Nos. 6,127,526 and 6,333,398 (Blank, G.) describe purifying proteins, such as anti-HER2 antibodies, by Protein A chromatography.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides a method for purifying a polypeptide from a composition comprising the polypeptide and contaminants. The composition is loaded onto an ion exchange resin with an equilibrium buffer having a first salt concentration. The ion exchange resin is washed with a wash buffer until a predetermined protein concentration is measured in the flowthrough. During the wash the salt concentration of the wash buffer increases from an initial, second salt concentration that is greater than the salt concentration of the equilibration buffer, to a final, third salt concentration. A fixed volume of wash buffer at the final, third salt concentration is then passed over the resin. Finally, the polypeptide is eluted from the ion exchange resin exchange resin with elution buffer that has a salt concentration that is greater than the final salt concentration of the wash buffer.

In one embodiment the ion exchange resin is an anion exchange resin. In another embodiment the ion exchange resin is a cation exchange resin. Preferably, the cation exchange resin comprises sulphopropyl immobilized on agarose.

In another embodiment the elution buffer has a higher conductivity than the equilibration buffer. In a particular embodiment the elution buffer comprises about 145 mM Na/HOAc and the equilibration buffer comprises about 70 mM Na/HOAc. In another embodiment the elution buffer comprises about 100 mM NaCl and the equilibration buffer comprises about 45 mM NaCl.

The wash buffer preferably comprises a mixture of equilibration buffer and elution buffer. Thus, in one embodiment the increase in the salt concentration of the wash buffer during step (b) is achieved by increasing the proportion of elution buffer in the wash buffer. The proportion of elution buffer in the wash buffer may be increased at a constant rate. In one embodiment the increase in the proportion of elution buffer causes the salt concentration of the wash buffer to increase at a constant rate of from about 1 mM to about 3 mM per column volume of wash buffer.

In another embodiment the percentage of elution buffer in the wash buffer increases at two or more different rates during the course of washing in step (b). For example, the percentage of elution buffer in the wash buffer increases at a first rate for a first segment of the washing, at a second rate for a second segment of the washing and at a third rate for a third segment of the washing.

In one embodiment the polypeptide that is purified is an antibody. In this case, the contaminant may be a deamidated