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ARTICLE

Interaction of α_{S1}-Casein with Curcumin and Its Biological Implications

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α_{S1}-Casein is one of the major protein components of the casein fraction of milk. Curcumin (diferuloyl methane), the major curcuminoid, constituting about 2−5% of turmeric (*Curcuma longa*) is the active ingredient with many physiological, biochemical, and pharmacological properties. On the basis of spectroscopic measurements, it is inferred that curcumin binds to α_{S1}-casein at pH 7.4 and 27 °C with two binding sites, one with high affinity [$(2.01 \pm 0.6) \times 10^6$ M^{-1}] and the other with low affinity [$(6.3 \pm 0.4) \times 10^4$ M^{-1}]. Binding of curcumin to α_{S1}-casein is predominantly hydrophobic in nature. The anisotropy of curcumin or conformation of α_{S1}-casein did not change on interaction. The stability of curcumin in solution at pH 7.2 was enhanced on binding with α_{S1}-casein. The chaperone-like activity of α_{S1}-casein gets slightly enhanced on its binding to curcumin. The ability of curcumin to protect erythrocytes against hemolysis was not affected due to curcumin− α_{S1}-casein interaction. The two binding sites of α_{S1}-casein for curcumin, along with enhanced solution stability on interaction, may offer an alternative approach in physiological and nutritional applications.

KEYWORDS: α_{S1}-Casein; curcumin stability; fluorescence titration; circular dichroism; chaperone activity

INTRODUCTION

Casein constitutes nearly 80% of total milk protein and is a mixture of four phosphoprotein fractions, namely, α_{S1}-casein, α_{S2}-casein, β-casein, and κ-casein (*1*). Casein is amphiphilic in nature. The presence of hydrophobic clusters and negatively charged regions along the peptide chain helps to form large colloidal aggregate leading to the formation of casein micelle complexes (*2*). These large protein complexes have strong affinity for the bivalent and trivalent cations, mainly to the phosphoserine residues (*3*). Caseins are mainly random coiled proteins characterized by the lack of folded structure. Due to lack of folded structure they have high intramolecular flexibility (*4*). Unlike their globular counterparts, caseins interact with different targets due to their flexibility (*5*). Although casein function is largely nutritional, recent studies provide insight about the functional properties of casein exhibiting chaperone-like activity and its ability to solubilize hydrophobically aggregated proteins. Casein acts as a chaperone in a manner similar to small heat shock proteins, protecting the substrate proteins against aggregation (*1*, *6*, *7*), including amyloid fibril formation (*8*), when placed under conditions of stress such as elevated temperature or reduced environment.

The most abundant fraction of casein is α_{S1}-casein, constituting 40% of total casein. A single polypeptide chain with 199 amino acid residues including 8−9 serine monophosphates, α_{S1}-casein has a molecular mass of 23619 (*9*). The structure of α_{S1}-casein contains a short hydrophilic amino-terminal portion, a segment of hydrophobic β-sheet, the phosphopeptide region, and a short portion of α-helix, which connects the N-terminal portion to the highly hydrophobic carboxy-terminal domain (residues 100−199) containing extended β-strands (residues 134−160 and 163−178) (*9*). The self-association of α_{S1}-casein monomer, in aqueous solution, is attributable to the highly hydrophobic C-terminus (*10*).

Curcumin (diferuloylmethane; 1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), a natural lipid-soluble yellow compound from the plant *Curcuma longa*, is a potent antioxidant, antitumorigenic, and anti-inflammatory molecule (*11*, *12*). Curcumin is reported to inhibit free radical-induced hemolysis of human red blood cells (*13*). There are recent reports in the literature on the modulation of chaperone activity associated with α-crystallin by curcumin (*14*, *15*). The major drawback that limits the utility of curcumin is its limited aqueous solubility. Efforts are in progress to improve the physiological functions of curcumin by bettering its solubility, stability, and bioavailability.

In the present study, the binding characteristics of curcumin with α_{S1}-casein have been followed by steady state fluorescence and circular dichroism measurements. The stability of curcumin, the chaperone activity of α_{S1}-casein, and the hemolytic activity of curcumin have been investigated.

MATERIALS AND METHODS

N-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), curcumin, dephosphorylated casein, 1-anilinonaphthalene-8-sulfonic acid (ANS), bovine serum albumin (BSA), phosphatidylcholine (PC) from egg yolk, carbonic anhydrase, catalase, and Tris base were

