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Conversion of Human α -lactalbumin to an Apo-like State in the Complexes with Basic Poly-Amino Acids: Toward Understanding of the Molecular Mechanism of Antitumor Action of HAMLET

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It was recently shown that α -lactalbumin associated with oleic acid (HAMLET) interacts with core histones thereby triggering apoptosis of tumor cells (*J. Biol. Chem.* **2003**, *278*, 42131). In previous work, we revealed that monomeric α -lactalbumin in the absence of fatty acids can also interact with histones and, moreover, with basic poly-amino acids (poly-Lys and poly-Arg) that represent simple models of histone proteins (*Biochemistry* **2004**, *43*, 5575). Association of α -lactalbumin with histone or poly-Lys-(Arg) essentially changes its properties. In the present work, the character of the changes in structural properties and conformational stability of α -lactalbumin in the complex with poly-Lys(Arg) has been studied in detail by steady-state fluorescence, circular dichroism, and differential scanning calorimetry. Complex formation strongly depends on ionic strength, confirming its electrostatic nature. Experiments with the poly-amino acids of various molecular masses demonstrated a direct proportionality between the number of α -lactalbumin molecules bound per poly-Lys(Arg) and the surface area of the poly-amino acid random coil. The binding of the poly-amino acids to Ca^{2+} -saturated human α -lactalbumin decreases its thermal stability down to the level of its free apo-form and decreases Ca^{2+} -affinity by 4 orders of magnitude. The conformational state of α -lactalbumin in a complex with poly-Lys(Arg), named α -Lactalbumin Modified by Poly-Amino acid (LAMP), differs from all other α -lactalbumin states characterized to date, representing an apo-like (molten globule-like) state with substantially decreased affinity for calcium ion. The requirement for efficient conversion of α -lactalbumin to the LAMP state is a poly-Lys(Arg) chain consisting of several tens of amino acid residues.

Keywords: α -lactalbumin • histones • poly-amino acids • electrostatics • thermal stability • calcium-binding

α -Lactalbumin is a small (Mr 14,200), acidic (pI 4–5) Ca^{2+} -binding protein that is very interesting from several points of view. First, it is one of two proteins comprising the lactose synthase enzyme complex (EC 2.4.1.22) that catalyzes the final step in lactose biosynthesis, which occurs in the Golgi lumen of the lactating mammary gland.¹ α -Lactalbumin is secreted from the mammary gland as a component of milk, where it may fulfill some other functions besides serving as just a food component. Second, α -lactalbumin has a single high affinity Ca^{2+} binding site,^{2,3} and it is frequently considered as a simple

model Ca^{2+} binding protein. Third, α -lactalbumin forms several partially folded intermediate states. At acidic pH and in the apo-state at elevated temperatures α -lactalbumin is the classic 'molten globule'.^{4,5} Fourth, it was found that some forms of α -lactalbumin can induce apoptosis in tumor cells^{6,7} and also possess bactericidal activity^{8,9} which suggests that it fulfills many important protective biological functions. Finally, it has been recently shown that α -lactalbumin is able to form amyloid fibrils at low pH, where it adopted the classical molten globule-like conformation.¹⁰

Recently, it was shown that α -lactalbumin complexed with oleic acid (HAMLET, human α -lactalbumin made lethal to tumor cells) interacts with core histones triggering apoptosis of tumor cells.¹¹ In our previous work, we showed that monomeric α -lactalbumin in the absence of fatty acids can also interact with both histones and basic poly-amino acids (i.e., poly-Lys and poly-Arg), which represent simple models of

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histone proteins.¹² Association of α -lactalbumin with histone or poly-Lys(Arg) was shown to change essentially the structural properties of the protein. The present work is devoted to the detailed examination of α -LA interaction with poly-L-Lys and poly-L-Arg and to the characterization of changes in structural properties and conformational stability induced in α -LA by interaction with these basic poly-amino acids. To this end, we characterize the α -lactalbumin complexed with basic poly-amino acids of varying chain lengths. An unusual, apo-like (molten globule-like) state of human α -lactalbumin was found in the complex with poly-Lys or poly-Arg. Even in the complex with calcium ions it had remarkably low thermal stability, which was close to that of native noncomplexed apo- α -LA, but in contrast to the intact apo-protein it had much lower affinity for calcium ions (i.e., decreased by 4 orders of magnitude). To distinguish this form from all other known states of α -lactalbumin, we have proposed classifying it as LAMPA (" α -lactalbumin modified by poly-amino acid").

