Determining Steric Layer Thickness and Conformation Using Dynamic Light Scattering

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Introduction

This application note summarizes measurement of the adsorbed layer thicknesses of β-lactoglobulin-dextran conjugates as a function of the change with dextran molecular weight. These measurements should yield information on the structure of this layer in terms of its thickness and orientation.

Caseins and whey proteins are widely used as emulsifiers in food, cosmetic and pharmaceutical industries. The efficiency of these emulsifiers is a function of their interfacial structure [1,2]. The caseins form a thick fluid interfacial layer which extends 13 to 15nm into the bulk aqueous phase [3]. Emulsions formed using casein are generally stable when the steric layer extends into the bulk. They become unstable upon the collapse of this steric layer as a result of lowering the pH or in the presence of ethanol [4].

The whey proteins form a thin (2 to 3nm) dense interface with little protein protruding into the bulk [4]. Whey protein emulsions are stabilized by electrostatic charge [5]. They flocculate upon removal of this charge when the pH approaches the isoelectric point or in the presence of electrolyte [3,5]. The stability of whey protein emulsions can be enhanced by attaching a steric layer through reaction with a polysaccharide via a carbodiimide linkage or the Maillard reaction [6]. Numerous studies report improved emulsion capacity and stability when β-lactoglobulin is chemically linked to carbohydrates [6-8]. Most studies examine emulsions with low protein contents (0.1 wt%) and the enhanced emulsion stability is measured using a turbimetric method [8, 9]. The enhanced emulsion stability observed using this method is more often related to particle size (emulsion capacity) rather than to changes in colloidal stability [6, 10, 11]. Therefore the question of "how bulky does the attached carbohydrate have to be in order to impart high steric stability to emulsions?" remains unanswered.

Good models for studying this phenomenon are protein-dextran conjugates, as the dextran is predominantly linear and readily available in defined molecular weights. There is little knowledge of the interfacial structure of protein-dextran conjugates with respect to its orientation and thickness as a function of molecular weight and its effect on emulsion stability. Dynamic light scattering (DLS) can be used to gain an understanding of the hydrodynamic thickness of the interface by examining emulsifiers adsorbed onto latex [12, 13]. DLS measures the time-dependent fluctuations in the intensity of scattered light from a suspension of particles undergoing random, Brownian motion. Analysis of these intensity fluctuations allows for the determination of the diffusion coefficients, which in turn yield the particle size through the Stokes-Einstein equation [14].

Experimental

Materials

β-Lactoglobulin (Lot No. 025K7017), and dextrans D35, D65 and D500 were obtained from Sigma Aldrich. The dextrans had average molecular weights (M_w) of 36, 60 and 440KDa respectively. Dextrans D20 and D150 were obtained from Pharmacosmos and had average molecular weights of 18.3 and 153KDa respectively. Polystyrene latex spheres (anionic, 166 and 60nm diameter) were obtained from Duke Scientific. All buffers and salts were analytical grade and were also obtained from Sigma Aldrich.

Conjugate Preparation

Extensive details of the preparation, evaluation of the extent of conjugation and purification of β-lactoglobulin-dextran conjugates can be found in the literature [15].

Latex Adsorption

Maillard conjugate adsorbed layer thickness was assessed by adsorbing the protein/purified conjugate onto monodisperse polystyrene latex beads and measuring the particle size using dynamic light scattering. Adsorbed layer thickness was estimated from the difference in the hydrodynamic diameter of the naked

latex bead and latex beads with adsorbed layers. All DLS measurements were carried out on a Zetasizer ® Nano ZS ^{TM} using a scattering angle of 173^{o}. Samples were measured after 5 minutes equilibration within the instrument at 25^{o}C and the results are reported as the average of 3 measurements. The error between measurements on different sized lattices was ±0.3nm. All samples were prepared in a buffer of 20mM Tris/HCl and 30mM NaCl which were filtered using a 0.45µm filter prior to use.