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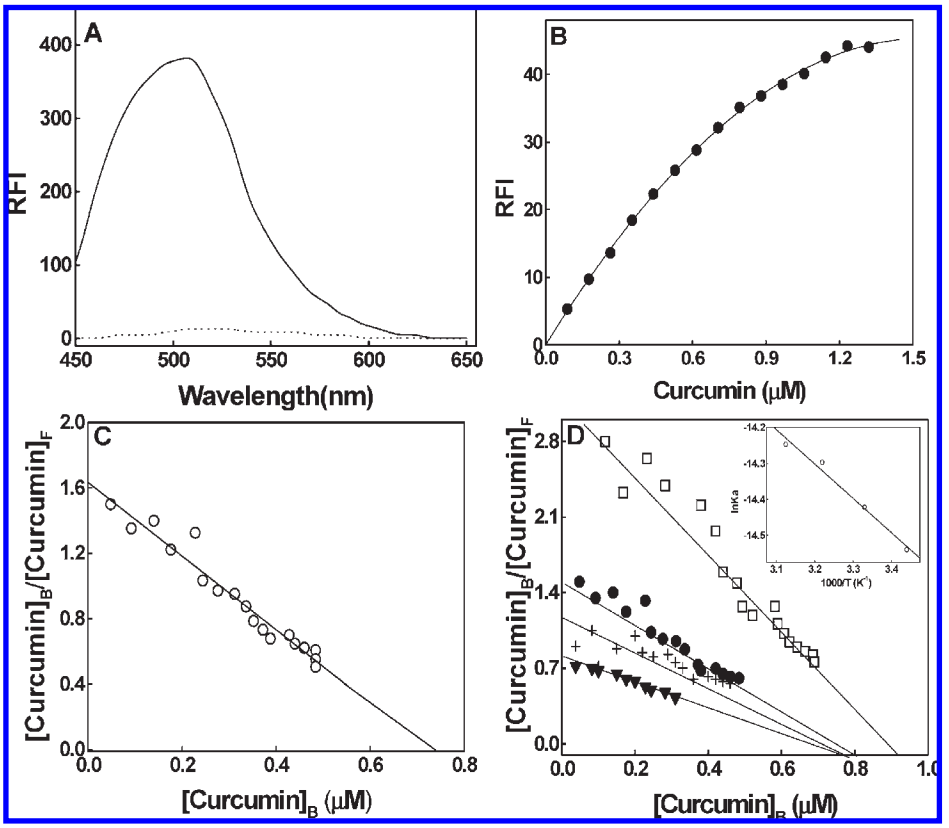

Figure 1. (**A**) Fluorescence spectra of 10 μM curcumin at pH 7.4 in the absence (· · ·) and in the presence of 30 μM α_{S1}-casein (—). Excitation wavelength was 430 nm. Excitation and emission slit widths were 5 and 10 nm, respectively. (**B**) Fluorescence enhancement of curcumin bound to α_{S1}-casein. A 2 μL aliquot of curcumin (from 0.11 mM stock in methanol) was titrated against 3 μM α_{S1}-casein in 10 mM HEPES buffer, pH 7.4. (**C**) Scatchard plot for determining binding of curcumin to α_{S1}-casein. (**D**) Effect of temperature: (□) 17 °C; (●) 27 °C; (+) 37 °C; (▼) 47 °C. (Inset) Variation of ln K_a for the formation of α_{S1}-casein−curcumin complex monitored at 510 nm. Line plot indicates the fit according to van't Hoff's plot.

procured from Sigma Chemicals (St. Louis, MO). 2,2′-Azobis-(2-amidinopropane hydrochloride) (AAPH) was from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of analytical grade.

Bovine milk α_{S1}-casein was purified to homogeneity as previously described (*16*). The concentration of α_{S1}-casein solution in 0.01 M HEPES buffer, pH 7.4, was determined spectrophotometrically ($E^{1\%}$ = 10.5 at 280 nm) after filtering through a 0.22 μm filter. Curcumin was dissolved in HPLC grade methanol for determining the concentration (ε_{425nm} = 54954 M^{-1} cm^{-1}). The concentration of alcohol never exceeded 1.5%. Its purity was ascertained by reverse phase high-performance liquid chromatography (RP-HPLC) using a C_{18} column with detection at 425 nm. The elution was carried out using 5% methanol and acetonitrile containing 0.1% trifluoroacetic acid (TFA) (*17*).

Fluorescence Measurements. Fluorescence measurements were carried out on a Shimadzu RF 5000 spectrofluorometer attached to a circulating Peltier thermostat. Excitation and emission slit widths were set to 5 and 10 nm, respectively. All of the measurements were made using 0.01 M HEPES buffer, pH 7.4, at 27 °C. A 2 μL aliquot of curcumin from 0.11 mM stock was titrated against protein (3 μM). Excitation and emission wavelengths were at 430 and 510 nm, respectively, or in the range of 465−600 nm in a 10 mm path length cuvette. The changes in fluorescence intensity due to curcumin were followed as a function of fixed concentration of casein and dephosphorylated casein. The binding parameters were calculated using the Scatchard procedure (*18*), which is based on the general equation

$$v/L = \sum_{i=1}^{n} n_i K_i / 1 + K_i L \quad (1)$$

where v is the moles of ligand bound per mole of protein, L is the molar concentration of free ligand, n is the number of ligand binding sites, and K is the association constant. The effect of temperature on the association constant of curcumin with α_{S1}-casein was determined in the range of 17−47 °C. The concentrations of α_{S1}-casein and curcumin were the same as given above.

For ANS binding studies, ANS stock solution (7.6 mM) was prepared in methanol, and 2 μL was titrated against casein (3 μM). Excitation and emission wavelengths were at 350 and 500 nm, respectively. Binding constants for phosphorylated and dephosphorylated caseins were calculated using a Scatchard plot. Appropriate blanks were subtracted to obtain the fluorescent enhancement caused by the probe.

For change in anisotropy measurements, curcumin (10 μM) was titrated with 10 μL increments of α_{S1}-casein (0−20 μM). The data were obtained by setting the excitation and emission wavelengths at 430 and 510 nm, respectively. For anisotropy measurements, intensities of horizontal and vertical components of the emitted light ($I_{\parallel}$ and $I_{\perp}$) were corrected for the contribution of scattered light as described (*19*).

