1. **General conjugation**

**Bioconjugation** is the process of coupling two biomolecules together in a covalent linkage[1]. Common types of bioconjugation chemistry are amine coupling of lysine amino acid residues (typically through amine-reactive succinimidyl esters), sulfhydryl coupling of cysteine residues (via a sulfhydryl-reactive maleimide), and photochemically initiated free radical reactions, which have broader reactivity. The product of a bioconjugation reaction is a bioconjugate.

The most common bioconjugations are coupling of a small molecule (such as biotin or a fluorescent dye) to a protein, or protein-protein conjugations, such as the coupling of an antibody to an enzyme. Other less common molecules used in bioconjugation are oligosaccharides, nucleic acids, synthetic polymers such as polyethylene glycol (a.k.a. PEG a.k.a. polyethylene oxide).[2] and carbon nanotubes.[3]

Antibody-drug conjugates such as Brentuximab vedotin and Gemtuzumab ozogamicin are also examples of bioconjugation, and are an active area of research in the pharmaceutical industry.[4]

References

2. Why Conjugation?

Proteins are biopolymers composed of polymeric chains containing many hydrophobic and hydrophilic domains, often giving the molecules amphipathic structures that are somewhat similar to those of polymeric surfactants. The unique functional properties and surface activities of proteins—including water solubility, surface absorption and binding, rheology modification, emulsifying activity and emulsion stabilization, gel formation, foam formation and stabilization, and fat absorption—arise from their large molecular weights and their amphipathic properties.

Nevertheless, to increase the industrial applications of proteins, many methods have been developed to improve their functional properties and surface activities. Notably, the chemical modification of proteins is one of the most important methods toward improving their surface activities—for example, to improve their emulsify power and wetting ability or to minimize the surface tensions of solutions.

Polysaccharide attachment is a very efficient method of increasing the stability of proteins toward denaturation. In other words, conjugation of a polysaccharide to a protein improves the heat stability of the protein. In addition, because of the poor emulsifying properties of proteins, many formulations usually require the addition of emulsifiers and stabilizers to facilitate the formation of stable emulsions.
Protein–polysaccharide conjugates could potentially combine the excellent emulsification properties of the protein with the stabilizing effect of the polysaccharide. The bonding between the surface-active protein and the polysaccharide could result in the stabilizing effect of the polysaccharide being targeted at the portion of the aqueous phase immediately adjacent to the emulsion interface.


3. What bioconjugates?

(1) To improve functional properties of proteins including their emulsifying ability, chemical modifications have often been made. Over the past few years, there has been growing interest in the modification of proteins with sugars through the maillard reaction; a complex network of non-enzymatic reactions between reducing sugars and protein amino groups.
At an early stage of the maillard reaction amino groups in proteins react with carbonyl groups of sugars to give 1-amino-1-deoxy-ketose, known as the amadori compound. The set of reactions that occurs thereafter results in the formation of both large protein aggregates and low molecular weight products that are believed to confer flavor, aroma, color, texture, and antioxidant characteristics to various foods.

Ref. J. Oleo Sci. 57, (10) 539-547 (2008) Bovine serum albumin-sugar conjugates through the maillard reaction: effects on interfacial behavior and emulsifying ability
In this study we prepared a series of gelatin–glucose conjugates through Maillard-type reactions (covalent coupling) between a modified gelatin hydrolysate and glucose. Our gelatin–glucose conjugates exhibited excellent surface activity properties, including reduced surface tension and high low-foaming and wetting power. In addition, these new surfactants displayed greater thermal stability and improved emulsifying properties relative to those of industrial standards. Finally, we used UV–Vis spectroscopy to study the interactions of direct dyes with the gelatin–glucose conjugates in aqueous solution.
(3) The binding reaction (panel A) and mode (panel B) for the formation of protein-galactomannan conjugates are proposed as shown in Fig. 1.

![Diagram](image)

Fig. 1. Schematic presentation of the binding mode of polysaccharide with protein through Maillard reaction (A) and the resulting protein-polysaccharide conjugate (B).

4. **Application range: food, pharmaceutics**

(1) Excellent Emulsifying Properties of Protein-Polysaccharide Conjugates

Both protein and polysaccharide have a role in the stabilization of oil in water emulsions. Proteins adsorb at the oil-water interface during emulsification to form a coherent viscoelastic layer, while polysaccharides confer colloid stability through their thickening...
and gelation behavior in the aqueous phase. Therefore, the protein-polysaccharide conjugates are expected to exhibit good emulsifying properties.

The conjugates of proteins with polysaccharide revealed much better emulsifying activity and emulsion stability than the control mixtures of proteins with polysaccharides. Since high salt conditions, acidic pH, and/or heating process are commonly encountered in industrial application, the protein-polysaccharide conjugate may be an ingredient suitable for use in food processing.

