(like [D-Lys]^{6}GnRH) was more affected by the chemical modification. Note that DMSO, which is a dipolar aprotic solvent, was used as a vehicle by Yahalom et al.; therefore, the barrier function of the skin might be decreased so that the increase in hydrophobicity did not affect the permeability of molecules.

Opposite to peptides, unfortunately, few studies have been conducted on chemical modification of proteins for skin delivery, especially by attachment of hydrophobic moieties. Protein lipidation is a natural process that occurs in the body either in the cytoplasm of cells or in the lumen of the secretory pathway. This process is important for the protein functionality. Fatty acids, isoprenoids, and cholesterol are some lipids that are used in lipid modification of proteins. Palmitoylation is a reversible process in which long-chain fatty acids like palmitic acid are covalently added to proteins. Palmitoylation promotes the association of soluble proteins with plasma membranes (Nadolski & Linder 2007).

Covalent attachment of palmitic acid to the lysine residues in interferon α2b (IFNα2b) was conducted by Foldvari et al. (1998). This process led to the production of different palmitoyl derivatives of IFNα2b with 10, 11, and 12 palmitoyl substitutions. Using full-thickness human breast skin in permeation studies, Foldvari et al. reported that cutaneous absorption of palmitoyl IFNα2b (p-IFNα2b) was five to six times higher than that of IFNα2b. The antiviral activity of IFNα2b decreased by 50% due to chemical modification. Interferon α2b, a hydrophilic protein, has a molecular weight of 19.3 kDa and 165 amino acids. The attachment of 10 to 12 palmitic acids to IFNα2b, which is already a large molecule, increases its molecular weight. Therefore, the increased partitioning of the protein due to lipidation is apparently able to compensate for molecular weight increments and therefore reduction of the diffusion coefficient.

This study was conducted in 1998, and so far, about 20 years later, no studies have been reported for the application of lipidation in skin absorption enhancement of proteins.

34.3.2 Peptide or Protein Conjugation with Cell-Penetrating Peptides

CPPs or protein transduction domains (PTDs) are short peptides (generally less than 30 residues in length) that are inherently able to cross cellular membranes without causing serious membrane damage (Bechara & Sagan 2013; Buckley et al. 2016; Guo et al. 2016). These types of peptides are usually highly cationic or/and amphipathic molecules that are capable of transporting biomolecules (e.g., protein, peptide, plasmid DNA, oligonucleotide, siRNA) across biological barriers (Brasseur & Davita 2010; Munyendo et al. 2012). CPPs can internalize into the cell by two main mechanisms: energy-dependent, endocytotic pathways and non-energy-dependent, nonendocytotic pathways (Zhang et al. 2016).

The mechanisms of CCPs for increasing skin penetration are not fully understood. The stratum corneum consists of keratin-containing dead cells embedded in a lipid matrix (Menegatti et al. 2016). The molecular weights of CCPs are mostly more than 1000 Da, and it has been argued that these molecules do not interact with the intercellular lamellar structure of the SC (Gennari et al. 2016). Using coadministration of five different CCPs with cyclosporine A (CsA) individually, Kumar et al. (2015) found that CCPs interacted with the skin proteins and led to changes in their secondary structure; as a result, they increased partitioning of CsA into the corneocytes. However, Gennari et al. (2016) reported that both the fluidity of intercellular lipids and extension of keratins were induced by the skin penetration enhancer heptapeptide.

CPPs are added to peptides and proteins through either physical complexation or covalent conjugation (Brasseur & Davita 2010; Kristensen et al. 2016). Most studies are about coadministration of CPPs with peptides and proteins, but since this chapter is about chemical modification, it continues to focus only on conjugation.

The successful topical delivery of CsA because of conjugation to short oligomers of arginine was reported by Rothbard et al. (2000). CsA, a cyclic peptide with 11 amino acids and molecular weight of 1202.6 Da, has poor skin penetration. CsA was conjugated to a heptamer of arginine through a