Materials and Methods

Materials. α -Lactalbumin (lot no. 60K7002, bovine) was purchased from Sigma Chemical Co. (St. Louis, MO) or isolated and purified from human milk as described earlier.^{13,14} Protein concentrations were evaluated spectrophotometrically, using an extinction coefficient $E_{1\%,280\text{ nm}}$ of 20.1 for bovine protein and $E_{1\%,280\text{ nm}}$ of 18.2 for human α -lactalbumin.^{13,15} Poly-L-lysine hydrobromide and poly-L-arginine hydrochloride were purchased from Sigma Chemical Co. Degrees of polymerization for poly-amino acids used throughout the text represent arithmetic average of the values, determined by viscosimetry and low angle light scattering (LALLS) methods. Calcium chloride standard solution bought from Fluka was used for Ca^{2+} -titrations. All solutions were prepared using nano-pure or distilled, deionized water.

Methods. Fluorescence studies were performed on a Cary Eclipse spectrofluorimeter (Varian Inc.) or on an automated lab-built instrument described previously.¹⁶ Protein fluorescence was excited at 280 nm. All spectra were corrected for the spectral sensitivity of the instrument and fitted to log-normal curves¹⁷ using nonlinear regression analysis.¹⁸ The positions of the fluorescence spectrum maxima (λ_{max}) were obtained from these fits. Temperature scans were performed stepwise, allowing the sample to equilibrate at each temperature for at least 5 min. The temperature was monitored directly inside the cell. The fraction of conversion from the native to thermally denatured protein state was calculated from the plots of temperature dependence of emission intensity at a fixed wavelength as previously described.^{19–21}

In all fluorescence experiments, the α -lactalbumin illumination time and UV irradiation power level were minimized to avoid UV-induced structural rearrangements.^{14,22}

Circular dichroism measurements were performed on an AVIV 62DS or Jasco J-500A spectropolarimeter. Typical instrument conditions were: scan rate, 5 nm/min; time constant, 8 s. The path length was 10 mm for the near-UV studies. All data were baseline corrected.

Scanning calorimetry measurements were carried out using VP-DSC differential scanning microcalorimeter (Microcal Inc., Northampton, MA) at a 1 K/min heating rate in 10 mM HEPES–KOH buffer, pH 7.8. An extra pressure of 30 psi was maintained in order to prevent possible degassing of the solutions during heating. α -Lactalbumin concentration was 1 mg/mL. The heat sorption curves were baseline corrected. The

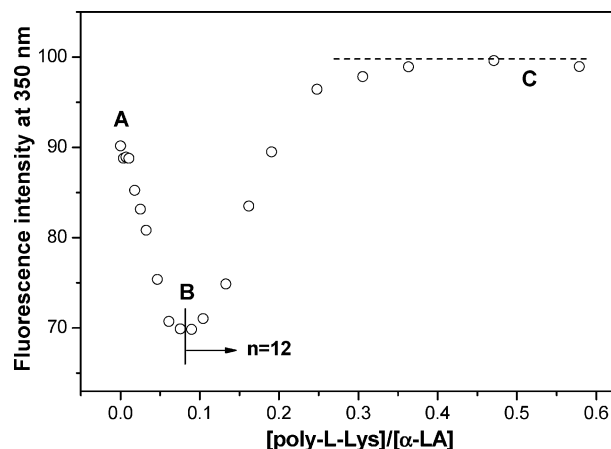


Figure 1. Spectrofluorimetric titration of Ca^{2+} -loaded human α -lactalbumin by poly-L-Lys ($N = 137$) at 5 °C (pH 7.7, 10 mM HEPES–KOH, 1 mM CaCl_2). The excitation wavelength was 280 nm. The α -LA concentration was 10 μM . Empty circles represent the fluorescence intensity at 350 nm in arbitrary units. **A**, **B**, and **C** denote characteristic levels of the titration curve. The stoichiometry of α -LA binding to poly-L-Lys, n , is determined as the value of $[\alpha\text{-LA}]:[\text{poly-L-Lys}]$ ratio, at which the fluorescence intensity reaches its minimal value. In this case, $n = [\alpha\text{-LA}]:[\text{poly-L-Lys}] = 12:1$.

contribution from the poly-amino acid was experimentally estimated and subtracted. α -Lactalbumin specific heat capacity (C_p) was calculated as described by Privalov & Potekhin.²³ Partial molar volume of the protein was calculated according to Hakel et al.²⁴ Temperature dependencies of C_p were fit to a *cooperative two-state scheme*, assuming that the difference between heat capacities of denatured and native protein states (ΔC_p) is independent of temperature (all values were normalized by protein molecular weight MW)

$$C_p = C_{p,D} - \Delta C_p / (1 + K) + \left[\frac{\Delta H_{\text{VH}} + \Delta C_p \cdot (T - T_0)}{T(1 + K)} \right]^2 \cdot K/R \quad (1)$$

where $K = \exp[(\Delta H_{\text{VH}} - \Delta C_p \cdot T_0)/R(1/T_0 - 1/T) + \Delta C_p/R \ln T/T_0]$, $R = 8.31/(n_c \cdot \text{MW})$.

