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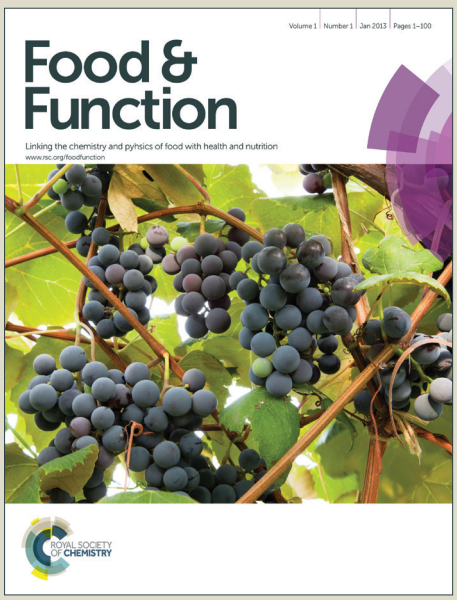

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Interaction of the dietary pigment curcumin with hemoglobin: Energetics of the complexation

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Abstract

The thermodynamics of the interaction of the chemotherapeutic and chemopreventive dietary pigment curcumin with hemoglobin was studied by isothermal titration calorimetry. The binding was characterized to be exothermic. At 293.15 K the equilibrium constant for curcumin-Hb complexation was found to be $(4.88\pm0.06)\times10^5$ M^{-1}. The binding stoichiometry was calculated to be 1.08±0.05 confirming a 1:1 complexation. The binding was driven by a large negative standard molar enthalpy change (ΔH^0=-118.45±0.05 kJ · $mole^{-1}$) and an unfavorable standard molar entropy change ($T\Delta S^0$=-86.53±0.01 kJ · $mole^{-1}$) at 293.15 K. Increasing the temperature favoured the binding and the magnitude of the negative standard molar heat capacity change suggested the involvement of significant hydrophobic forces in the binding process. With increasing salt concentration the magnitude of the equilibrium constant decreased slightly and the complexation mostly involved non-polyelectrolytic forces that contributed to about 92-94% of the standard molar Gibbs energy change. DSC studies revealed that curcumin binding caused partial unfolding of the protein.

1. Introduction

Curcumin (Fig. 1) is a lipophilic polyphenolic compound obtained from the rhizome of the herb *Curcuma longa*. It is the pharmacologically active substance of the food spice turmeric which has been used as a folk medicine for centuries.[1] Curcumin can exist in 1,3-diketo form as well as two equivalent enolic forms. This enolic form is more stable in the solid phase and in solution owing to intramolecular hydrogen-bonding. Curcumin has been used as a herbal medicine for ages due to its nontoxic nature and myriad therapeutic potential. It possesses a wide variety of therapeutic activities and the same has been documented in many recent reviews.[2-5]

Human hemoglobin (Hb) is an iron-containing respiratory blood protein. It is mostly present in the red blood cells of vertebrate erythrocytes and is responsible for carrying oxygen from the lungs to different respiring tissues. The structure of Hb and the mechanisms of its carrying oxygen and transferring electron are well known.[6,7] The enzymatic and antioxidant activities of this respiratory blood protein are also well documented. [6,7]

Hb molecule consists of two α and two β subunits which are linked non-covalently with the erythrocytes as a tetramer. Each α-chain consists of 141 amino acid residues and each β-chain comprises of 146 amino acid residues.[8,9] It is often referred to as $\alpha_2\beta_2$ where α-Trp^{14}, β-Trp^{15} along with β-Trp^{37} are situated.[7,10] These subunits are of almost uniform size and structurally identical. Each of these subunits of Hb has a molecular weight of about 17,000 Da making the total molecular weight of the tetrameric Hb around 68,000

Da.[7] The amino acids in Hb predominantly form alpha helices which are interconnected by short non-helical segments. The helical sections inside Hb are mostly stabilized by hydrogen bonding interactions that leads to the folding of the polypeptide chains into a particular shape.[7]