Circular Dichroism (CD) Measurements. CD measurements were recorded on a Jasco J-810 automatic recording spectropolarimeter at 27 °C. Spectra were collected from 500 to 340 nm using a rectangular 1 cm path length quartz cell. An average of three scans at a speed of 20 nm/min, with a bandwidth of 1 nm and a response time of 1 s, were recorded. CD of the curcumin−α_{S1}-casein complex was corrected by subtracting the spectra of protein-free ligand solutions. α_{S1}-Casein was titrated against 2 μL aliquots of 2.0 mM stock curcumin solution. The association constant, K_a, was calculated as reported (*19*).

Stability Measurements. Mixed PC micelles were prepared according to the method of Began et al. (*20*), in 50 mM Tris-HCl, pH 7.2. The stability of free curcumin, α_{S1}-casein-bound curcumin, BSA-bound curcumin, and mixed PC micelles bound curcumin was established by incubating curcumin in 50 mM Tris-HCl, pH 7.2, or in the presence of other above-mentioned components at 30 °C with intermittent mixing. Concentrations of proteins and PC were 1 mg/mL and 1 mM, respectively. Incubated sample (20 μL) containing 65 μM curcumin was injected to the HPLC column (C_{18} column, 250 × 4.5 mm, 5 μm, Waters) at 0−7 h time

intervals with detection at 425 nm by applying the gradient as described above for curcumin purity.

Chaperone Activity. Thermally induced aggregation of carbonic anhydrase and catalase was studied by measuring the increase in absorbance of the enzyme solutions on a Shimadzu UV1601 double-beam spectrophotometer, with an attached Shimadzu TCC Peltier temperature control. The extent of aggregation was measured by recording the apparent absorbance at 400 nm as a function of time as described earlier (*6*). The reference cuvette contained all of the components except the substrate proteins.

Inhibition of Hemolysis by α_{S1}-Casein-Bound Curcumin. Inhibition of hemolysis by α_{S1}-casein-bound curcumin was carried out as described (*13*). The extent of hemolysis was determined spectrophotometrically at 540 nm. In the case of antihemolysis experiments, free curcumin and/or α_{S1}-casein-bound curcumin (in the ratio 1:1) was incubated along with the RBC suspension, 0.5 h before the addition of AAPH. The results obtained from three individual sets of experiments were found to be reproducible with 5% deviation.

Statistical Analysis. Data are presented as means ± standard deviation. For all of the measurements, a minimum of three to four replicates was taken for data analysis. Using the software Origin 6.1, all of the values were averaged and plotted.

RESULTS AND DISCUSSION

Commercially available curcumin is a mixture of three naturally occurring curcuminoids with curcumin as the main constituent. The purity of curcumin, as reported under Materials and Methods, was ascertained by RP-HPLC. The retention time of the purified compound was 5.9 min. The homogeneity of α_{S1}-casein was confirmed by SDS-PAGE.

Fluorescence Measurements. Curcumin fluorescence in aqueous solution is very weak. The spectrum reveals a broad maxima at ~530 nm when excited at 430 nm. Addition of small increments of α_{S1}-casein results in a sharper fluorescence peak with increased intensity of ~25 times that of curcumin alone (**Figure 1A**). The emission maximum of curcumin shifts toward blue to 510 nm and remains constant during titration; emission intensity was enhanced with increasing curcumin concentration (**Figure 1B**). The fluorescence of curcumin is sensitive to the polarity of its surrounding environment. These results suggest that curcumin is transferred from a hydrophilic to a more hydrophobic environment. The Scatchard plot derived using eq 1 is given in **Figure 1C**. The association constant (K_a) estimated from the plot is $2.01 \pm 0.6 \times 10^6$ M^{-1}.

With dephosphorylated α_{S1}-casein, the association constant was found to be $(2.3 \pm 0.5) \times 10^6$ M^{-1}, which is very similar to that of phosphorylated α_{S1}-casein. The similarity in association constants suggests that the phosphate groups are not involved in the binding process. The surface hydrophobicity of α_{S1}-casein did not change due to dephosphorylation. This was ascertained by ANS binding measurements with α_{S1}-casein and dephosphorylated α_{S1}-casein. The binding constant for ANS with α_{S1}-casein was $(1.8 \pm 0.4) \times 10^4$ M^{-1}, and with dephosphorylated α_{S1}-casein, the binding constant was $(2 \pm 0.4) \times 10^4$ M^{-1} (data not shown). The hydrophobicities of phosphorylated and dephosphorylated α_{S1}-casein are similar. The anisotropy of curcumin obtained from fluorescence polarization measurements did not change on binding to α_{S1}-casein.

The driving force for the binding of curcumin with α_{S1}-casein was estimated by following the changes in the K_a values in the temperature range from 17 to 47 °C. The results are given in **Figure 1D**. The results show an increase in K_a with a decrease in temperature. The free energy change, $\Delta G°$ at 27 °C, calculated from the association constant obtained from the slope of the curve, was −8.65 kcal/mol. The van't Hoff plot is given in the inset of **Figure 1D**. The $\Delta H°$ and $\Delta S°$ for the binding reaction were estimated to be −1.28 kcal/mol and 24.7 cal/mol, respectively.

