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Short communication

A differential scanning calorimetric study of the influence of copper and dodecyl trimethyl ammonium bromide on the stability of bovine α-lactalbumin

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Abstract

Bovine α-lactalbumin (α-LA) has been studied by differential scanning calorimetry (DSC), fluorescence spectroscopy and viscometry with various concentrations of Cu^{2+} and DTAB to elucidate the effect of these ligands on its thermal properties. The DSC profile of dialyzed form of α-lactalbumin (m-α-LA) contrary to the undialyzed form (holo-form, h-α-LA) shows two temperature induced heat absorption peaks. The m-α-LA is not a new form of α-LA. It contains mixture of the apo (a-α-LA) and holo (h-α-LA) forms of α-LA at low and high temperatures, respectively. Therefore, these two states of α-LA (apo and holo) are equilibrating with together after dialyze experiment. The Cu^{2+} as a metal ion and DTAB as a non metal ion alter the two heat-absorption peaks, in such a manner that, the addition of Cu^{2+} to the m-α-LA increases partial molar heat capacity and enthalpy change values of the h-α-LA form at high temperature because the molecular population of the a-α-LA form changes into the h-like-α-LA. On the contrary, the interaction between the DTAB and the m-α-LA increases these thermodynamic values for the a-α-LA at low temperature. However, DTAB bound to m-α-LA prevents from Ca^{2+} binding to protein, because there are positive charges repulsion between them. The high temperature peak occurs at the same temperature as the unfolding of the h-α-LA, while the low temperature peak lies within the temperature range associated with the unfolding of the a-α-LA. The R_s values of m-α-LA, h-α-LA and a-α-LA forms confirmed the folding and unfolding of the m-α-LA during the addition of Cu^{2+} and DTAB at different concentration, respectively.

Keywords: Ion binding protein; α-Lactalbumin; Apo and holo forms; Differential scanning calorimetry; Thermodynamics; Structure changes

1. Introduction

The α-lactalbumin (α-LA) is a 14300 Da acidic milk protein, which is the specifier component of lactose synthase in the lactating mammary gland [1,2]. Its physical characteristics and folding properties are significantly affected by specific interactions with Ca^{2+}. It is a metal-binding protein, which binds Ca^{2+} and Na^{+} ions competitively to one specific site, giving rise to a large conformational change of the protein. The removal of bound calcium greatly decreases the thermal stability of α-LA but the protein retains essentially the same folded conformation [3,4]. One of the most interesting properties of α-LA is its transition to the molten globule state [5–7]. At elevated temperatures above the thermal denaturation transition (ca. $T = 323$ K for apo-protein), at intermediate denaturant concentrations (3–4 M urea), or when Ca^{2+} dissociates from α-LA at acidic pH (<3), the protein adopts the molten globule conformation, which has been described as a compact state containing a significant degree of the secondary structure present in the native pro-

Abbreviations: a-α-LA, apo form of α-LA; DSC, differential scanning calorimetry; DTAB, dodecyl trimethyl ammonium bromide; h-α-LA, holo (undialyzed) form of α-LA; α-LA, α-lactalbumin; m-α-LA, dialyzed form of α-LA (mixture of apo-like and holo-like forms); R_s, Stoke radius

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tein, but with fluctuating tertiary structure [8]. At neutral pH and in the presence of calcium, α-LA unfolds cooperatively at high temperatures, with significant increase of enthalpy and heat capacity [9]. At lower pH, α-LA unfolds in two stages [9,10]. The first stage is highly cooperative and proceeds with significant and sharp heat absorption, but at this stage the heat capacity does not reach the value expected for the fully unfolded polypeptide chain [8]. This value is reached at the second stage, which proceeds without a significant heat absorption peak. It is therefore unclear whether this second stage represents a cooperative process with a small enthalpy, or is a gradual process [10]. Analysis of the change of optical properties associated with these two stages, of their dependence on the presence of calcium and comparison with the unfolding of the structurally related equine lysozyme, permits the assignment of the first cooperative stage to the unfolding of the β-domain of α-LA and the second stage to the unfolding of the α-domain [9,10]. The presence of domains that have temperature-dependent unfolding was also verified by fluorescence studies [11]. However, it was unclear as to how independent are these domains, i.e., whether one can fold and be stable without the other. This point needs to be clarified in order to understand the nature of the intermediate state of α-LA, which is usually regarded as a liquid-like "molten globule" state [12,13], rather than a partly unfolded state of a two-domain protein with one retained domain.