Results and Discussion

The hydrodynamic thickness of β-lactoglobulin-dextran Maillard conjugate interfaces was assessed using dynamic light scattering. The adsorbed layer thickness was estimated from the difference in size between latex spheres and those with protein adsorbed onto their surface. The final thickness of the conjugate layer was taken from the plateau region of protein concentration versus layer thickness curves. There is some difference in layer thickness depending on how the conjugate was applied (figure 1). Layers formed by sequential addition of conjugate to the one batch of latex had a higher plateau thickness than those prepared fresh at each concentration (using fresh latex and a single aliquot of solution). This may be because the orientation of sequentially adsorbed conjugates is affected by the nature of the initial adsorbed conjugate layer. This may result in uneven packing at the interface and hence an artificially higher layer thickness (figure 2). Therefore, all subsequent adsorption profiles were prepared fresh at each protein concentration.

Figure 3 shows the adsorbed layer thickness versus protein concentration (not conjugate concentration) of β-lactoglobulin and of β-lactoglobulin-dextran Maillard conjugates (βlg-D20, βlg-D35, βlg-

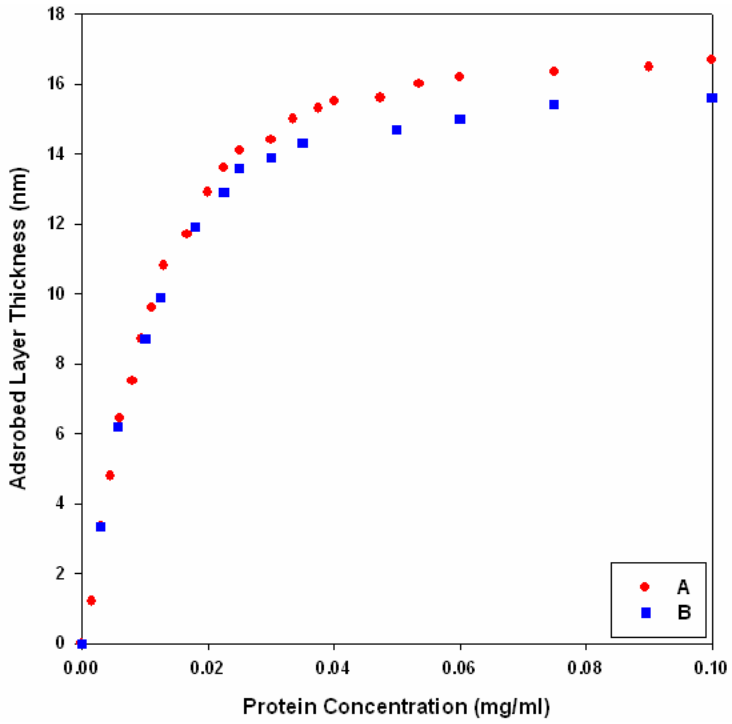

Figure 1: Effect of addition sequence history on the adsorbed layer thickness of β-lactoglobulin-dextran (M_W = 65kDa) conjugate adsorbed onto latex measured by (A) sequential addition to the one batch of latex (●) and (B) fresh preparation at each concentration (■)

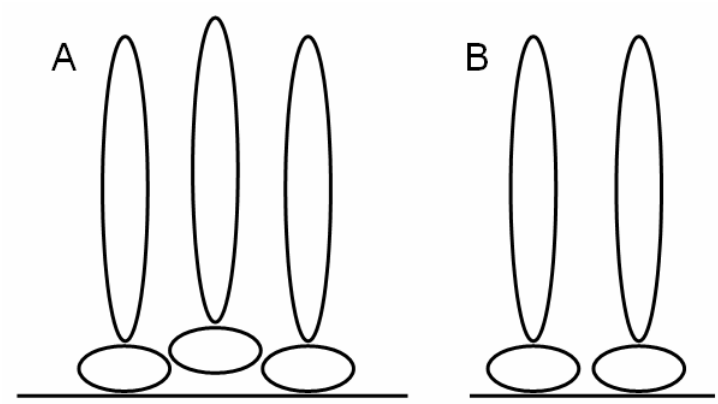

Figure 2: Cartoon representing uneven packing that might result from sequential addition of conjugate