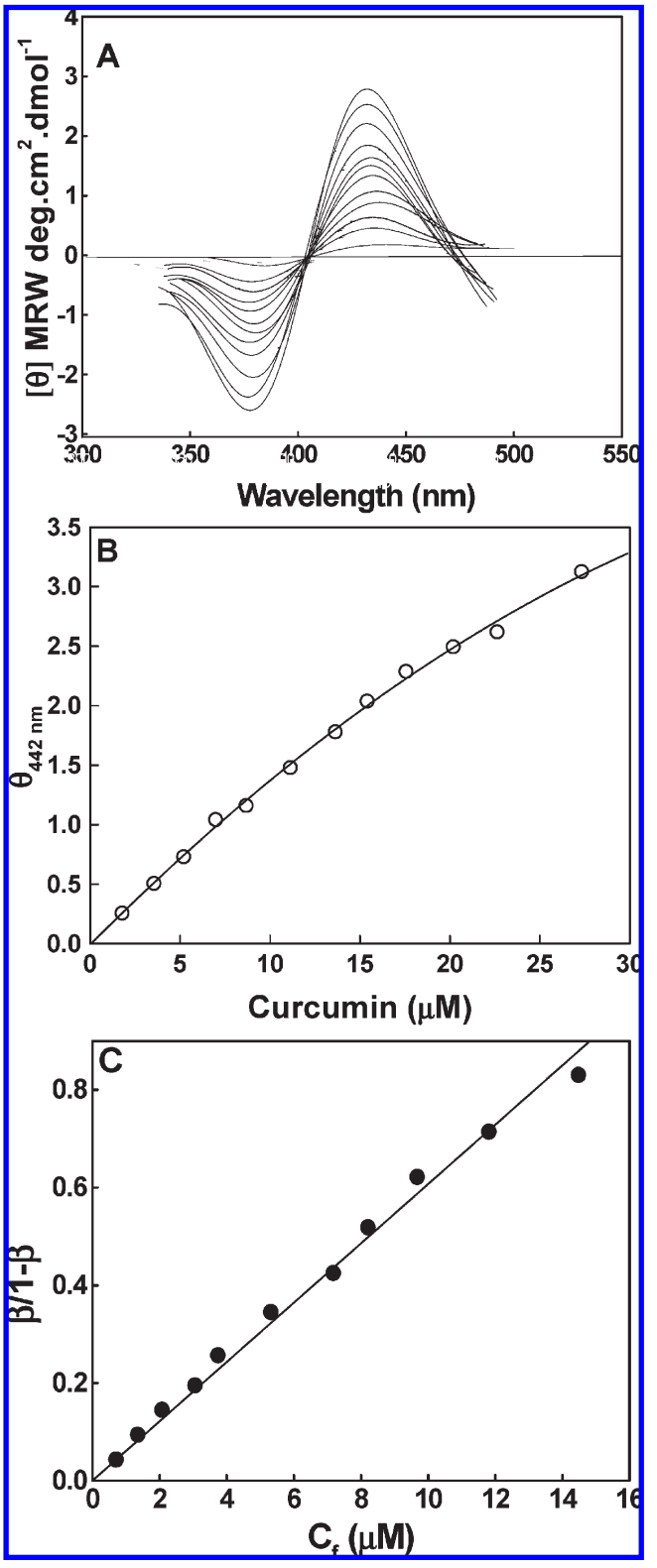

Figure 2. (**A**) Induced circular dichroism spectra of curcumin−α_{S1}-casein complex. α_{S1}-Casein concentration in 10 mM HEPES buffer, pH 7.4, was 20 μM. Curcumin concentrations were in the range of 2−27 μM. (**B**) Variation of ellipticity values at 442 nm as a function of curcumin concentration. (**C**) Plot of $\beta/(1-\beta)$ versus C_f for α_{S1}-casein−curcumin complex.

The obtained thermodynamic parameters reveal that interaction is driven by an increase in entropy and that the enthalpy of binding is very small. A positive $\Delta S°$ value is taken as evidence for