Here, $C_{p,D}$ is a specific heat capacity of the denatured protein, linearly extrapolated to the transition region. Fitting parameters ΔC_p , ΔH_{VH} (van't Hoff's enthalpy of protein denaturation), T_0 (mid-transition temperature) and n_c (cooperativity coefficient) were estimated using Origin 5.0 software (MicroCal Inc.).

Calcium-selective electrode measurements were performed at room temperature with an Elit 8041 (NICO2000 Ltd.) calcium-selective electrode and EVL-1M3 reference electrode, on a cpX-2 pH-meter/ion-meter (IBI RAS, Pushchino). Changes in the free calcium level upon addition of poly-amino acids to α -lactalbumin solution were determined from a calibration curve.

Results and Discussion

The interaction of α -lactalbumin with basic poly-amino acids can be easily monitored by the changes in α -LA intrinsic fluorescence.¹² Figure 1 demonstrates a typical titration experiment. Addition of poly-L-Lys ($N = 137$) to Ca^{2+} -loaded human α -LA solution at 5 °C (where even apo- α -LA is in the native state) causes a sharp decrease in the fluorescence quantum yield from the initial level **A** down to a minimum level (level **B**) followed by an increase up to level **C**. The complicated shape

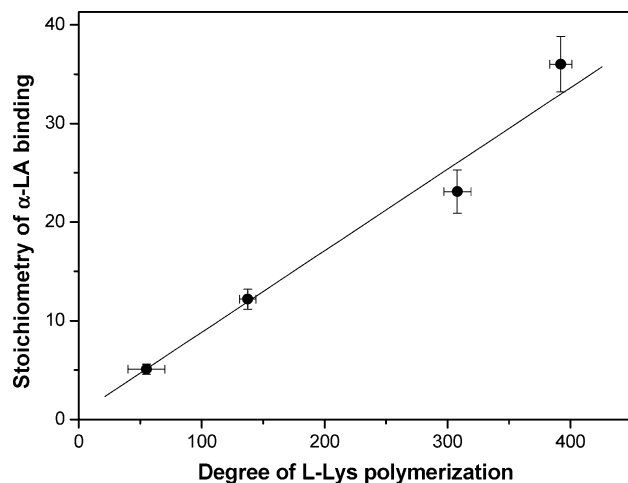


Figure 2. Stoichiometry of α -LA binding to poly-L-Lys determined as a number of human α -lactalbumin molecules bound per a poly-L-Lys molecule, n , versus chain length of the polymer, as measured by intrinsic α -LA fluorescence at 5 °C (pH 7.7, 10 mM HEPES–KOH, 1 mM CaCl_2). See Figure 1 for the definition of n .

of the titration curve is explained by the existence of several processes. The initial level **A** corresponds to the fluorescence intensity of free α -LA. Addition of poly-L-Lys results in the gradual formation of α -LA–poly-L-Lys complex, which is totally saturated by α -LA molecules at fairly low poly-L-Lys concentrations. Further addition of poly-L-Lys decreases the concentration of free α -LA molecules until level **B** is reached, which corresponds to the stoichiometric limit of α -LA:poly-L-Lys complex (in this case α -LA:poly-L-Lys = 12:1). A further increase in poly-L-Lys concentration results in the redistribution of α -LA molecules (i.e., presaturation) over an excess number of poly-L-Lys molecules. Finally, each α -LA molecule is bound to its own poly-L-Lys molecule (level **C**). It should be noted that super-saturation of poly-L-Lys with α -LA molecules is accompanied by a protein aggregation, which reversibly disappears upon increasing poly-L-Lys concentration.