	Material Properties		β-Lactoglobulin-Dextran Conjugate Properties	
	Molecular Weight (kDa)	Hydrodynamic Diameter (nm)[a]	Adsorbed Layer Thickness (nm)[b]	Dextran Steric Layer Thickness (nm)[c]
β-lactoglobulin	18.3	5.6 (dimer)	2.9	0
D20	18.2	5.0	8.2	5.3
D35	36.0	8.0	12	9.1
D65	60.3	10	15.7	12.9
D150	132	14.9	20	17
D440	440	18	23	20.1

Table 1: Summary of the measured and calculated hydrodynamic dimensions of β-lactoglobulin and dextran in solution and conjugates as adsorbed layers. [a] taken as the volume average diameter from DLS measurements in 50mM NaCl, [b] taken as the plateau thickness from the measurements presented in figure 3 and [c] taken as the adsorbed layer thickness minus 2.9nm (thickness of β-lactoglobulin layer)

D65, βlg-D150 and βlg-D500). This figure serves to determine the concentration where a plateau in the layer thickness is obtained. The reproducibility of these plateau thicknesses between different preparations on the two different sized latex spheres (60 and 160nm), was found to be about 0.6nm. The adsorption of β-lactoglobulin results in the formation of a 2.9nm thick layer on the latex. This thickness measurement corresponds with that obtained by other authors [16]. The adsorption profiles shown in figure 3 indicate that conjugation of dextran to β-lactoglobulin increases layer thickness. Table 1 summarizes the measured and calculated hydrodynamic dimensions of β-lactoglobulin and dextran in solution and conjugates as adsorbed layers. These results show that the thickness of the layer is influenced by the dextran molecular weight M_w.

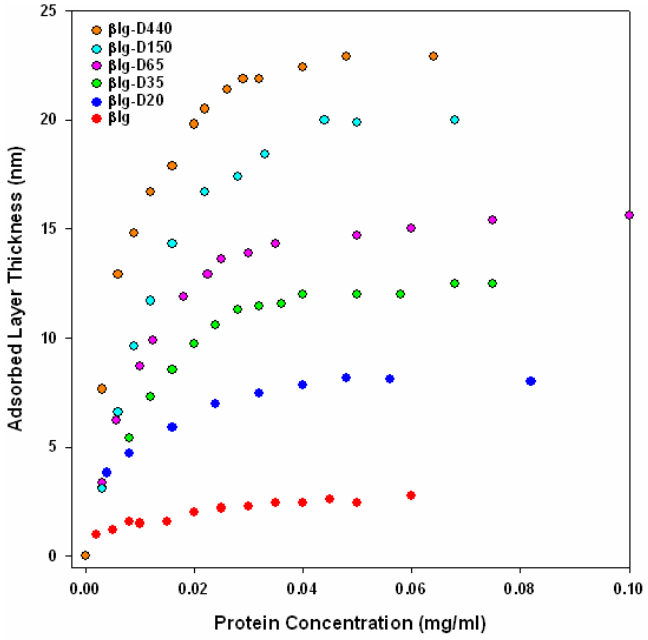

Figure 3: Adsorbed layer thickness of β-lactoglobulin and β-lactoglobulin-dextran Maillard conjugates of medium molecular weight: βlg = β-lactoglobulin, βlg-D20 = β-lactoglobulin-dextran (M_w = 20kDa), βlg-D35 = β-lactoglobulin-dextran (M_w = 35kDa), βlg-D65 = β-lactoglobulin-dextran (M_w = 65kDa), βlg-D150 = β-lactoglobulin-dextran (M_w = 150kDa) and βlg-D440 = β-lactoglobulin-dextran (M_w = 440kDa). Adsorbed layer thickness taken from the difference between naked and covered polystyrene latex.