Yutani et al. [14] have studied calorimetrically the calcium-free a-α-LA, which is supposed to be in the molten globule state in low ionic strength solutions: they did not observe any excess heat effect upon heating. It was therefore assumed that this state of α-LA is close to the unfolded state [7]. However, Relkin et al. [15] studied α-LA at low concentrations of calcium using a Perkin-Elmer scanning calorimeter: they observed two heat absorption peaks upon heating the solution. The first peak was attributed to the thermal denaturation of the a-α-LA form and the second one to the h-α-LA form.

This partly unfolded state occurs because removal of Ca^{2+} results in the appearance of strong repulsive forces between uncompensated negative charges at the calcium-binding site. In the presence of monovalent salts a-α-LA has a native like structure that unfolds cooperatively upon heating with significant heat absorption, although at much lower temperatures than the h-α-LA [3,9]. The Ca^{2+} binding is necessary for the native folding of α-LA, and the structure of the metal ion-depleted form is a typical molten globule [16–19]. Although the structure of a-α-LA has been frequently investigated, the structure of Zn^{2+}-h-α-LA in solution is not well known. Berliner and co-workers suggested that Zn^{2+} binding to Ca^{2+}-bound α-LA (h-α-LA) shifts the structure towards a new apo-like conformer [20–25]. The intrinsic fluorescence spectrum of h-α-LA is shifted to that of a-α-LA by the second ion binding. The binding constant of the hydrophobic fluorescent probe for h-α-LA also increased when Zn^{2+} was bound to h-α-LA. On the other hand, results from X-ray crystallography revealed that the crystal structure of Zn^{2+}-bound h-α-LA is not significantly different from that of h-α-LA [26].

In this paper we demonstrate, using sensitive scanning microcalorimetry techniques, that a solution of α-LA at pH 8.0 in dialyzed condition represents a mixture of apo-α-LA (a-α-LA) and holo-α-LA (h-α-LA) forms that do not readily interconvert, and therefore unfold at different temperatures. The interaction between α-LA and ions (Cu^{2+} and DTAB) show that these ions change the partial heat capacity and enthalpy change values of a-α-LA and h-α-LA forms. These calorimetric data permit a reliable determination of the molecular population partition for a-α-LA and h-α-LA forms interaction with Cu^{2+} and DTAB, a quantity difficult to obtain by other methods.

2. Materials and methods

2.1. Materials

Bovine α-LA was obtained from Merck Chemical Co. Purity of the protein was monitored by PAGE under native and denatured conditions. The concentration of protein solution was measured spectrophotometrically using an extinction coefficient of $E^{\%1}$ (280 nm) = 20.9 [27] with correction for light-scattering effects. A-α-LA was prepared by previously described procedures [28]. The concentrations of a-α-LA and m-α-LA were measured by Bradford assay [29] and Stoschek method's [30]. Dodecyl trimethyl ammonium bromide (DTAB) and copper sulfate (Cu^{2+}) were obtained from Sigma and Merck, respectively. Visking membrane dialysis tubing (molar mass cut-off 10,000–14,000 g mol^{-1}) was obtained from SIC, Eastleigh, Hampshire, UK. All other materials and reagents were of analytical grade, and solutions were made in double-distilled water. Tris solution of concentration 10 mmol dm^{-3}, pH 8.0 was used as a buffer.

Visking tubing, as the semipermeable membrane, was boiled three times, each time for 15 min in ethylendiaminetetraacetic acid (EDTA) and sodium bicarbonate and then washed several times with distilled water and stored in (0.2 C_2H_5OH + 0.8 H_2O). Bovine α-LA solution was dialyzed against buffer (Tris, 10 mM, pH 8.0). The buffer had been changed with new buffer every 8 h, m-α-LA was ready after 24 h.

2.2. Methods

2.2.1. Fluorescence measurements

Fluorescence measurements were made on Jasco SP-6200 spectrofluorometer at an excitation wavelength of 280 nm (while identical spectral line shapes were observed over the excitation wavelength range 280–295 nm, it was experimentally preferable to use a 280 nm excitation to reduce any light scattering problems in the emission spectra on this instruments). Trp fluorescence for apo-α-LA (a-α-LA) and holo-α-LA (h-α-LA) forms was followed at 335 and 325 nm,