It is reasonable to expect a direct proportionality between the number of α -LA molecules bound per poly-amino acid molecule and the surface area of the poly-amino acid random coil. Strictly speaking, the term “surface” with respect to a random coil is quite illusive. However, it still may be considered as an imaginary surface of sphere with radius, corresponding to the hydrodynamic radius of the random coil. The hydrodynamic size of the random coil is known to be proportional to the square root of the chain length N , regardless of the random coil model used (for review, see ref 25). Consequently, the area of the random coil surface should be directly proportional to N . Thus, the stoichiometry of α -LA binding to basic poly-amino acids should be proportional to their chain lengths. Indeed, our fluorescence measurements of human α -LA bound to a polymer with various chain lengths at 5 °C confirm this dependence within experimental accuracy (Figure 2). Analogous data were obtained for bovine α -LA (data not shown). Use of logarithmic scale reveals some deviation from this law for poly-L-Lys sample with $N = 10$. In fact, the mentioned above considerations are valid only for fairly long polymer chains.

The saturation of Ca^{2+} -loaded human α -lactalbumin with poly-L-Lys by the addition of equimolar concentration of the poly-amino acid ($N = 119$) results in more than a 40 °C decrease in the melting temperature of α -LA; i.e., down to a value characteristic of the apo-form (Figure 3). The results of

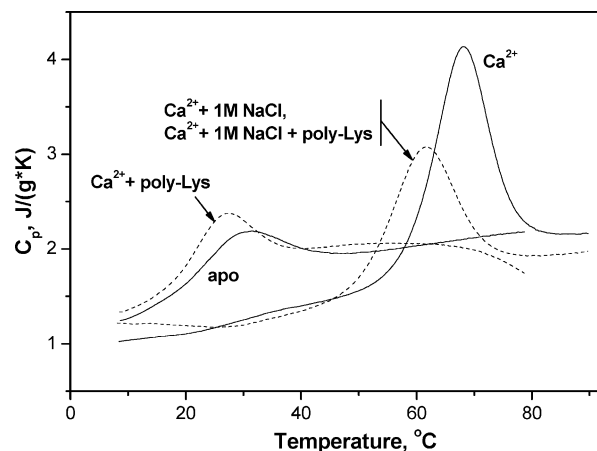


Figure 3. Specific heat capacity of human α -lactalbumin in the complex with poly-L-Lys ($N = 119$), estimated from scanning microcalorimetry data (pH 7.8, 10 mM HEPES–KOH). Concentration of calcium ions was controlled by addition of either 1 mM CaCl_2 (Ca^{2+}) or 1 mM EDTA (*apo*). Concentration of α -lactalbumin was 1 mg/mL. Poly-L-Lys was added in equimolar concentration. Heating rate was 1 K/min.

Table 1. Results of Fitting of the Scanning Microcalorimetry Data Presented in Figure 3, According to the Cooperative Two-State Scheme¹

human α -LA state	$t_{1/2}$, °C	ΔH_{VH} , $J/(g \cdot K)$	n
apo-state	25.4	10.0	0.93
Ca^{2+} -loaded + poly-L-Lys	24.0	8.8	1.53

fitting of the scanning microcalorimetry data to a cooperative two-state scheme (1) are presented in Table 1. α -LA complexed with poly-L-Lys is slightly less thermostable than apo- α -LA (by about 1.4 °C). At the same time, while the thermal denaturation of intact apo- α -LA can be fairly well described by a simple two-state scheme (cooperativity coefficient $n_c = 0.93$), thermal denaturation of the complex seems to be a more complicated process, demonstrating positive cooperativity ($n_c = 1.53$). The same conclusion can be drawn from fluorescence thermal melting experiments (data not shown). Furthermore, the cooperativity coefficient grows with increasing α -lactalbumin:poly-L-Lys molar ratio. For example, ~ 4 -fold molar excess of α -LA over poly-L-Lys gave rise to an increase in the cooperativity coefficient n up to 2.05. This observation suggests that the positive cooperativity arises due to some interactions between α -LA molecules within their complex with the poly-amino acid.

Analogous experiments in the presence of 1 mM EDTA were accompanied by significant protein aggregation, which did not permit reliable DSC data collection under these conditions. Only under the conditions of much lower protein concentration, while detecting the thermal transition by intrinsic α -lactalbumin fluorescence, we were able to find that the thermal stability of apo- α -LA in a poly-L-Lys complex was lowered by ~ 5 –7 °C.

It should be noted that the interaction of Ca^{2+} -loaded human α -lactalbumin with poly-L-Arg causes an even more pronounced drop in thermal stability compared with poly-L-Lys. The resulting complex melts at ~ 15 °C, precluding accurate DSC measurements.

