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# Effect of synthetic polymers on polymer–protein interaction

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ABSTRACT

Synthetic polymers are often used for delivery of therapeutic drugs and proteins. We report the binding of milk β-lactoglobulin (β-LG) with poly(ethylene glycol) (PEG), methoxypoly(ethylene glycol) polyamidoamine (mPEG-PAMAM-G-3) and polyamidoamine (PAMAM-G4) nanoparticles in aqueous solution at pH 7.4, using Fourier Transform infrared (FTIR), circular dichroism (CD), fluorescence spectroscopic methods, transmission electron microscopy (TEM) and molecular modeling. Structural analysis showed that polymers bind β-LG *via* both hydrophilic and hydrophobic contacts with overall binding constants $K_{PEG\text{-}8000\text{-}\beta\text{-}LG} = 4.8\ (\pm 0.4) \times 10^4\ M^{-1}$ and $K_{mPEG\text{-}PAMAM\text{-}G3\text{-}\beta\text{-}LG} = 5.8\ (\pm 0.6) \times 10^5\ M^{-1}$ and $K_{PAMAM\text{-}G4\text{-}\beta\text{-}LG} = 6.7\ (\pm 0.9) \times 10^4\ M^{-1}$. The number of binding sites were occupied by polymers on protein (n) was 0.3 for PEG-8000, 0.4 for mPEG–PAMAM-G3 and 0.4 for PAMAM-G4. The order of binding is mPEG-PAMAM-G3 > PAMAM-G4 > PEG-8000. Transmission electron microscopy showed significant changes in protein morphology as polymer–protein complexation progressed with major increase in the diameter of the protein aggregate (180%). Furthermore, modeling showed several H-bonding systems between PEG and different amino acids stabilize polymer–β-LG complexes. mPEG-PAMAM-G3 is a stronger protein binder than PAMAM-G4 and PEG-8000.



## 1. Introduction

Synthetic polymers play a major role in therapeutic drug, protein and gene delivery [1–7]. Among synthetic polymers, poly(ethylene glycol) and its derivatives show potential applications in gene and drug delivery due to their solubility, nontoxicity and biocompatibility [8,9]. PEGylation of protein and peptide causes biopolymer structural changes [10]. It has been shown that PEG induces significant changes in DNA and protein solubility and structure under given conditions [8–15]. PEGylation of synthetic polymers such as dendrimers is shown to reduce toxicity and increase biocompatibility and DNA transfection [7,8,10]. Similarly, the effect of PEGylation on the toxicity and permeability of natural polymers such as chitosan has been recently reported [16,17]. Even though, the interactions of PEGylated dendrimers with DNA and RNA are well characterized [18,19]. Detailed structural analysis of PEG, mPEG and mPEG–dendrimers complexes with protein is less investigated [20,21]. Therefore, it was of interest to study the interaction of PEG, mPEG-PAMAM-G3 and PAMAM-G4 nanoparticles (Scheme 1) with protein, using spectroscopic methods, electron microscopy and modeling in order to evaluate the efficacy of these nanoparticles in protein delivery.

β-Lactoglobulin contains several high affinity binding sites for fatty acids, lipids, aromatic compounds, vitamins and polyamines [22–30]. There are two potential binding sites in β-lactoglobulin for small hydrophobic molecules. One is located in the interior cavity and the other on the surface cleft of the β-lactoglobulin structure [31,32]. The structure of this protein is well known and at neutral pH, β-LG exists as a mixture of monomers and dimers of which the equilibrium ratio depends on the association constant of the dimer and on the protein concentration [33]. Each monomer consists of 162 amino acid residues and has a molecular mass of 18 kDa [33,34]. As a member of the lipocalin family, β-LG is a small globular protein folded into a calyx formed by eight antiparallel β-strands and a α-helix located at the outer surface of the β-barrel [34]. Several spectroscopic and X-ray crystallographic reports examined the binding sites of hydrophobic ligands on β-LG [35]. Competitive binding studies based on fluorescence spectroscopy showed different binding sites for hydrophobic compounds with β-LG [32].

*Abbreviations:* PEG, poly(ethylene glycol); mPEG, methoxypoly(ethylene glycol); PAMAM, poly(amidoamine); β-LG, beta-lactoglobulin; FTIR, Fourier transform infrared; CD, circular dichroism; TEM, transmission electron microscopy.