Hemoglobin is safe and inert within the red blood cells but becomes reactive and toxic upon hemolysis under many diseased conditions and also by the usage of some drugs. Such conditions may lead to exposure of free hemoglobin accessible to small molecules in the plasma. Many drug molecules may cross the erythrocyte membrane and binds to hemoglobin, altering its modulation and the release of ATP.[11] Furthermore, Hb is also found outside red blood cells and their progenitor lines. It is present in A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, the protein has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism consistent with iron chelator activity which has relevance to anticancer activity of curcumin. How curcumin binds to the protein may be interesting to understand this property of curcumin. Hemin, a degradation product of hemoglobin, is released by the lysis of red blood cells in hemorrhagic stroke, is a highly reactive compound and a toxic molecule. Therefore, to counteract the damage induced by hemin improving of the antioxidant potential of brain cells is necessary. Taking into account the antioxidant properties of curcumin and the oxidant-mechanisms involved in the toxicity induced by heme groups, curcumin may be able to attenuate the damage induced by hemin.

Recent studies showed that curcumin nanoformulations appear to be very compatible with erythrocytes and have low serum protein binding characteristics, which suggests that they may be suitable for application in the treatment of malignancy.[12] Furthermore, from the stand point of ligand-protein interaction, hemoglobin offers a target protein with significant attraction in terms of its structure as a heme bound protein. The Hb molecule is one of the most well-studied protein systems. Our knowledge of structure–function relations of Hb is by and large provided by structural and spectroscopic studies. Recently a number of studies on small molecules, including curcumin, binding to Hb have been reported.[7,9,11,13,14] However, one of the most important aspects of the curcumin-Hb association process viz. the energetics have not yet been investigated. A complete knowledge of the binding characteristics of curcumin with Hb is essential to understand its therapeutic potential. Thermodynamic characterization of the complexation process enables complete understanding of the molecular aspects of the interaction and also allows us to correlate the structural aspects which may be helpful to realize the complete therapeutic potential of curcumin. Furthermore, thermodynamic data lends valuable insights into the pharmacological aspects which determine the effectiveness of curcumin as a therapeutic agent. In the present paper, we report the energetics of the interaction of curcumin with Hb using microcalorimetric techniques.

2. Experimental

2.1. Materials

Hemoglobin and curcumin (≥0.94 mass fraction purity, M = 368.38 Da) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The commercial Hb sample was purified by diethyl-aminoethanol (DEAE)-sepharose Fast Flow anion exchange chromatography. The purity of Hb sample was over 99% as revealed from reversed phase-high performance liquid chromatography (RP-HPLC) performed on an Agilent 1100 HPLC system with a ZORBAX® 300SB-C8, column (Agilent, 5 µm, 300 Å, 4.6 × 250 mm). The purified sample was dialyzed into the experimental buffer. The nativeness of the sample was checked by optical melting and DSC experiments as described by Hazra *et al*.[14] All experiments were conducted in filtered 10 mM citrate-phosphate (CP) buffer, pH 7.4, containing 5 mM Na_2HPO_4. All the solutions were prepared in deionised and triple distilled water. The pH of the buffer solution was adjusted with citric acid. The concentration of Hb was determined spectrophotometrically by using a molar extinction coefficient (ε) value of 1.79×10^5 $M^{-1} \cdot cm^{-1}$ at 405 nm.[9,15] Due to poor solubility of curcumin in aqueous buffer, a highly concentrated stock solution of curcumin was prepared in ethanol and its concentration was determined spectrophotometrically using a ε value of 5.50×10^4 $M^{-1} \cdot cm^{-1}$ at 430 nm.[16,17] All buffer salts and other reagents were of analytical grade. Solutions were freshly prepared each day in the buffer and kept protected in the dark at room temperature to avoid any light induced photochemical changes.