The sharp drop in thermal stability of Ca^{2+} -loaded human α -lactalbumin was observed for poly-L-lysine of chain lengths

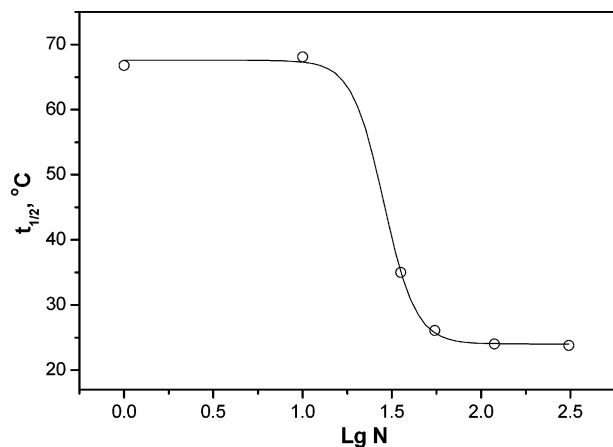


Figure 4. Dependence of half-transition temperature ($t_{1/2}$) for human α -lactalbumin complexed with poly-L-Lys on chain length of the polymer (N) (pH 7.8, 10 mM HEPES–KOH, 1 mM CaCl_2). The solid curve represents fit of experimental points (empty circles) by sigmoidal function.

exceeding 54 residues, and no measurable changes were observed for N values below 11 (Figure 4). The sigmoidal curve depicted in Figure 4 was fit to a mid-transition at $N = 28$. Thus, several tens of Lys residues are required to induce a significant decrease in α -LA thermal stability. Nevertheless, poly-L-lysine with lower molecular weights, despite inability to affect α -lactalbumin thermal stability, is actually capable of high affinity binding to α -LA. For example, fluorescence measurements performed for poly-L-lysine with $N = 10$ reveal binding of at least two α -LA molecules per a poly-L-Lys molecule.

The fact that gradual addition of poly-L-Lys shifts the thermal transition of Ca^{2+} -loaded human α -lactalbumin and that no distinct low- or high-temperature components were found implies that the rate of exchange between free α -LA molecules and the poly-amino acid complex was significantly faster than the heating rate in the thermal denaturation experiment.

Molar concentrations of monovalent salts eliminate the decrease in α -LA thermal stability upon complexation with poly-L-Lys, as shown in Figure 3 in the presence of NaCl (or KCl). The results suggest that electrostatic screening prevents formation of the complex, confirming the electrostatic nature of the α -lactalbumin – poly-L-Lys interaction.

Although the nature of these interactions appear to be similar for both human and bovine α -lactalbumin, the effect on the thermal stability of bovine α -LA¹² is quantitatively different than that observed for human α -LA. The thermal stability of Ca^{2+} -loaded bovine α -lactalbumin complexed with poly-L-Arg and poly-L-Lys decreases to 45 °C or 56 °C, respectively. This decrease is much less than the stability drop observed with the human protein ($t_{1/2} \approx 15$ °C or 25.4 °C, respectively). The most striking differences were observed with apo- α -LAs. Bovine apo- α -LA showed a 5 °C or 11 °C *increase* in thermal stability upon the binding to poly-amino acids, whereas the same interaction *destabilized* the human apo- α -LA. The thermodynamic behavior of bovine α -lactalbumin in poly-amino acid complexes resembles that of the desMet-form of D87N bovine α -LA.²⁶ That is, the removal of a negative charge at the carboxyl group of Asp87 in the Ca^{2+} -binding loop of α -LA does not change structural properties of the protein, yet results in changes in thermal stability, which can be rationalized in terms of compensation of unfavorable excessive negative charge at the Ca^{2+} coordination site of α -LA and a concomitant decrease in

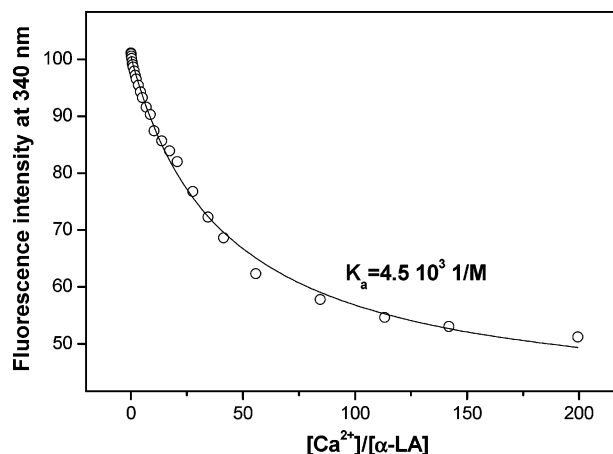


Figure 5. Spectrofluorimetric titration of human α -lactalbumin in the complex with poly-L-Lys ($N = 119$) by calcium ions at 5 °C (pH 7.7, 10 mM HEPES–KOH). The excitation wavelength was 280 nm. The α -LA concentration was 6 μM . Poly-L-Lys was added in equimolar concentration. Empty circles represent the fluorescence intensity at 340 nm in arbitrary units. The solid curve fitted to the experimental points is calculated according to the single site binding scheme.