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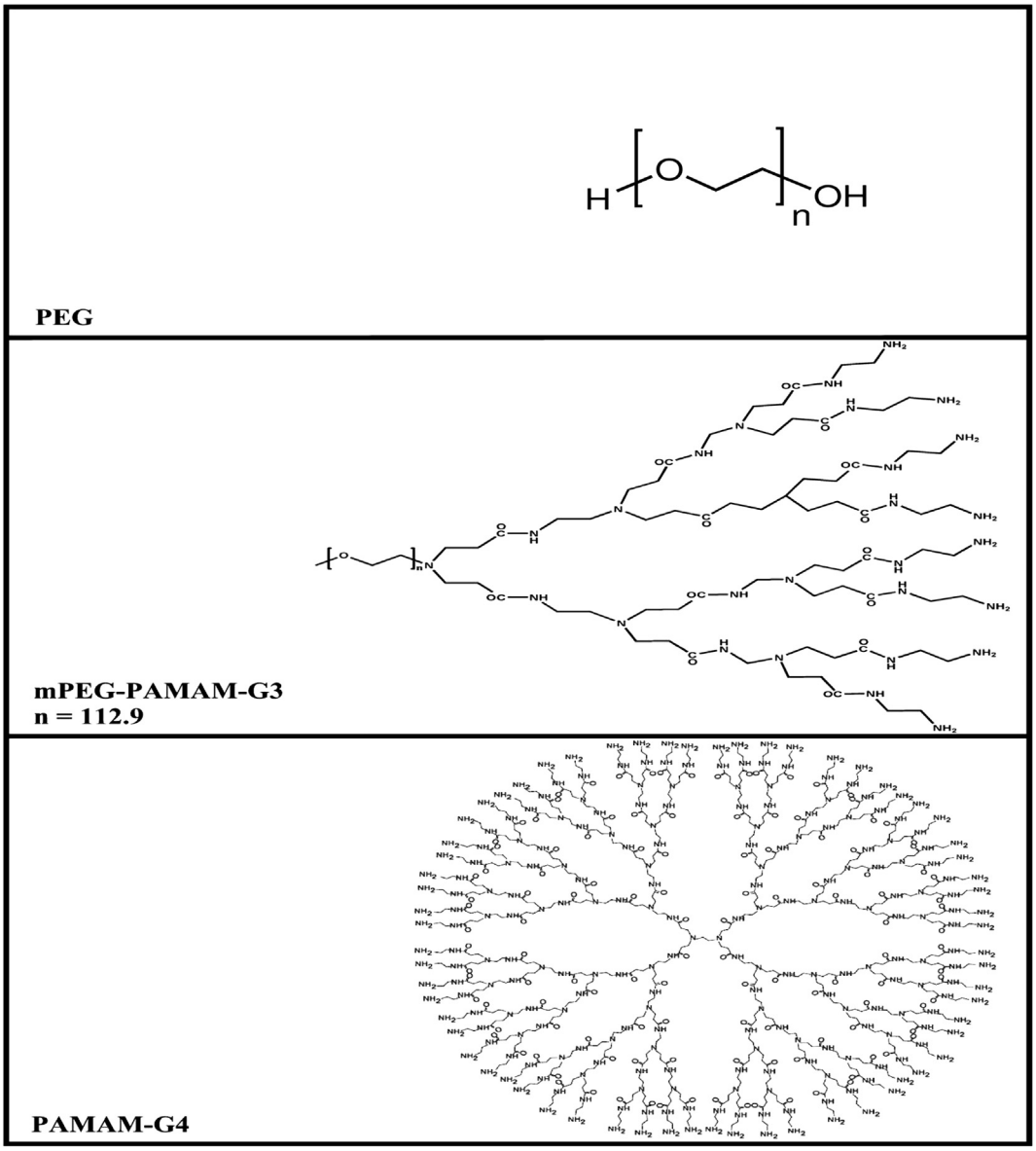


**Scheme 1.** Chemical structures of PEG, mPEG-PAMAM-G3 and PAMAM-G4.

However, the exact binding sites of the hydrophobic ligands such as synthetic polymers on β-LG are not fully investigated. Therefore, it was of interest to determine the binding sites of synthetic polymers with β-LG and the effects of polymer complexation on the protein stability and conformation.

Fluorescence quenching is considered as a useful and reliable technique for measuring binding affinities [36]. Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with quencher molecule [37]. Therefore, it is possible to use quenching of the intrinsic tryptophan fluorescence of Trp-61 and Trp-19 in β-LG [22,23] as a tool to study the interaction of synthetic polymers with β-LG in an attempt to characterize the nature of polymer–protein interaction.

We report spectroscopic analysis, TEM and molecular modeling of the complexes of β-LG with PEG-8000, mPEG-PAMAM-G3 and PAMAM-G4 in aqueous solution at pH 7.4, using constant protein concentration and various polymer contents. Structural analysis regarding protein binding sites and the effect of polymer–protein complexation on the β-LG stability and morphology is reported. Furthermore, the affinity of each polymer as a protein binder is discussed here.

## 2. Materials and methods

### 2.1. Materials

β-Lactoglobulin (A variant, purity > 90%), PEG-8000 and PAMAM-G4 ($M_w$ = 14,214 g/mol) were purchased from Aldrich Chemical Co. and used as supplied. mPEG-PAMAM-G3 ($M_w$ 13,423 g/mol) (mPEG block has a molecular weight of 5000 g/mol) was synthesized according to published methods [8,38]. Other