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Structural Aspects of β -Lactoglobulin during Self-assembly into Amyloid-like Fibrils

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Anant Chandrakant Dave

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Abstract

This study explores the structural characteristics of β -lactoglobulin (β -Lg) during its self-assembly into long amyloid-like fibrils on heating at low pH and low ionic strength. β -Lg (1%, w/v) was heated at 80°C, pH 2 and low ionic strength and the different processes occurring during self-assembly were characterized using a variety of techniques including circular dichroism spectroscopy, sodium dodecyl sulfate polyacrylamide gel electrophoresis, and mass spectrometry.

The results of this study indicate that fibril formation from β -Lg self-assembly consists of four processes: 1) protein unfolding; 2) heat- and acid- induced protein hydrolysis 3) nuclei formation and; 4) growth of nuclei into mature fibrils by peptide self-assembly. It was found that the heat-induced unfolding of β -Lg and its acid hydrolysis promoted self-assembly by removing structural constraints and generating assembly-capable peptides. The peptides from the N-terminal region (1-53) of β -Lg were found to play an important role during nucleation and may form the core of the fibrils. The characterization of fibril composition strongly indicated the presence of disulfide bonding in fibrils and the native disulfide bond Cys66-Cys160 in β -Lg appeared to be conserved during fibril formation.

The substitution of amino acid residues in β -Lg variants A, B and C did not significantly affect the kinetics of different self-assembly processes. The fibrils from β -Lg A, B and C had similar morphology, but were slightly different in their peptide compositions. The latter may be explained on the basis of sites of genetic substitutions, in particular, the Asp64 of β -Lg A that is Gly in variants B and C. In comparison, glucosylation and lactosylation of β -Lg strongly inhibited fibril formation primarily by inhibition of peptide self-assembly due to the steric conformational

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restrictions. The inhibitory effect of glycation varied with the type of sugar and the degree of glycation. Lactosylation produced a stronger effect than glucosylation, but glycation with either sugar did not appear to have any effect on the morphology of fibrils.

The modification of the aqueous phase composition by glycerol and sorbitol (0-50 % w/v) greatly decreased the rate of β -Lg self-assembly and the effect of glycerol and sorbitol on β -Lg self-assembly was concentration-dependent. Sorbitol inhibited the self-assembly by stabilizing β -Lg against unfolding and acid-hydrolysis, resulting in fewer fibril-forming peptides, whereas glycerol inhibited peptide self-assembly without affecting unfolding and acid-hydrolysis. Although, both polyols increased the viscosity of the solutions, viscosity did not affect the self-assembly of peptides, indicating that, under these conditions, the self-assembly was not diffusion-limited.

The effects of β -casein on β -Lg self-assembly were investigated by heating β -Lg- β -casein mixtures (molar ratios 1:0.0625 to 1:1). Heating under fibril-forming conditions resulted in acid hydrolysis of both proteins at approximately equal rate. β -Casein produced a small but consistent effect in inhibiting β -Lg self-assembly in heated β -Lg- β -casein mixtures. The transmission electron microscopy images of solutions showed irregular, coiled and ribbon-like structures co-existing with the β -Lg fibrils. These aggregates were absent in respective heated control samples of either protein indicating that β -Lg assembly-competent peptides have alternate competing pathway during self-assembly. The limited effect of β -casein on β -Lg self-assembly may be explained by the aggregation of β -casein peptides by a separate alternate pathway which competed with their interaction with β -Lg peptides.

Overall, the findings of this study have advanced our understanding of the mechanisms of self-assembly of globular proteins and provided insights into the ways to decouple self-assembly processes. This may help to design protocols for the control of globular protein self-assembly and extend the functionality and applications of protein fibrils.

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- Dave, A. C., Loveday, S. M., Anema, S. G., Loo, T. S., Norris, G. E., Jameson, G. B., & Singh, H. (2013). β -Lactoglobulin self-assembly: Structural changes in early stages and disulfide bonding in fibrils. *Journal of Agricultural and Food Chemistry*, 61(32), 7817-7828.
- Dave, A. C., Loveday, S. M., Anema, S. G., Jameson, G. B., & Singh, H. (2014). Modulating β -lactoglobulin self-assembly into nanofibrils at pH 2 using polyols. *Biomacromolecules*, 15(1), 95-103.
- Dave, A. C., Loveday, S. M., Anema, S. G., Jameson, G. B., & Singh, H. (2014). Glycation as a tool to probe the mechanism of β -lactoglobulin self-assembly. *Journal of Agricultural and Food Chemistry*. 62(14), 3269-3278.
- Dave, A. C., Loveday, S. M., Anema, S. G., Jameson, G. B., & Singh, H. ***In press***. Formation of nano-fibrils from the A, B and C variants of β -lactoglobulin. *International Dairy Journal*.