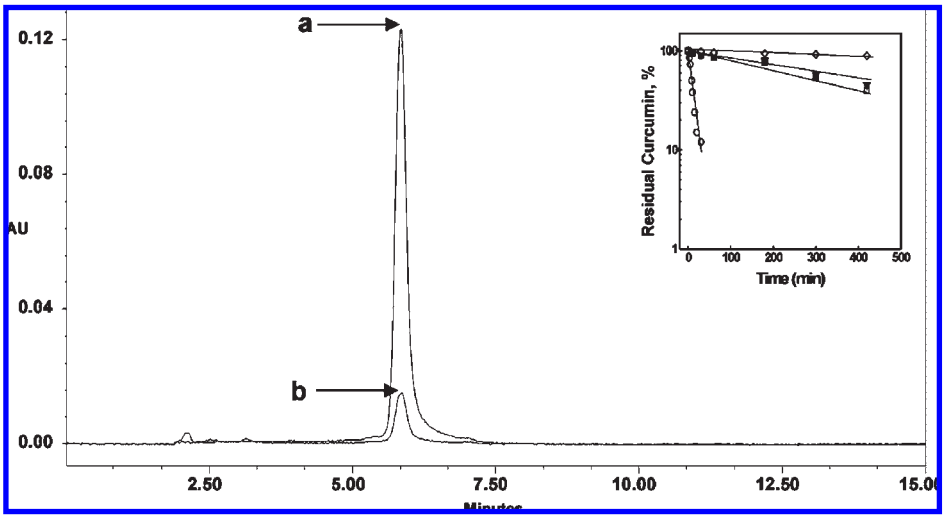

Figure 3. HPLC profile to show curcumin in buffer at (a) 0 min and (b) degraded curcumin after 30 min. (Inset) Semilogarithminc plot to ascertain the stability of curcumin in different conditions: (○) Tris-HCl, 50 mM, pH 7.2; (□) α_{S1}-casein; (▼) bovine serum albumin; (◇) mixed phosphatidylcholine micelles. The data are normalized to 100 at 0 time. The data points are fitted as first-order linear regression, and the rate constants (k) were calculated from the linear fit data.

hydrophobic interaction (*21*). Curcumin remains un-ionized under the experimental conditions (pH 7.4, pK_a value = 8.28). Hence, electrostatic interactions can be excluded from the binding process. Therefore, hydrophobic interactions are largely responsible, as evidenced by thermodynamic measurements. Similar results have been reported for curcumin−human serum albumin (HSA) interactions (*22*).

Circular Dichroism Measurements. Far-UV CD studies of α_{S1}-casein show no change in the secondary structure upon addition of curcumin, indicating the preservation of structure. Neither curcumin nor casein exhibits any CD band in the region of 340−500 nm. However, curcumin became optically active on binding to α_{S1}-casein, exhibiting CD bands in the region of 340−500 nm, suggesting the asymmetric environment of curcumin with the emergence of induced bands in the characteristic absorption region. The CD spectra of α_{S1}-casein in the presence of various concentrations of curcumin are given in **Figure 2A**. Addition of curcumin, in small increments, to α_{S1}-casein resulted in the appearance of two oppositely signed weak, extrinsic CD bands with a zero crossover point at 407 nm. The longer wavelength band at 442 nm was positive, whereas the shorter wavelength band, at 388 nm, was negative. The existence of two equal-intensity opposite bands is probably due to the excited state interaction called exciton coupling due to the mutual rotation caused by the two feruloyl moieties around the central methylene group (*23*). The weak induced CD signals suggest the binding of curcumin to the flexible region on the protein, rather than to a small, confined, binding pocket. **Figure 2B** shows the variation in the molar ellipticity value at 442 nm, as a function of curcumin concentration. The mass action plot is given in **Figure 2C**. The association constant was calculated to be $(6.3 \pm 0.4) \times 10^4$ M^{-1}. The above spectroscopic result indicates that curcumin binds to α_{S1}-casein at two different binding sites, characterized by association constants of $(2.01 \pm 0.6) \times 10^6$ and $(6.3 \pm 0.4) \times 10^4$ M^{-1}. The two different association constants obtained indicate two binding sites on α_{S1}-casein, one saturated at 2 μM (from fluorescence data) and the other site saturated at 25 μM curcumin (from CD data). Curcumin has a β-diketone moiety, flanked by two phenolic groups, that helps bind to proteins through hydrophobic interactions. The carboxyl-terminal of α_{S1}-casein (100−199 residues) predominantly contains hydrophobic amino acids, which may be involved in the binding process. Residues 14−24 in α_{S1}-casein are hydrophobic in nature and form a surface "patch" of hydrophobicity capable of interacting with fluorescent probes in solution (*24*). Curcumin may probably be binding at these two sites, with two different ranges of affinity through hydrophobic interaction.

Table 1. Half-Lives ($t_{1/2}$) and Rate Constants (k) of Curcumin in Different Solutions

curcumin medium[a]	rate constant (k) ($\times 10^{-3}$ min^{-1})	$t_{1/2}$ (min)	R	SD
buffer	78.5	8.8	0.9718	0.08852
α_{S1}-casein	2.04	340	0.9884	0.02426
BSA	1.86	373	0.9892	0.02138
mixed PC micelles	0.25	2779	0.9793	0.00418

[a] Tris-HCl buffer, pH 7.2.

Stability of α_{S1}-Casein-Bound Curcumin. The stability of curcumin in solution was followed in 50 mM Tris buffer, pH 7.2. The improved stability of curcumin in the presence of α_{S1}-casein, BSA, and mixed PC micelles was followed by RP-HPLC. Curcumin in buffer undergoes rapid degradation when incubated at 30 °C. **Figure 3** shows the HPLC pattern for the degradation of curcumin in buffer at zero time and 30 min. At zero time, a single peak is detected at 425 nm, with a retention time of 5.9 min. With progress in time, several small peaks are seen at lower retention times with concomitant decrease in the native curcumin peak at 5.9 min (**Figure 3**). About 90% of the curcumin is decomposed rapidly in buffer at the end of 30 min. At this pH, a proton is removed from the phenolic group, thereby leading to loss in the structure of curcumin molecule (*25*). The inset of **Figure 3** shows the semilogarithmic plot of residual curcumin (%) versus time (min) under the various test conditions. In the presence of α_{S1}-casein, about 45% of curcumin remains undegraded at the end of 6 h of incubation. The half-life of curcumin was calculated from the graph, and the results are given in **Table 1**. Curcumin in buffer has a half-life of 8.8 min. In the presence of α_{S1}-casein, the half-life is increased ~39-fold to 340 min. The stability in the presence of BSA is similar to that in the presence of α_{S1}-casein. The maximum stability of curcumin is seen in the presence of mixed PC micelles (half-life = 2779 min). The structure of curcumin is protected in the presence of α_{S1}-casein, thus enhancing the stability of curcumin in vitro. Curcumin is reported to

