in a decrease in total system entropy. In order to maximize the system entropy, the hydrophobic residues orient away from the water resulting in an increase in bulk water entropy and hence an increase in system entropy. Thus, removing the water in the reversed phase elution buffer weakens the entropic driving forces for the so-called hydrophobic bond (Tanford, 1980), and the stronger hydrophobic interactions will require higher concentrations of organic modifiers. Therefore a gradient of organic solvent will release proteins from the column matrix in the order of their hydrophobic interaction strengths.

Initially this method was used to separate small organic molecules, and has been successfully used to separate peptides that are generated after specific proteolysis using enzymes such as trypsin (Fullmer & Wasserman, 1979). This chromatography coupled with mass spectrometry has enabled peptide mapping whereby residues in large proteins that are chemically modified can be identified. Although RP-HPLC has been used successfully to analyze small proteins such as insulin (Seino, Funakoshi, Fu, & Vinik, 1985), growth hormone (Kohr, Keck, & Harkins, 1982), and human relaxin (Cipolla & Shire, 1991), especially for Met oxidation products, the resolution of larger intact proteins is limited. Specifically, in larger proteins the effect of the chemical alterations on the elution profile is smaller than for peptides and small proteins, and thus this chromatographic assay has limited utility in analyzing degradation in large proteins. Although this is generally the case, appropriate optimization of the RP-HPLC can be done to investigate chemical changes such as Trp oxidation in mAbs (Yang, Wang, Liu, & Raghani, 2007). The use of RP-HPLC for analysis of small rDNA-derived proteins has been reviewed (Frenz, Hancock, Henzel, & Horvath, 1990).

Electrophoretic methods

Reduced and nonreduced sodium dodecyl sulfate electrophoresis

In this method, an electric field is applied over a gel-based matrix, usually consisting of a polyacrylamide slab gel, and this assay is termed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). The buffer system of this gel matrix contains SDS and samples are incubated at higher temperature in a loading buffer solution that also contains SDS. Proteins will unfold in the typical SDS concentrations used, and thus will migrate as simple linear polypeptide chains (with disulfide cross-linking in the absence of reducing agent). After loading samples, usually with an added dye to visualize the transport into the gel, an electric field is applied. The pore size of the matrix, which is generated during the polymerization process, dictates the rate at which the unfolded protein molecules transport during electrophoresis. Charge is not a predominant factor because most proteins bind a constant ~1.4 g of SDS/g of protein resulting in SDS complexes with similar mass to negative charge ratio, and thus the protein samples separate on the basis of their size. The SDS binds to the protein through its hydrophobic portion, and thus membrane-bound proteins that have large hydrophobic regions may bind greater amounts of SDS resulting in incorrect determination of size. The use of reducing agents readily shows if protein covalent aggregates