(2) Heat Stability of Protein-Polysaccharide Conjugates

The attachment of polysaccharide may cause proteins to form a stable structure against heating. It seems likely that the protein denaturation process may be reversible in the protein-polysaccharide conjugates, because of the inhibition of the unfolded protein-protein interaction due to the attached polysaccharide.

(3) Polymannosyl Lysozyme Constructed by Genetic Modification

To further elucidate the molecular mechanism of the improvement of functional properties of proteins by attachment with polysaccharide, genetic glycosylation of lysozyme was successfully attempted using the yeast expression system (Nakamura et al., 1993a, 1993b; Kato et al., 1994; Shu et al., 1998; Kato et al., 1996). In yeast cells, the proteins having an Asn-X-Thr/Ser sequence are N-glycosylated in the endoplasmic reticulum and the attached oligosaccharide chain can be elongated with further extension of a large polymannose chain in the Golgi apparatus. Therefore, construction was attempted of a yeast expression plasmid carrying the mutant cDNA of hen egg white
lysozyme having an N-glycosylation signal sequence (Asn-X-Thr/Ser) at the molecular surface.

(4) Antimicrobial Action of Lysozyme-Polysaccharide Conjugates

Many attempts have been made to develop food preservatives having a superior antimicrobial effect without toxicities; the antimicrobial action of lysozyme-polysaccharide conjugates was observed in both Gram-positive and Gram-negative bacteria (Nakamura et al., 1991). The living cells were dramatically decreased with heating time at 50°C in the presence of lysozyme-polysaccharide conjugate. Thus, it was concluded that the lethal effect was effectively induced by exposing the cells to lysozyme-polysaccharide conjugate.

These results indicate that conjugates are very effective in inhibiting the growth of the bacterium and are promising for use in industrial applications, because the conjugates had excellent emulsifying properties and solubility, especially at neutral pH, in addition to antimicrobial action.

(5) Masking of Allergen Structure of Proteins

The allergen structure of proteins can be masked by conjugation with polysaccharide. Soy protein is a well-known allergenic protein, and the 34 kDa protein is a well identified allergenic protein in soy protein (Ogawa et al., 1993). Most patients who can not take soy protein are sensitive to 34 kDa protein. It has been reported that the allergenic protein of soybean reacts easily with polysaccharide by Maillard reaction. Therefore, it was expected that the allergen structure might be masked by the attachment of polysaccharide.
As described here, Maillard-type protein-polysaccharide conjugates showed excellent emulsifying properties which were superior to conventional commercial emulsifiers, heat stability, and antimicrobial activity. Therefore, the conjugates can be useful for industrial applications as natural emulsifiers and antimicrobial agents devoid of toxicity. It has also been proposed that conjugation of the allergen protein with polysaccharides may be effective in reducing the allergenicity.


(6)

Curcumin, a yellow bioactive component of Indian spice turmeric, is known to have a wide spectrum of biological applications. In spite of various astounding therapeutic properties, it lacks in bioavailability mainly due to its poor solubility in water. In this work, we have conjugated curcumin with silica nanoparticles to improve its aqueous solubility and hence to make it more bioavailable. Conjugation and loading of curcumin with silica nanoparticles was further examined with transmission electron microscope (TEM) and thermogravimetric analyzer. Cytotoxicity analysis of synthesized silica:curcumin conjugate was studied against HeLa cell lines as well as normal fibroblast cell lines. This study shows that silica:curcumin conjugate has great potential for anticancer application(cited from JAFC, 2013).
Protein modification: Shelf-life

- Enhancing antioxidant activity, affecting gas exchange, antimicrobial activity or by stabilization of emulsion or foam-based food products (del Rosario Moreira et al., 2011; Emmambux et al., 2004; Mendis et al., 2005).
- Nanobiocomposites of maize prolamin protein zein have also been employed as a gas barrier by coating tomatoes (Park et al., 1994), and apples (Bai et al., 2003).
- Nisin is a potent antibacterial 34 amino acid peptide containing a number of uncommon amino acids: Nisin has been employed as an approved food preservative in cheese (Martins et al., 2010), fish, meat, and beverages (reviewed in Lubelski et al., 2008).
- ε-poly-L-lysine exhibits antimicrobial activity against bacteria and fungi (reviewed in Hamano, 2011).
- Mixture of oregano oil and whey protein isolate showed inhibition of growth of lactic acid bacteria, reduction of pseudomonads, total flora and growth rates when applied to fresh beef (Zinoviadou et al., 2009).

(7) Sensory: color, odor, flavor, texture

- Sensory aspects of food products include sensation of flavor, odor, color, and texture. These factors play a large role in consumer acceptation of food products and the effects of various types of protein chemical engineering and their applications will be discussed in this paragraph. Many types of modification target the amino groups of lysine residues, including succinylation, lipiddation and glycosylation through the Maillard reaction.