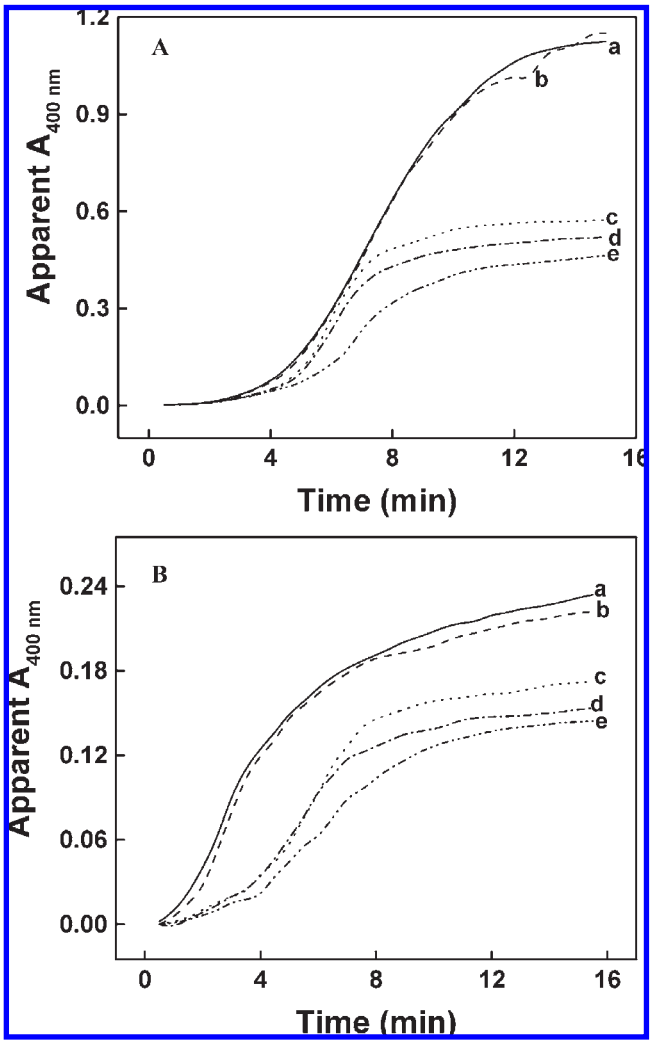

Figure 4. Thermal aggregation assay: (**A**) (a) carbonic anhydrase (0.22 mg/mL), (b) carbonic anhydrase and curcumin (15 μM), (c) carbonic anhydrase and α_{S1}-casein (0.15 mg/mL), (d) carbonic anhydrase, α_{S1}-casein, and curcumin (7.5 μM), (e) carbonic anhydrase, α_{S1}-casein, and curcumin (15 μM); (**B**) (a) catalase (1 mg/mL), (b) catalase and curcumin (15 μM), (c) catalase and α_{S1}-casein (0.2 mg/mL), (d) catalase, α_{S1}-casein and curcumin (7.5 μM), (e) catalase, α_{S1}-casein, and curcumin (15 μM). Values are the means of triplicates.

strongly bind at the hydrophobic moieties of HSA and fibrinogen, and this prevents its hydrolysis (*26*). Curcumin is relatively stable in the cell culture medium containing 10% fetal calf serum and in human blood (*25*).

Chaperone Activity. In vitro assay methods for molecular chaperone activity are generally followed by the prevention of thermal aggregation in substrate proteins. Previously, it has been reported that casein prevents the thermal aggregation of many substrate proteins by providing hydrophobic surfaces to the unfolding proteins (*6*). We have investigated the effect of curcumin binding on the chaperone activity of α_{S1}-casein. A solution of carbonic anhydrase or catalase is used as substrate protein. On heating, the solution becomes turbid, indicating the formation of aggregates. **Figure 4** shows the kinetic traces of the apparent absorbance of carbonic anhydrase and catalase, at 400 nm, in the presence of α_{S1}-casein. As can be seen from this figure, the chaperone activity of α_{S1}-casein is enhanced. Curcumin alone does not prevent aggregation of carbonic anhydrase or catalase. The concentration of casein was limited to prevent aggregation by ~40−50%. With the addition of 7.5 or 15 μM curcumin to form