2.2. Isothermal titration calorimetry

The interaction of curcumin with Hb was studied on a VP-ITC microcalorimeter (MicroCal, Northampton, MA,USA). The titration data was analyzed with the dedicated MicroCal Origin (version 7.0) software to obtain the thermodynamic quantities. The curcumin and Hb samples were extensively degassed prior to titration on the MicroCal's Thermovac unit. This ensured that no bubbles were formed in the calorimeter cell. The heat released due to the interaction of curcumin with Hb was detected by a thermoelectric device between the sample and reference cells. The titrations were performed by injecting 10 µl of curcumin solution from a rotating syringe into 1.4235 mL of Hb solution kept in the calorimeter cell. The duration of each injection was 10 seconds and the delay time between each injection was 240 seconds. The initial delay before the first injection was 60 seconds. The corresponding control experiments to determine the heat of dilution of curcumin alone was performed by injecting equal volumes of the same concentration of curcumin into the buffer. Each injection produced a heat burst curve (micro joule per second versus time). The resulting thermograms after appropriate control corrections were analyzed using the single set of binding sites model of Levenberg-Marquardt inbuilt with the software of the unit. The resulting corrected injection heats were plotted as a function of the molar ratio (χ) of curcumin/Hb, fitted with a model for one set of binding sites to obtain the equilibrium constant (K), the binding stoichiometry (N) and the standard molar enthalpy change (ΔH^0). The standard molar Gibbs energy change (ΔG^0) and the standard molar entropic contribution ($T\Delta S^0$), where ΔS^0 is the calculated standard molar entropy, were calculated using the following relationships

$\Delta G^0 = -RT \ln K$ (1)

where R is the gas constant (R = 8.314472 J · K^{-1} · mole^{-1}) and T is the temperature in kelvins.

$T\Delta S^0 = \Delta H^0 - \Delta G^0$ (2)

The calorimeter was periodically calibrated electrically and verified with water–water dilution experiments as per criteria of the manufacturer that the mean energy per injection was <5.46 μJ and standard deviation was <0.063 μJ.

2.3. Differential scanning calorimetry

To obtain insights into the temperature dependent transitions in Hb, excess heat capacities were measured as a function of temperature on a MicroCal VP-differential scanning calorimeter (DSC) (MicroCal, Inc., Northampton, MA, USA). In a series of DSC scans, both the calorimeter cells were loaded with the citrate phosphate buffer solution, equilibrated at T = 303.15 K for 20 min. and scanned within the range T = (303.15 to 373.15) K at a scan rate of 60 K^{-1} h^{-1}. The buffer scans were repeated until the base line was reproducible (noise specification <2.1 μJ · K^{-1} and repeatability specification <5.46 μJ · K^{-1}. In a typical DSC experiment around 10-12 scans were required to obtain baseline stability. On cooling, the sample cell was rinsed and loaded with the protein and then with the curcumin–Hb complex and scanned in the same range. Each experiment was repeated twice with separate fillings. The DSC thermograms of excess heat capacity versus temperature were analyzed using the Origin 7.0 software.

3. Results and discussion

3.1. Isothermal titration calorimetric studies

Isothermal titration calorimetry (ITC) is the most effective tool to thermodynamically characterize the binding of small molecules to biomacromolecules.[9,17-19] This is more advantageous over other techniques as from a single titration an estimate of all the thermodynamic quantities can be obtained including the binding affinity and the stoichiometry. Fig. 2 presents the ITC thermogram for the titration of curcumin to Hb at 293.15 K. Top panel of the figure shows the primary data from the titration of curcumin into a solution of Hb. Each heat burst spike in the figure represents the heat generated in a single injection. These injection heats were corrected by subtracting the corresponding control heats obtained from the injection of identical amounts of curcumin into the citrate-phosphate buffer alone (shown in the upper panel, curves off set for clarity). In the bottom panel of Fig. 2 the resulting corrected injection heats plotted against the corresponding molar ratios of curcumin/Hb is depicted. The data points in this panel correspond to the actual experimental points and the continuous solid line is the calculated best fit to the experimental data. The insertion of the phenyl rings of the curcumin molecules into the hydrophobic cavities of Hb causes hydrophobic interactions which is an exothermic process.[20] The removal of high energy water molecules from the hydrophobic cavities of Hb to the bulk buffer solution is an exothermic process.[21] The binding was characterized to be exothermic resulting in negative peaks in the plot of power *versus* time. The data were fit to a single site model as the integrated heat data showed only one binding event. The equilibrium constant (K) for curcumin-Hb complexation obtained from ITC at 293.15 K was $(4.88\pm0.06)\times10^5$ M^{-1}, which is in close agreement with that obtained from spectroscopic