its affinity to Ca^{2+} ions. The fact that bovine α -LA bound to positively charged polymers demonstrates very similar thermodynamic behavior suggests that the Ca^{2+} -binding loop may be involved into this interaction. At the same time, the observed differences in poly-amino acid induced changes in bovine and human α -lactalbumins thermal stability would suggest that the binding of poly-L-Lys(Arg) distorts the structure of different α -LA to different extents; i.e., while bovine α -LA remains substantially unaffected, human α -lactalbumin is significantly destabilized.

As the thermal stability of Ca^{2+} -loaded human α -lactalbumin drops down to the level of its apo-form upon binding to poly-L-Lys, one might assume that this phenomenon is caused by the release of Ca^{2+} ion from the strong cation-binding site. To examine the role of calcium ions, a spectrofluorimetric titration of α -LA – poly-L-Lys complex (in equimolar concentrations) by Ca^{2+} ions was performed at 5 °C (Figure 5). This temperature was chosen because α -LA is sufficiently far from its thermal transition at 5 °C (see Figure 3). Saturation of human α -LA (within its complex with poly-L-Lys) with Ca^{2+} results in about a 2-fold decrease in its fluorescence quantum yield along with a 10 nm spectral blue shift, from 337 nm to ~ 327 nm. These observations would reflect the ‘filling’ of the strong Ca^{2+} -binding site. A fit of the experimental data to a single site binding scheme gave the apparent Ca^{2+} -association constant $K_a = 4.5 \times 10^3$ 1/M (Figure 5). Additionally, a 5-fold increase in the molar excess of poly-L-Lys over α -LA molecules had no effect on this K_a value. Analogous titrations, performed in the presence of poly-L-Lys of various molecular weights gave remarkably similar K_a values, ranging from 4.5×10^3 1/M to 1.4×10^4 1/M.

Overall, these experiments show that conformational modification of α -lactalbumin caused by the binding of poly-L-Lys results in more than 4 orders of magnitude drop in its Ca^{2+} -affinity. This conclusion was also confirmed by direct free calcium measurements with a Ca^{2+} -selective electrode at room temperature (data not shown). The 1 mM Ca^{2+} ion concentrations used in the melting experiments was sufficient to ensure that α -LA was mostly Ca^{2+} -loaded. However, even under these

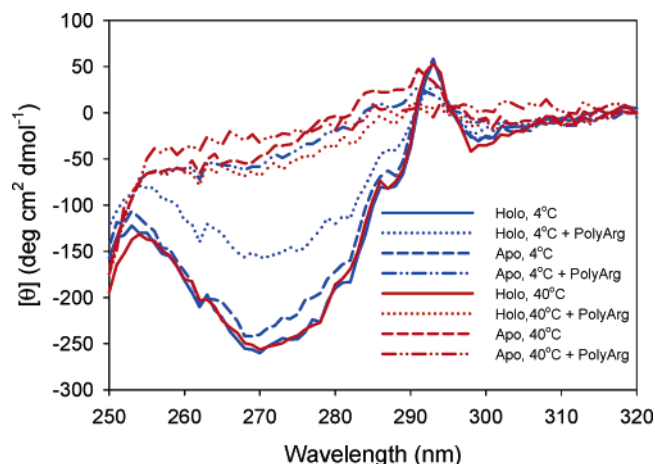


Figure 6. Near-UV CD spectra of noncomplexed human α -LA and α -LA–poly-Arg complexes (in equimolar concentrations) measured at 4 °C & 40 °C, in the presence (1 mM CaCl_2) or absence (1 mM EDTA) of Ca^{2+} (pH 7.8, 10 mM HEPES–KOH). All measurements were carried out at a protein concentration of 0.5 mg/mL.

conditions α -LA complexed with poly-L-Lys melts at lower temperature than nonbound α -LA (see Figure 3). Thus, calcium ion binding to α -LA:poly-L-Lys complexes is not sufficient to restore the native thermal stability of the protein and it still behave similarly to the apo-form.