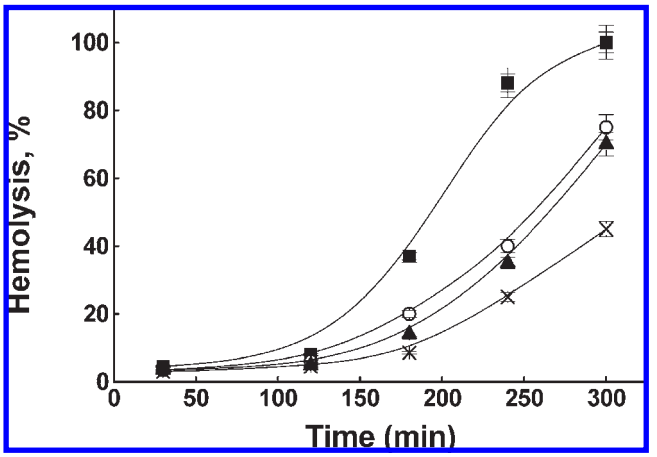

Figure 5. Inhibition of AAPH-induced hemolysis of erythrocytes by curcumin and α_{S1}-casein-bound curcumin: (■) 50 mM AAPH; (○) 10 μM α_{S1}-casein; (▲) 10 μM curcumin; (×) 10 μM each of α_{S1}-casein and curcumin. Data points are expressed as means (±SE) of triplicates.

the curcumin−α_{S1}-casein complex, decreases in aggregation by 11 ± 1 and 22 ± 2%, respectively, of carbonic anhydrase, were seen. The presence of 7.5 or 15 μM curcumin in the curcumin−α_{S1}-casein complex resulted in protection against aggregation of catalase by 12 ± 1 and 20 ± 1%, respectively. Curcumin may provide more hydrophobic surface, favor ing the interaction of α_{S1}-casein to the partially unfolded proteins through its solvent-exposed hydrophobic surfaces. Kumar et al. (*14*) have reported the modulation of chaperone activity of α-crystallin by curcumin in diabetic rat lens. Curcumin, a powerful antioxidant, is reported as a strong inducer of the heat shock response (*27*). In the concentration range of 3−10 μM, curcumin acts as a co-inducer for heat shock proteins (*28*).

Inhibition of Hemolysis by α_{S1}-Casein-Bound Curcumin. Erythrocytes have been used as a model to investigate oxidative damage in biomembranes, due to their susceptibility to free radical mediated peroxidation (*29*). Erythrocyte hemolysis is initiated by AAPH, a water-soluble azo compound, which is a peroxyl radical initiator that generates free radicals by itself, decomposing to alkyl radicals, at physiological temperature. In this study, the biological property of curcumin, in preventing hemolysis, after binding to α_{S1}-casein was investigated. The inhibitory effect of curcumin is shown in **Figure 5**. About 30% inhibition of hemolysis is seen when 10 μM curcumin alone is used. α_{S1}-Casein (10 μM), by itself, also shows 25% inhibition toward AAPH-induced RBC hemolysis by an unknown mechanism. On the binding of curcumin to α_{S1}-casein, the inhibition is 57%. The above result indicates the additive effect in protecting hemolysis. On the binding of curcumin to α_{S1}-casein, the antioxidant property of curcumin is retained. Curcumin and α_{S1}-casein independently contribute to antihemolysis action. Curcumin is a known inhibitor of free radical-induced hemolysis of RBC (*13*). Curcumin per se either scavenges the free radicals or may induce the endogenous antioxidant enzymes promoting the inhibition of hemolysis (*30*).

To conclude, curcumin binds to α_{S1}-casein with two binding sites, one with high affinity and one with low affinity, and binding is predominantly hydrophobic. The stability of curcumin is enhanced due to interaction. The chaperone-like activity of α_{S1}-casein is slightly enhanced on binding to curcumin. The inhibition of hemolytic activity by curcumin is not affected.

ABBREVIATIONS USED

ANS, 1-anilinonaphthalene-8-sulfonic acid; BSA, bovine serum albumin; PC, phosphatidylcholine; CD, circular dichroism; AAPH, 2,2′-azobis(2-amidinopropane hydrochloride); HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; TFA, trifluoroacetic acid; HSA, human serum albumin.

