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Acidic residue modifications restore chaperone activity of β-casein interacting with lysozyme

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ABSTRACT

An important factor in medicine and related industries is the use of chaperones to reduce protein aggregation. Here we show that chaperone ability is induced in β-casein by modification of its acidic residues using Woodward's Reagent K (WRK). Lysozyme at pH 7.2 was used as a target protein to study β-casein chaperone activities. The mechanism for chaperone activity of the modified β-casein was determined using UV–vis absorbencies, fluorescence spectroscopy, differential scanning calorimetry and theoretical calculations. Our results indicated that the β-casein destabilizes the lysozyme and increases its aggregation rate. However, WRK-ring sulfonate anion modifications enhanced the hydrophobicity of β-casein resulting in its altered net negative charge upon interactions with lysozyme. The reversible stability of lysozyme increased in the presence of WRK-modified β-casein, and hence its aggregation rate decreased. These results demonstrate the enhanced chaperone activity of modified β-casein and its protective effects on lysozyme refolding.

1. Introduction

Casein belongs to a family of phosphoproteins, which are suitable for use in a variety of food products with protective roles in milk and in the mammary gland [1–3]. Caseins exhibit unique structural characteristics. They are neither globular nor fibrous proteins in nature and, they don't have well-defined secondary and tertiary structures [4]. Caseins are extremely flexible and are essentially unfolded [5]. There are specific differences among the four main caseins including charge, hydrophobicity, and calcium sensitivity [6]. β-casein and α_s-casein have molecular chaperone-like properties [7,8]. Caseins are also shown to decrease turbidity of whey proteins under stress conditions [9]. α_s-casein exhibits a considerable anti-aggregation activity [4,7,8]. However, the mechanisms involved, and more specifically the contribution of charge–charge interactions, in chaperone activity needs further investigation. The primary structure of β-casein has a highly amphiphilic character [10], which is crucial for its function in aggregation and micellization processes [11]. β-casein also acts as a surfactant molecule in solution [3] and is considered a natural detergent [3]. There is a short N-terminal hydrophilic polar domain in β-casein chain, which carries most of the protein's net charge (mainly negative), and a prominent C-terminal hydrophobic domain [12,13]. Recently, a novel function for β-casein has been reported, namely a molecular chaperone that protects many proteins against heat and chemical induced aggregation [14–17]. The β-casein hydrophilic and hydrophobic domains enhance solubility in aqueous medium and allow binding of hydrophilic molecules, respectively [18,19].

The approach applied throughout this study was that of the chemical modification of β-casein, especially that of carboxyl residues (aspartate and glutamate residues on the surface), which were specifically selected for their effects on diminishing lysozyme aggregation. The carboxylic side chains of glutamate and aspartate residues on the β-casein surface were modified using Woodward's Reagent K (WRK), an isoxosolium salt [20–28]. The reaction of WRK with a carboxylate is outlined in Scheme 1 [29].

Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; DTT, dithiothreiotol; WRK, Woodward's reagent K; EP, electrostatic potential; ASA, accessible surface area.

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