Figure 4 shows that there is a correlation between the increase in layer thickness and dextran solution hydrodynamic diameter. However, as the molecular weight of the dextran increases, there is a difference between the solution hydrodynamic diameter and the steric layer thickness. The difference could be because of artifact generated by the assumption that the particle is spherical. In solution, dextran behaves as an ellipsoid with one axis longer than the other two. However, DLS treats dextran as a sphere and averages all three axes, resulting in a smaller apparent hydrodynamic diameter. When dextran is conjugated to β-lactoglobulin, it is possible that the long axis of dextran is perpendicular to the latex surface. In this case, the size that is measured is the long axis and this gives rise to the difference in size between the two measurements. Alternatively, the difference could result from a change in dextran conformation caused by the conjugate adsorbing onto the latex. When the conjugate is adsorbed onto latex, the osmotic pressure of the microenvironment of the dextran is higher than that of dextran in the bulk. This might cause the dextran to elongate to increase solvent interaction.

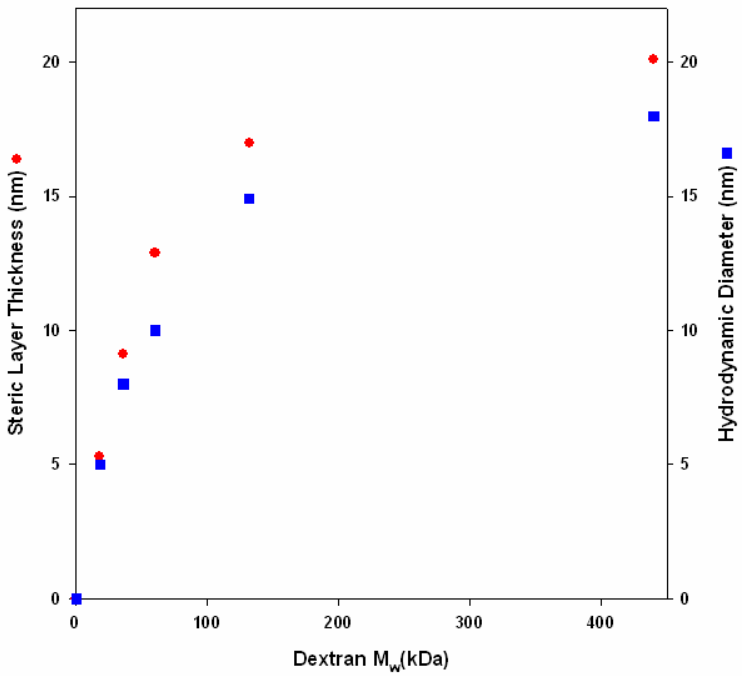

Figure 4: Correlation between dextran molecular weight and dextran steric layer thickness and hydrodynamic diameter. Dextran hydrodynamic diameters were determined by DLS measurements on 5mg/ml solutions in 50mM NaCl. The dextran steric layer thickness was defined as the increase in adsorbed layer thickness above that of β-lactoglobulin (2.9nm)

Conclusions

Dynamic light scattering is a useful technique for studying the adsorption of molecules onto the surface of particles. The work summarized in this application note has measured the effect of dextran attachment to β-lactoglobulin as a function of dextran molecular weight on the interfacial thickness of these emulsifiers. The results show that the attachment of dextran to β-lactoglobulin increased steric layer thickness. There was a high correlation between the increase in steric layer thickness and dextran solution hydrodynamic diameter suggesting that there was minimal change to dextran structure upon attachment.

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Zetasizer Nano

The Zetasizer Nano system from Malvern Instruments is the first commercial instrument to include the hardware and software for combined static, dynamic, and electrophoretic light scattering measurements. The wide range of sample properties available for measurement with the Nano system include, particle size, molecular weight, and zeta potential.

The Zetasizer Nano system was specifically designed to meet the low concentration and sample volume requirements typically associated with pharmaceutical and biomolecular applications, along with the high concentration requirements for colloidal applications. Satisfying this unique mix of requirements was accomplished using a backscatter optical system and a novel cell chamber design. As a consequence of these features, the Zetasizer Nano specifications for sample size and concentration exceed those for any other commercially available dynamic light scattering instrument, with a size range of 0.6nm to 6µm, and a concentration range of 0.1ppm to 40% w/v.

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