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LITERATURE CITED

(1) Morgan, P. E.; Treweek, T. M.; Lindner, R. A.; Price, W. E.; Carver, J. A. Casein-proteins as molecular chaperones. *J. Agric. Food Chem.* **2005**, *53*, 2670–2683.
(2) Schmidt, D. G. The association of α_{s1}-casein B at pH 6.6. *Biochim. Biophys. Acta* **1970**, *207*, 130–138.
(3) Baumy, J. J.; Guenot, P.; Sinbandhit, S.; Brule, G. Study of calcium binding to phosphoserine residues of β-casein and its phosphopeptide (1−25) by ^{31}P NMR. *J. Dairy Res.* **1989**, *46*, 277–280.
(4) Tompa, P. Intrinsically unstructured proteins. *Trends Biochem. Sci.* **2002**, *27*, 527–533.
(5) Wright, P. E.; Dyson, H. J. Intrinsically unstructured proteins: re-assessing the protein structure−function paradigm. *J. Mol. Biol.* **1999**, *293*, 321–331.
(6) Bhattacharyya, J.; Das, K. P. Molecular chaperone-like property of an unfolded protein, α_S-casein. *J. Biol. Chem.* **1999**, *274*, 15505–15509.
(7) Zhang, X.; Fu, X.; Zhang, H.; Liu, C.; Jiao, W.; Chang, Z. Chaperone-like activity of β-casein. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 1232–1240.
(8) Thorn, D. C.; Meehan, S.; Sundae, M.; Rekas, A.; Gras, S. L.; MacPhee, C. E.; Dobson, C. M.; Wilson, M. R.; Carver, J. A. Amyloid fibril formation by bovine milk κ-casein and its inhibition by the molecular chaperones α_s- and β-casein. *Biochemistry* **2005**, *44*, 17027–17036.
(9) Kumosinski, T. F.; Brown, E. M.; Farrell, H. M. Jr. Three-dimensional molecular modeling of bovine caseins: α_{S1} casein. *J. Dairy Sci.* **1991**, *74*, 2889–2895.
(10) Holt, C. Structure and stability of bovine casein micelles. *Adv. Protein Chem.* **1992**, *43*, 63–133.
(11) Aggarwal, B. B.; Bhatt, I. D.; Ichikawa, H.; Ahn, K. S.; Sethi, G.; Sandur,S. K.; Sundaram, C.; Seeram, N.; Shishodia, S. In *Turmeric − The Genus Curcuma*; Ravindran, P., Nirmalbabu, K., Sivaraman, K., Eds.; Taylor and Francis Press: London, U.K., 2007; pp 298−348.
(12) Joe, B.; Vijaykumar, M.; Lokesh, B. R. Biological properties of curcumin − cellular and molecular mechanisms of action. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 97–111.
(13) Deng, S. L.; Chen, W. F.; Zhou, B.; Yang, L.; Liu, Z. L. Protective effects of curcumin and its analogues against free radical-induced oxidative haemolysis of human red blood cells. *Food Chem.* **2006**, *98*, 112–119.
(14) Kumar, P. A.; Suryanarayana, P.; Yadagiri Reddy, P.; Bhanuprakash Reddy, G. Modulation of α-crystallin chaperone activity in diabetic rat lens by curcumin. *Mol. Vis.* **2005**, *11*, 561–568.
(15) Pfeiffer, E.; Hoehle, S. I; Walch, S. G; Riess, A; Solyom, A. M; Metzler, M. Curcuminoids form reactive glucuronides in vitro. *J. Agric. Food Chem.* **2007**, *55*, 538–544.
(16) Rasmussen, L. K.; Hojrup, P.; Petersen, T. E. Disulphide arrangement in bovine caseins: localization of intrachain disulphide bridges in monomers of κ- and α_{s2}-casein from bovine milk. *J. Dairy Res.* **1992**, *61*, 485–493.
(17) Jayprakasha, G. A.; Rao, L. G. M.; Sakariah, K. K. Improved HPLC method for the detection of curcumin, demethoxycurcumin and bisdemethoxycurucmin. *J. Agri. Food Chem.* **2002**, *50*, 3668–3672.
(18) Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660–673.
(19) Mahesha, H. G.; Singh, S. A.; Srinivasan, N.; Appu Rao, A. G. A spectroscopic study of the interaction of isoflavones with human serum albumin. *FEBS J.* **2006**, *273*, 451–467.
(20) Began, G.; Sudharshan, E.; Udaya Sankar, K.; Appu Rao, A. G. Interaction of curcumin with phosphatidylcholine: a spectrofluorometric study. *J. Agric. Food Chem.* **1999**, *47*, 4992–4997.
(21) Ross, P. D.; Subramanian, S. Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry* **1981**, *20*, 3096–3102.
(22) Pulla Reddy, A. C.; Sudharshan, E.; Rao, A. G. A.; Lokesh, B. R. Interaction of curcumin with human serum albumin − a spectroscopic study. *Lipids* **1999**, *34*, 1025–1029.
(23) Berova, N.; Gargiulo, D.; Derguini, F.; Nakanishi, K.; Harada, N. Unique UV−vis absorption and circular dichroic exciton-split spectra of a chiral biscyanine dye. Origin and nature. *J. Am. Chem. Soc.* **1993**, *115*, 4769–4775.
(24) Creamer, L. K.; Zoerb, H. F.; Olson, N. F.; Richardson, T. Surface hydrophobicity of α_{S1}-I, α_{S1}-casein A and B and its implications in cheese structure. *J. Dairy Sci.* **1982**, *65*, 902–906.
(25) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. Stability of curcumin in buffer solutions and characterization of its degradation products. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867–1876.
(26) Leung, M. H. M.; Kee, T. W. Effective stabilization of curcumin by association to plasma proteins: human serum albumin and fibrinogen. *Langmuir* **2009**, *25*, 5773–5777.
(27) Calabrese, V.; Scapagnini, G.; Colombrita, C.; Ravagna, A.; Pennisi, G.; Giuffrida Stella, A. M.; Galli, F.; Butterfield, D. A. Redox regulation of heat shock protein expression in aging and neurodegenarative disorders associated with oxidative stress: a nutritional approach. *Amino Acids* **2003**, *25*, 437–444.
(28) Ohtsuka, K.; Kawashima, D.; Gu, Y.; Saito, K. Inducers and co-inducers of molecular chaperones. *Int. J. Hyperthermia* **2005**, *21*, 703–711.
(29) Liu, F.; Ng, T. B. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sci.* **2000**, *66*, 725–735.
(30) Pulla Reddy, A. C.; Lokesh, B. R. Alterations in lipid peroxidation in rat liver by dietary n-3 fatty acids: modulation of antioxidant enzymes by curcumin, eugenol and vitamin E. *Eur. J. Nutr. Biochem.* **1994**, *229*, 181–188.

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