studies.[7] The binding stoichiometry (N) was calculated to be 1.08±0.05 indicating 1:1 complexation of curcumin to Hb. The binding reaction was driven by large favorable negative standard molar enthalpic contribution (ΔH^0=-118.45±0.05 kJ · mole^{-1}) and unfavorable standard molar entropic contributions ($T\Delta S^0$=-86.53±0.01 kJ · mole^{-1}). This negative value of standard molar enthalpy substantiates the existence of electrostatic interaction as well as hydrogen bonding interactions between the aromatic rings of curcumin and the amino acid residues of Hb.[22] The standard molar enthalpic contribution being more negative overcomes the unfavorable entropic contribution to make the overall standard molar Gibbs energy change (ΔG^0) negative, i.e., the reaction becomes feasible. The standard molar Gibbs energy change for curcumin-Hb complexation process was calculated to be – 31.92±0.06 kJ · mole^{-1} at 293.15 K. To obtain further insights into the molecular forces governing the complexation phenomenon, isothermal calorimetric experiments were performed at different temperatures and salt concentrations.

3.2. Determination of heat capacity changes from temperature dependence of the calorimetric data

To obtain additional information about the forces driving the molecular interaction, curcumin-Hb complexation was examined as a function of temperature in the range 283.15-303.15 K. No variation in pH of the solutions was observed in the temperature range studied. The thermodynamic parameters obtained from the temperature dependent ITC studies are depicted in table 1. Overall, as the temperature enhanced, the equilibrium constant values increased and the standard molar enthalpies became more negative with their magnitudes increasing. The binding stoichiometry varied in the range 1.23-1.08

indicating 1:1 complexation between Hb-curcumin irrespective of the temperature. The negative standard molar enthalpies of complexation at all temperatures indicated favorable exothermic binding interaction. The entropy contribution became more and more unfavorable with increasing temperature and the binding reaction became increasingly enthalpy driven with rise in temperatures. Therefore, there are significant differences in the energetics of the interaction at different temperatures which indicates differences in the molecular forces that govern the binding of curcumin to Hb. Variation of the standard molar enthalpy change as a function of temperature provides an estimate of the standard molar heat capacity changes (ΔC_p^0) of the binding. The standard molar heat capacity change, the first derivative of the temperature dependence of enthalpy change, was calculated using the following relationship

$$\Delta C_p^0 = \partial \Delta H^0 / \partial T \qquad (3)$$

Standard molar heat capacity changes serve as a link between the structural and energetic data and may be used as a tool to understand hydration-dehydration effects that occur during the complexation process. The standard molar enthalpy change (ΔH^0) varied linearly (Fig. 3) in the temperature range studied indicating that there is no measurable shift in the pre-existing equilibrium between the conformational states of Hb. The standard molar heat capacity change obtained from the variation of the standard molar enthalpy change with temperature yielded a value of -2.25±0.03 kJ · mole^{-1} K^{-1} (Fig. 3). This non-zero negative value of standard molar heat capacity change suggested that the binding is specific and accompanied by burial of non polar surface area.[23,24] Such negative standard molar heat

capacity values have been reported for many small molecules binding to nucleic acids and proteins.[9,17,25-29] Such non-zero negative ΔC_p^0 values are interpreted in terms of the favourable involvement of several hydration contributions in the complexation process.[30-33] The change in the solvent accessible surface area also accounts for a large component of ΔC_p^0.[33-36] The removal of water from hydrophobic interfaces induced by electrostatic interaction may be also responsible for the negative value of ΔC_p^0. This negative ΔC_p^0 value is further associated with changes in hydrophobic or polar group hydration and is indicative of the involvement of a strong hydrophobic component in the binding process. A graphical representation of the variation in thermodynamic parameters with temperature is depicted in Fig. 4.

3.3. Dependence of the binding on the ionic strength of the medium and parsing of the standard molar Gibbs energy

The thermodynamic parameters for the binding of curcumin to Hb was determined from ITC experiments conducted at four different Na^+ concentrations viz. 10, 20, 30 and 50 mM. All the thermodynamic quantities calculated from the salt dependent ITC studies are presented in table 2. The equilibrium constant (K) values decreased from $(4.88\pm0.06)\times10^5$ M^{-1} to $(4.36\pm0.06)\times10^5$ M^{-1} to $(3.97\pm0.05)\times10^5$ M^{-1} on increasing the salt concentration from 10 to 20 to 30 mM. On further increasing the Na^+ concentration to 50 mM the equilibrium constant further decreased to $(3.29\pm0.05)\times10^5$ M^{-1}. Thus, the magnitude of equilibrium constant decreased around 1.5 folds on increasing the Na^+ concentration from 10 to 50 mM. Irrespective of the salt concentration the binding stoichiometry was calculated to be around 1 indicating that 1:1