To retrieve more information on the effect of polypeptide binding on structural properties of human α -LA, we analyzed the near-UV CD spectrum of α -LA–poly-L-Arg complex (in equimolar concentrations). Figure 6 compares near-UV CD spectra of noncomplexed and complexed human α -LA measured at 4 °C and 40 °C in the presence or absence of Ca^{2+} . It can be seen that in the absence of poly-L-Arg, human holo- α -LA showed intensive and well defined near-UV CD spectrum at both temperatures, suggesting that aromatic amino residues are in the asymmetric environment; i.e., noncomplexed holo-protein possesses rigid 3-D structure at 4 °C and 40 °C. This is in a good agreement with the calorimetric analysis (see Figure 3, which shows that much higher temperatures are required to induce denaturation of human holo- α -LA). Figure 6 shows that the structure of α -LA at 4 °C is not affected by the removal of Ca^{2+} , as the near-UV CD spectrum measured under these conditions is almost indistinguishable from those measured for the holo-protein. However, at 40 °C apo-form of human α -LA does not have rigid 3-D structure, as it follows from the complete diminishing of near-UV CD signal. Once again, this is in a good agreement with microcalorimetry studies that revealed that apo- α -LA, being native at 4 °C, is almost completely denatured at 40 °C.

Importantly, the behavior of complexed α -LA was dramatically different. Figure 6 shows that the only conditions where α -LA complexed with poly-L-Arg preserved some asymmetric environment of its aromatic amino acid residues (i.e., had some rigid structure) are 4 °C in the presence of Ca^{2+} . Being involved into the complex formation with poly-L-Arg, both holo-protein at 40 °C and apo- α -LA at 4 °C and 40 °C do not have rigid 3-D structure, as it follows from the lack of near-UV CD ellipticity. Interestingly, the interaction of holo- α -LA with poly-L-Arg at 4 °C resulted in noticeable (almost 2-fold) decrease in the intensity of near-UV CD spectrum. This strongly suggests that the formation of α -LA–poly-L-Arg complex does significantly affects the structure of holo-protein even at 4 °C.

In summary, results presented in this paper show that human α -lactalbumin complexed with poly-L-Lys(Arg) is characterized by unique properties: its thermal stability is comparable to that for the noncomplexed apo- α -LA, even in the complex with calcium ion. But in contrast to the noncomplexed apo-protein, it has a substantially decreased Ca^{2+} -affinity. In these senses, this state differs from all the other α -LA states reported to date. To distinguish it from all the other known forms of α -lactalbumin, we propose to call it LAMPA (“ α -lactalbumin modified by poly-amino acid”).

Abbreviations: α -LA, α -lactalbumin; MAL, multimeric α -LA; HAMLET, human α -lactalbumin made lethal to tumor cells; BAMLET, bovine α -lactalbumin made lethal to tumor cells; LAMPA, α -lactalbumin modified by poly-amino acid; N, degree of polymerization of poly-amino acid; UV, ultra violet; CD, circular dichroism; LALLS, low angle light scattering.

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References

- Hill, R. L.; Brew, K. Lactose synthetase. *Adv. Enzymol. Relat Areas Mol. Biol.* **1975**, *43*, 411–490.
- Hiraoka, Y.; Segawa, T.; Kuwajima, K.; Sugai, S.; Murai, N. α -Lactalbumin: a calcium metalloprotein. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1098–1104.
- Permyakov, E. A.; Yarmolenko, V. V.; Kalinichenko, L. P.; Morozova, L. A.; Burstein, E. A. Calcium binding to α -lactalbumin: structural rearrangement and association constant evaluation by means of intrinsic protein fluorescence changes. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 191–197.
- Dolgikh, D. A.; Gilmanshin, R. I.; Brazhnikov, E. V.; Bychkova, V. E.; Semisotnov, G. V.; Venyaminov, S. Y.; Ptitsyn, O. B. α -Lactalbumin: compact state with fluctuating tertiary structure? *FEBS Lett.* **1981**, *136*, 311–315.
- Kuwajima, K. The molten globule state of α -lactalbumin. *FASEB J.* **1996**, *10*, 102–109.
- Hakansson, A.; Zhivotovsky, B.; Orrenius, S.; Sabharwal, H.; Svanborg, C. Apoptosis induced by a human milk protein. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8064–8068.
- Svensson, M.; Hakansson, A.; Mossberg, A. K.; Linse, S.; Svanborg, C. Conversion of α -lactalbumin to a protein inducing apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4221–4226.
- Hakansson, A.; Svensson, M.; Mossberg, A. K.; Sabharwal, H.; Linse, S.; Lazou, I.; Lonnerdal, B.; Svanborg, C. A folding variant of α -lactalbumin with bactericidal activity against *Streptococcus pneumoniae*. *Mol. Microbiol.* **2000**, *35*, 589–600.
- Pellegrini, A.; Thomas, U.; Bramaz, N.; Hunziker, P.; von Fellenberg, R. Isolation and identification of three bactericidal domains in the bovine α -lactalbumin molecule. *Biochim. Biophys. Acta* **1999**, *1426*, 439–448.
- Goers, J.; Permyakov, S. E.; Permyakov, E. A.; Uversky, V. N.; Fink, A. L. Conformational Prerequisites for α -Lactalbumin Fibrillation. *Biochemistry* **2002**, *41*, 12546–12551.
- Düringer, C.; Hamiche, A.; Gustafsson, L.; Kimura, H.; Svanborg, C. HAMLET interacts with histones and chromatin in tumor cell nuclei. *J. Biol. Chem.* **2003**, *278*, 42131–42135.
- Permyakov, S. E.; Pershikova, I. V.; Khokhlova, T. I.; Uversky, V. N.; Permyakov, E. A. No need to be HAMLET or BAMLET to interact with histones: binding of monomeric α -lactalbumin to histones and basic poly-amino acids. *Biochemistry* **2004**, *43*, 5575–5582.
- Kaplanas, R. I.; Antanavichyus, A. I. Isolation and purification of α -lactalbumin, a component of lactose synthetase. *Biochemistry (Mosc.)* **1975**, *40*, 493–495.

