Conformational Changes of α-Lactalbumin and Its Fragment, Phe³¹–Ile⁵⁹, Induced by Sodium Dodecyl Sulfate

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Conformational changes of bovine α-lactalbumin in sodium dodecyl sulfate (SDS) solution were studied with the circular dichroism (CD) method using a dilute phosphate buffer of pH 7.0 and ionic strength 0.014. The proportions of α-helix and β-structure in α-lactalbumin were 34% and 12%, respectively, in the absence of SDS. In the SDS solution, the helicity increased to 44%, while the β-structure disappeared. In order to verify the structural change from β-structure to α-helix, the moiety, assuming the β-structure in the α-lactalbumin, was isolated by a chymotryptic digestion. The structure of this α-lactalbumin fragment, Phe³¹–Ile⁵⁹, was almost disordered. However, the fragment adopted a considerable amount of α-helical structure in the SDS solution. On the other hand, the tertiary structure of α-lactalbumin, detected by changes of CD in the near-ultraviolet region, began to be disrupted before the secondary structural change in the surfactant solution. Dodecyl sulfate ions of 80 mol were cooperatively bound to α-lactalbumin. Although the removal of the bound dodecyl sulfate ions was tried by the dialysis against the phosphate buffer for 5 days, 4 mol dodecyl sulfates remained per mole of the protein. The remaining amount agreed with the number of stoichiometric binding site, determined by the Scatchard plot, indicating that the stoichiometric binding was so tight.

KEY WORDS: α-Lactalbumin, protein fragment, sodium dodecyl sulfate, secondary structure, circular dichroism.

1. INTRODUCTION

The conformation of α-lactalbumin has been the focus of much recent research (Kuwajima et al., 1990; Lala and Kaul, 1992). It has been noted that this protein is a Ca²⁺-binding metalloprotein and has a similar amino acid sequence to chicken egg-white lysozyme (Hiraoka et al., 1980; Kuwajima et al., 1985). Recently, we have found that the helicity of α-lactalbumin increases, while the β-structure almost disappears in a solution of sodium dodecyl sulfate (SDS) (Takeda and Moriyama, 1990). Few attempts have been conducted to investigate conformational changes of the protein in surfactant solutions. However, many studies have been made on the conformational changes of proteins due to the interaction with surfactants (Steinhardt and Reynolds, 1969; Jones, 1975; Lapanje, 1978; Takeda et al., 1987). The denaturing action of the surfactants, such as SDS, arises from their ability to weaken or disrupt the original hydrophobic interactions in a protein structure (Bruning and Holtzer, 1961; Nozaki and Tanford, 1963; 1970; Herskovit et al., 1978).

In the present study, the conformational change of α-lactalbumin in the SDS solution was examined in detail. This paper shows that the β-structure-to-α-helix transition occurs in a particular moiety of the protein on the basis of the examination of a fragment containing the segment which originally adopts the β-structure.

2. MATERIALS AND METHODS

Crystalline α-lactalbumin (L6010) and α-chymotrypsin (C4129) were obtained from Sigma. A
phosphate buffer of pH 7.0 and ionic strength 0.014 was exclusively used in the measurements.

2.1. Chymotryptic Digestion of α-Lactalbumin and Isolation of the Fragment

Incubation of α-lactalbumin with α-chymotrypsin was performed for 30 min at pH 4.0 and stopped by the addition of diisopropyl fluoro phosphate of pH 7.0. The digests were dialyzed against the phosphate buffer and then concentrated using a freeze drier. The isolation of the desired fragment was carried out with a fast protein liquid chromatography (FPLC) system (Pharmacia) in two stages. First, the digests were applied to an ion exchange column, Mono QHR-5/5 (Pharmacia). The procedure was repeated using an autoinjector, because the FPLC system had a low loading capacity. Four major peaks were obtained in the elution pattern. When the fractions were numbered according to increasing retention time, the second fraction contained the desired fragment (the first and the fourth fractions corresponding to the intact α-lactalbumin and α-chymotrypsin, respectively). Second, the fragment was purified by applying the second fraction to a gel filtration column, Superose 12 (Pharmacia).

2.2. Determination of N- and C-Terminals of the Fragment by Amino Acid Analysis

The analysis of plural amino acid residues was made on sequences at the N-terminal and C-terminal of the fragment. The N-terminal amino acids were analyzed by repeating the Edman method (Fraenkel-Conrat et al., 1954). Amino acids released by the method are in the phenylthiohydantoin (PTH) form. The PTH amino acids were analyzed using a high-performance liquid chromatograph (Hitachi HPLC 655A) connected to a LiChroCART manufix 250-4 column (Merck). Amino acids at the C-terminal were cleaved with carboxypeptidases A and B (Fraenkel-Conrat et al., 1954). The resulting free amino acids were then analyzed using HPLC with an ion exchange column for amino acid analysis (Hitachi 2617F). The location of the fragment was finally determined to be Phe31–Ile59 in the entire sequence of α-lactalbumin. The extinction coefficient of the fragment was determined by weight using the lyophilized fragment sample of mass 50–100 mg. The extinction coefficient was determined to be 3200 at 280 nm.

2.3. CD and Absorbance Measurements

CD and absorbance measurements were made at 25°C with a JASCO J-600 spectropolarimeter and Hitachi spectrophotometer U-3200, respectively. Throughout the measurements, the concentrations of α-lactalbumin and fragment were kept at 10 μM. The relative proportions of secondary structures were calculated by the curve-fitting method of CD spectrum (Greenfield and Fasman, 1969; Chen et al., 1974; Yang et al., 1986). The CD spectrum was simulated in the wavelength region 200–240 nm at 1-nm intervals (Takeda et al., 1987; 1988a–c), using the reference spectra for α-helix, β-structure, and unordered structure as determined by Chen et al. (1974).

2.4. Binding Isotherm

Binding isotherm was obtained using HPLC connected to a GL-W550 column (Hitachi) (Takeda et al., 1988b). The SDS concentration was determined by the colorimetry of the methylene blue-dodecyl sulfate complex extracted with chloroform (Takeda et al., 1981, 1988b).

3. RESULTS AND DISCUSSION

About 80 mol of dodecyl sulfate ions were cooperatively bound to α-lactalbumin. In order to compare CD data, the progress of dodecyl sulfate binding against 10 μM protein is shown in Fig. 1A as a function of the total concentration of SDS. The amount of bound dodecyl sulfates increased stepwise. The first binding was completed until 5 mM SDS and the saturated binding was attained around 8 mM SDS. The saturation of the binding occurred at relatively high SDS concentrations because of low concentrations of salts composing the buffer. The binding profile in a low SDS concentration range was examined by the application of the Scatchard equation.

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r/C = K_n - K_r
\]

where \( r \) is the number of moles of surfactant ions bound per mole of protein, \( C \) is the free concentration of surfactant ion, \( n \) is the number of equivalent binding sites per protein, and \( K \) is the intrinsic bind-