2 Liquid chromatography of biomolecules

Proteins, peptides, DNA, RNA, lipids, and organic cofactors have various characteristics such as electric charge, molecular weight, hydrophobicity, and surface relief. Purification is usually achieved by using methods that separate the biomolecules according to their differences in these physical characteristics, such as ion exchange (Sect. 2.1), gel filtration (Sect. 2.2), and affinity chromatography (Sect. 2.3).

2.1 Ion exchange chromatography

In ion exchange chromatography, the stationary solid phase commonly consists of a resin with covalently attached anions or cations. Solute ions of the opposite charge in the liquid, mobile phase are attracted to the ions by electrostatic forces. Adsorbed sample components are then eluted by application of a salt gradient which will gradually desorb the sample molecules in order of increasing electrostatic interaction with the ions of the column (Figs. 2.1 – 2.3). Because of its excellent resolving power, ion exchange chromatography is probably the most important type of chromatographic methods in many protein preparations.

The choice of ion exchange resin for the purification of a protein largely depends on the isoelectric point, pI, of the protein. At a pH value above the pI of a protein, it will have a negative net charge and adsorb to an anion exchanger. Below the pI, the protein will adsorb to a cation exchanger. For example, if the pI is 4 then in most cases it is advisable to choose a resin which binds to the protein at a pH > 4. Since at pH > 4 this protein is negatively charged, the resin has to be an anion ion exchanger, e.g., DEAE. One could also use a pH < 4 and a cation exchanger, but many proteins are not stable or aggregate under these conditions. If, in contrast, the protein we want to purify has a pI = 10, it is positively charged at usually suitable conditions for protein ion exchange chromatography, i.e., at a pH around 7. Thus, in general for this protein type we have to choose a cation ion exchange resin, e.g., CM, which is negatively charged at neutral pH.

The capacity of the resin strongly depends on the pH and the pI of the proteins to be separated (Fig. 2.4; Table 2.1), but also on the quality of the resin, the applied pressure, and the number of runs of the column (Fig. 2.5). To improve the life of the resin, it should be stored in a clean condition in the appropriate solvent and not be used outside the specified pH range and pressure limit.

For the separation of some enzymes which may lose their activity by contact with metals in the wall of stainless steel columns, glass-packed columns may be more appropriate. The chromatographic resolution mainly depends on the type of biomolecules, type and quality of the resin, ionic strength gradient during elution, temperature, and the geometry of the column.

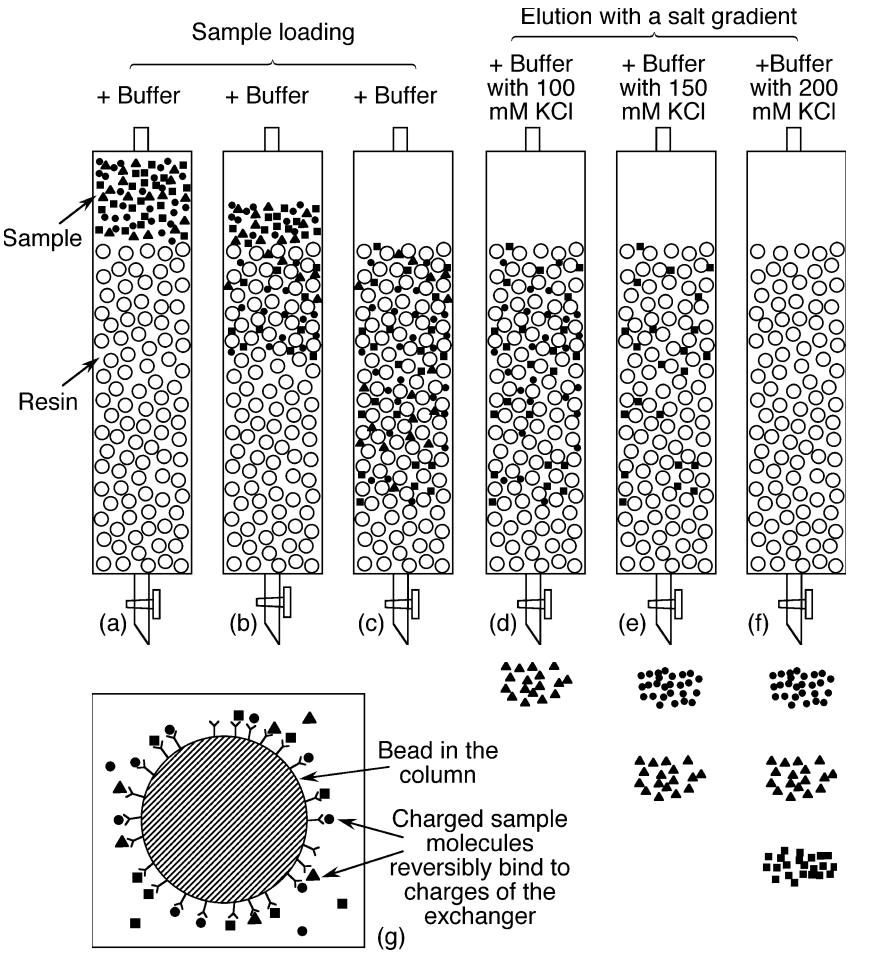

Fig. 2.1 Example of ion exchange chromatography. (**a**)–(**c**) Loading the column: mobile anions (or cations) are held near cations (or anions) that are covalently attached to the resin (stationary phase). (**d**)–(**f**) Elution of the column with a salt gradient: the salt ions weaken the electrostatic interactions between sample ions and ions of the resin; sample molecules with different electrostatic properties are eluted at different salt concentrations, typically between 0–2 M. (**g**) Interaction of sample molecules with ions attached to the resin: at a suitable pH and low salt concentration, most of the three types of biomolecules to be separated in this example reversibly bind to the ions of the stationary phase