- (14) Vanhooren, A.; Devreese, B.; Vanhee, K.; Van Beeumen, J.; Hanssens, I. Photoexcitation of Tryptophan Groups Induces Reduction of Two Disulfide Bonds in Goat α -Lactalbumin. *Biochemistry* **2002**, *41*, 11035–11043.
- (15) Nozaka, M.; Kuwajima, K.; Nitta, K.; Sugai, S. Detection and characterization of the intermediate on the folding pathway of human α -lactalbumin. *Biochemistry* **1978**, *17*, 3753–3758.
- (16) Permyakov, E. A.; Burstein, E. A.; Sawada, Y.; Yamazaki, I. Luminescence of phenylamine residues in superoxide dismutase from green pea. *Biochim. Biophys. Acta* **1977**, *491*, 149–154.
- (17) Burstein, E. A.; Emelyanenko, V. I. Log-normal description of fluorescence spectra of organic fluorophores. *Photochem. Photobiol.* **1996**, *64*, 316–320.
- (18) Marquardt, D. W. An algorithm for least-squares estimation of nonlinear parameters. *J. Soc. Indust. Appl. Math.* **1963**, *11*, 431–441.
- (19) Permyakov, E. A.; Burstein, E. A. Some aspects of studies of thermal transitions in proteins by means of their intrinsic fluorescence. *Biophys. Chem.* **1984**, *19*, 265–271.
- (20) Permyakov, E. A.; Burstein, E. A. Some aspects of studies of thermal transitions in proteins by means of their intrinsic fluorescence. *Biophys. Chem.* **1984**, *19*, 265–271.
- (21) Permyakov, E. A. *Luminescent Spectroscopy of Proteins*; CRC Press: Boca Raton, Ann Arbor, London, Tokyo, 1993.
- (22) Permyakov, E. A.; Permyakov, S. E.; Deikus, G. Y.; Morozova-Roche, L. A.; Grishchenko, V. M.; Kalinichenko, L. P.; Uversky, V. N. Ultraviolet illumination-induced reduction of α -lactalbumin disulfide bridges. *Proteins* **2003**, *51*, 498–503.
- (23) Privalov, P. L.; Potekhin, S. A. Scanning microcalorimetry in studying temperature-induced changes in proteins. *Methods Enzymol.* **1986**, *131*, 4–51.
- (24) Hackel, M.; Hinz, H. J.; Hedwig, G. R. *Biophys. Chem.* **1999**, *82*, 35–50.
- (25) Cantor, C. R.; Schimmel, P. R. *Biophysical Chemistry*; W. H. Freeman and Company: San Francisco, 1980.
- (26) Permyakov, S. E.; Uversky, V. N.; Veprintsev, D. B.; Cherskaya, A. M.; Brooks, C. L.; Permyakov, E. A.; Berliner, L. J. Mutating aspartate in the calcium-binding site of α -lactalbumin: effects on the protein stability and cation binding. *Protein Eng.* **2001**, *14*, 785–789.

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