barrier although some exceptions exist. Importantly, most small molecules show oral bioavailability which peptides and proteins do not. Thus, one disadvantage of protein and peptides compared with small molecules is that they need to be injected. Administration by injection necessitates that the drug is stable in formulation which may be challenging as many endogenous peptides and proteins lack sufficient chemical or physical stability to be manufactured in an aqueous formulation. Alternatively, they may be provided as lyophilized products that must be dissolved prior to use which is less convenient when, for example, daily injections are needed.

Likewise, in contrast to small molecules, both proteins and peptides may form insoluble aggregates or fibrils upon quiescent storage which in the long run may provoke an immune response and the formation of antibodies. Antibody formation is also a concern when a protein sequence is changed from that of the native peptide or protein which may lead to a loss of drug's effect or reduced efficacy.

9.2 PEPTIDE AND PROTEIN PROTRACTION

As previously discussed, major challenges in the development of biopharmaceuticals as therapeutics are rapid clearance and thus a very short blood residence time. The reason for this is partly due to fast renal elimination of peptides and small proteins, and also proteolytic plasma clearance as well as receptor clearance.

9.2.1 Polymer Extension

The most important determinant of renal clearance is the size or the hydrodynamic volume of the peptide or protein. While there are several ways to increase the volume of a peptide or protein, one common approach is to add a random polymer that simply increases the size of the resulting polymer–protein conjugate. In addition to reducing clearance, such modification appears to provide a shielding effect that lowers the antigenicity of the conjugate and protect against proteolysis. It is important to keep in mind, however, that such shielding may also affect access to the target protein and thus impact the potency.

9.2.1.1 PEGylation

The most successful polymer has so far been polyethylene glycol (PEG) (Figure 9.1a). PEGylation of peptides and proteins originated in the 1970s and was one of the very first protein engineering strategies to be used. Pegademase bovine, a modified enzyme used for enzyme replacement therapy, was the first to reach the market after approval in 1990, and several other PEGylated proteins have since been approved, including pegaspargase, 1994, peginterferon alfa-2b, 2000, peginterferon alfa-2a, 2002, pegfilgrastim, 2002, pegvisomant, 2002, pegaptanib, 2004, methoxy polyethylene glycol-epoetin beta, 2007, and certolizumab pegol, 2008.

Most of these drugs were randomly PEGylated through amidation to surface exposed lysines. The main reason for PEGylation was to increase the hydrodynamic volume and thereby reduce renal clearance, but in many cases it also increased stability toward proteolysis which also contributed to a longer circulation time. The molecular weight cut-off for glomerular filtration is approximately 60 kDa, but interestingly renal clearance of PEGylated peptides had a cut-off at about 20–30 kDa. This was explained by the tendency of PEG to bind water and the tendency to form a random coiled noncharged polymer with a larger hydrodynamic radio compared to a charged compact and structured protein of similar molecular weight. By attachment of PEG several other benefits include reduced immunogenicity, antigenicity, and in several cases also improved solubility which was of importance for drug product formulation. The first applications used PEGs of less than 12 kDa and with rather high degree of polydispersity due to the polymerization process in manufacturing the PEGs. The process technology has since improved and today various PEGs are available that offer higher molecular weights and a narrower polydispersity index. The conjugation technology has also