complexation takes place at all salt concentrations. The binding reaction becomes less exothermic with increasing salt concentration as manifested by the decrease in the magnitude of the negative enthalpy values. The standard molar Gibbs energy also decreases by 0.96 kJ · mole^{-1} (in absolute values) on increasing the salt concentration from 10 to 50 mM. This shows that the binding reaction becomes less favorable with increasing ionic strength of the medium. The dependence of binding on the ionic strength is due to the binding of the cations of the buffer salt to the charged amino acid residues of Hb. Manning's polyelectrolytic theories based on counter ions condensation model provide a basis for interpreting this salt dependent ITC data.[37] From the polyelectrolytic theory, a relationship was derived linking the equilibrium constant (K) to the Na^+ concentration in the medium. The slope of the plot of log K versus log [Na^+] is related to the counter ion release by the following relation[31]

$$\Delta_r N(\text{ion}) = \left(\frac{\partial \log K}{\partial \log[\text{Na}^+]}\right)_{T,P} = -z\varphi \qquad (4)$$

where $\Delta_r N(\text{ion})$ is the number of ions released upon binding of a ligand, z is equivalent to the apparent charge of the bound ligand and φ is the fraction of [Na^+] bound per Hb molecule. The plot of log K vs log [Na^+] (Fig. 5A) afforded a straight line with a slope of -0.240. In order to obtain valuable insights into the molecular forces governing the binding reaction, the observed standard molar Gibbs energy change was partitioned between the polyelectrolytic (ΔG^0_{pe}) and non-polyelectrolytic (ΔG^0_t) components (Fig. 5B) according to the following equation

$$\Delta G^0 = -RT \ln K = \Delta G^0_t + \Delta G^0_{pe} \qquad (5)$$

The magnitude of ΔG^0_t gives an idea about the molecular contributions arising from H-bonding, van der Waals interaction and hydrophobic transfer whereas the magnitude of ΔG^0_{pe} gives a measure of the contribution arising from the coupled polyelectrolytic forces. This ΔG^0_{pe} term (polyelectrolytic contribution to the standard molar Gibbs energy) can be quantitatively measured from the relationship[38,39]

$$\Delta G^0_{pe} = -z\varphi \, RT \ln([Na^+]) \qquad (6)$$

where -zφ is equivalent to the number of ions released upon complex formation. The magnitude of ΔG^0_{pe} was calculated to be -2.69±0.06, -2.29±0.06, -2.05±0.05 and -1.75±0.05 kJ · mole^{-1}, respectively, at 10, 20, 30 and 50 mM Na^+ concentration (table 2). The non-polyelectrolytic contribution to the standard molar Gibbs energy at 10, 20, 30 and 50 mM Na^+ concentration was -29.23±0.06, -29.36±0.06, -29.37±0.05 and -29.21±0.05 kJ · mole^{-1}, respectively. This shows that the non-polyelectrolytic contribution (ΔG^0_t) is dominant and almost invariant irrespective of the salt concentration. The ΔG^0_t contribution accounts for almost 94-92% of the total standard molar Gibbs energy change. Thus, hydrophobic interactions like π-π stacking, H-bonding and van der Waals interaction predominates the binding phenomenon irrespective of the salt concentration.

3.4. Differential scanning calorimetric studies

The effect of curcumin on the thermal stability of Hb was studied by differential scanning calorimetry (DSC). The melting profile of Hb alone and complexed with curcumin exhibited single transition with melting temperatures of 334.82 and 331.27 K, respectively (Fig. 6). The ratio between the calorimetric enthalpy (ΔH_{cal}) and the van't Hoff enthalpy (ΔH_v) obtained for

the thermal unfolding of Hb is not unity indicating that the melting is not exhibiting a simple two state unfolding behavior. A decrease in the melting temperature of Hb (ΔT_m) by 3.55 K indicated that the binding of curcumin destabilizes the Hb structure leading to secondary structural changes.

