α-to-β Structural Transformation of Ovalbumin: Heat and pH Effects

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Ovalbumin is an important member of the serpin superfamily without inhibitory activity. The heat- and pH-induced α-to-β structural transformations of ovalbumin were investigated by means of circular dichroism and binding of ANS and Congo red dyes. The native ovalbumin shows a mixture of α-helix and β-sheet, while both the heat and alkali treatments are able to transform the native protein into a predominance of β-sheet secondary structure. The free energy changes during transitions to the unfolded state are 5.19 kcal/mol from the native state and 4.00 kcal/mol from the heat-treated one. The binding abilities of the heat-treated and the alkali-treated forms to ANS and Congo red suggest that the altered forms exhibit hydrophobic exposure and intermolecular interaction. The results substantiate that the altered protein forms bearing increased β-sheet structures are prone to aggregation, which is implicated in the pathogenesis of some conformational diseases.

KEY WORDS: Ovalbumin; structural transformation; aggregation; heat; pH.

1. INTRODUCTION

The thermodynamic hypothesis of protein folding holds that the native conformation of a protein is at a free energy minimum, that is, a protein under a physiological condition must maintain a substantial conformation, and the unique structure of the protein directly governs its biological function. Recently, research has concentrated on the kinetic pathway that may be involved in controlling the folding processes (Baker and Agard, 1994). Serpins,¹ which inhibit the serine proteinase which functions to regulate coagulation and fibrinolysis, provides a system to address the problem. Folding of a serpin protein is a process under kinetic control (Wang et al., 1996). The conformation of the reactive center loop varies strikingly. Proteolytic cleavage can result in a dramatic structural transformation. The cleaved reactive loop may switch to a β-strand inserting into the preexisting β-sheet structure (Wright, 1996).

Ovalbumin is a noninhibitory serpin, despite homology to the serpin superfamily both in sequence and three-dimensional structure. It is the major globular protein of chicken egg white. The molecular weight is ca. 45 kDa (43 kDa deduced from the amino acid sequence) including carbohydrates and a cystine disulfide (C73/C120). The crystal structure shows that the native ovalbumin is mainly comprised of a mixture of α-helix and β-sheet, and a flexible loop-helix-loop motif constitutes the reactive center (Stein et al., 1991). Interestingly, the native ovalbumin can spontaneously transform into the S-form during storage of eggs. The S-form can also be obtained by heating the native protein at 56°C and pH 10 (Huntington et al., 1995). Recent study suggests that

¹ Abbreviations: AL-form, alkali-treated form; ANS, 1-anilinonaphthalene-8-sulfonic acid; ASA, accessible surface area; BSA, bovine serum albumin; CD, circular dichroism; Cm, midpoint; CR, Congo red; GdmCl, guanidinium chloride; H-form, heat-treated form; I-form, inhibitory form; N-form, native form; serpin, serine proteinase inhibitor; S-form, storage form; Tris, 2-amino-2 (hydroxymethyl)-1,3-propane-diol; U-form, unfolded form.

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heating can transform it into an inhibitory ovalbumin (I-form), which is a potent reversible competitive inhibitor of some proteinases (Mellet et al., 1996).

Here we report extensive research on the novel α-to-β transition of ovalbumin induced by heat and pH treatment and compare the structural properties of these variant forms. The results may provide insight into the mechanism of structural transformation and aggregation of proteins.

2. MATERIALS AND METHODS

2.1. Materials

Ovalbumin (chicken egg albumin) was purchased from Sigma (Grade VII, S-ovalbumin free). Congo red (CR) and bovine serum albumin (BSA) were also from Sigma. Ultra guanidinium chloride (GdmCl) was purchased from USB. ANS was a product of BDH. All other reagents were of analytical quality.

2.2. Sample Preparation

Ovalbumin was dissolved in a Tris-HCl buffer (25 mM Tris, 50 mM NaCl, pH 7.4) for all experiments except where indicated. The concentration was determined by the Bradford method with BSA as a standard. The extinction coefficient of the native ovalbumin (N-form) at 280 nm is 2.21 (mg/ml)\(^{-1}\). The heat-treated form (H-form) was prepared by heating a 1.0 mg/ml aqueous solution (pH 6.6) at 80\(^\circ\)C for 30 min and then rapidly cooling it on an ice bath. No precipitation occurred in administering the protein solution. After centrifugation, its concentration was unchanged. In order to avoid nonspecific precipitation probably prompted by high ionic strength, aqueous solution without salt was required in all the heating procedures. The stable H-form ovalbumin was then diluted with the Tris-HCl buffer for further experiments. The pH-treated forms were prepared by adjusting the aqueous solution to acidic (pH 2.0) or alkaline (pH 12.0) condition. After incubation at 25\(^\circ\)C for more than 1 h, the samples were readjusted to a central pH and diluted with Tris-HCl buffer to a final pH 7.4.

2.3. Circular Dichroism Spectra

Far-UV CD measurements were performed on a JASCO-715 spectropolarimeter with a computer data processor. Spectra were recorded over 190–250 nm using a cuvette of 1 mm pathlength at a scan speed of 10 nm/min and a time constant of 0.125 sec. Data were further processed for noise reduction, baseline subtraction, and signal averaging when needed. All data were presented as mean residual molar ellipticities \([\theta]\). The secondary structure contents were estimated from the CD spectra according to Yang’s method (Yang et al., 1986). Near-UV CD spectra were recorded over 320–250 nm with 10 nm pathlength.

2.4. Equilibrium Unfolding

The protein was incubated with different concentration of GdmCl for 1 h at 25\(^\circ\)C to reach equilibrium. According to the two-state hypothesis, the fractions of the folded and the unfolded state can be obtained by normalizing the CD ellipticities. We calculated the unfolding constant \(K_u\) and the Gibbs free energy change \(\Delta G^{\text{H}_2\text{O}}\) of the unfolding process \(\Delta G = -RT \ln K_u\).

2.5. Fluorescence Measurements

Aliquots of the native or the altered form of ovalbumin were titrated to an ANS solution. The fluorescence enhancement of ANS by binding to different forms of ovalbumin was determined by a fluorophotometer (Hitachi F-4500) at 25\(^\circ\)C. The excitation wavelength was 350 nm, and the emission fluorescence at 480 nm was recorded.

2.6. Binding with CR

The protein samples with different concentrations were incubated with a 40 \(\mu\)M CR solution at 25\(^\circ\)C for 30 min. The maximum absorbance of free CR was 490 nm and that of the protein-bound CR shifted to 541 nm. We recorded the absorbance at 490 and 541 nm and calculated the ratio of \(A_{541}/A_{490}\) for evaluating the binding ability (Klunk et al., 1999).

3. RESULTS

3.1. CD Spectra Showing Secondary Structures

Figure 1A shows the far-UV CD spectra of the native form (N-form) and the heat-treated form (H-form) of ovalbumin. The N-form shows a double-peak spectrum in which the negative peak at 222 nm is slightly larger than that at 208 nm, suggesting that the spectrum originates from a mixture of α-helix and β-sheet structures. The H-form, however, gives a broad negative peak around