4. Conclusions

The energetics of the interaction of the chemotherapeutic dietary pigment curcumin with Hb using microcalorimetric techniques is presented. The equilibrium constant for the 1:1 complexation of curcumin to Hb at 293.15 K was evaluated to be $(4.88\pm0.06)\times10^5$ M^{-1}. The binding was spontaneous and driven by large negative enthalpy and unfavorable negative entropy contributions. The binding affinity enhanced with temperature in the range 283.15-303.15 K. The negative standard molar heat capacity changes of the binding suggested the dominant role of hydrophobic forces in the complexation. Increasing the salt concentration led to small decrease of the affinity, suggesting the involvement of weak electrostatic interactions that was further confirmed from the small magnitude of Na^+ ions released. Furthermore, at all the [Na^+], the non-polyelectrolytic contribution to the binding was remarkably dominant and accounted for about 92-94% of the Gibbs energy change. Curcumin binding led to an unfolding of the hemoglobin conformation causing decrease of its thermal stability. The interesting thermodynamics of the interaction of curcumin-Hb interaction may prove useful in developing curcumin based therapeutics.

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Figure legends

Fig. 1. Molecular structure of curcumin.

Fig. 2. Representative ITC profile for the sequential titration of successive aliquots of curcumin to Hb (curve at the bottom), along with the dilution profiles (curves on the top offset for clarity). The top panel represents the raw data and the bottom panel shows the integrated heat data after correction of the heat of dilution. The symbols (■) represent the data points that were fitted to a one-site model and the solid line represent the best-fit data.

Fig. 3. Plot of variation of standard molar enthalpy change (ΔH^0) with temperature for the complexation of curcumin with Hb.

Fig. 4. Variation of ΔH^0 (black), $T\Delta S^0$ (hatched) and ΔG^0 (white) for the complexation of curcumin with Hb as derived from temperature dependent ITC studies.

Fig. 5. (A) The slope of the plot of log K versus log [Na^+] for curcumin-Hb complexation. (B) Partitioned polyelectrolytic (ΔG^0_{pe}) (white) and non-polyelectrolytic (ΔG^0_t) (black) contributions to the standard molar Gibbs energy at different salt concentrations.

Fig. 6. DSC thermograms of (A) Hb and (B) curcumin-Hb complex.

Table 1

Thermodynamic parameters for the association of curcumin with Hb from ITC at different temperatures.[a]

T/K	10^{-5} K/M^{-1}	N	ΔH^0/(kJ · mole^{-1})	$T\Delta S^0$/(kJ · mole^{-1})	ΔG^0/(kJ · mole^{-1})	ΔC_p^0/(kJ · K^{-1} · mole^{-1})
283.15	2.60±0.04	1.23±0.07	-98.91±0.03	-69.56±0.01	-29.35±0.04	
293.15	4.88±0.06	1.08±0.05	-118.45±0.05	-86.53±0.01	-31.92±0.06	-2.25±0.03
298.15	8.11±0.06	1.12±0.05	-133.41±0.04	-99.68±0.01	-33.73±0.05	
303.15	14.70±0.05	1.09±0.05	-143.09±0.04	-107.30±0.01	-35.79±0.04	

[a]All the data in this table are derived from the ITC experiments conducted and are the average of four determinations. T is the temperature in kelvins (K), K, the equilibrium constant, N, the stoichiometry and ΔH^0, the standard molar enthalpy change which were determined from ITC profiles fitting to Origin 7.0 software as described in the text. The values of ΔG^0, standard molar Gibbs energy change and $T\Delta S^0$, the standard molar entropy contribution were determined using the equations $\Delta G^0 = -RT\ln K$, and $T\Delta S^0 = \Delta H^0 - \Delta G^0$. R is the gas constant. ΔC_p^0 is the standard molar heat capacity change. All the ITC profiles were fit to a model of single binding sites. The overall uncertainties in K, N, ΔH^0, $T\Delta S^0$, ΔG^0 and ΔC_p^0 are 5, 5, 4, 1, 5 and 3%, respectively.

Table 2

Thermodynamic parameters for the association of curcumin with Hb from ITC at different salt concentrations.[a]

c(NaCl)/mM	10^{-5} K/M^{-1}	N	ΔH^0/(kJ · mole^{-1})	$T\Delta S^0$/(kJ · mole^{-1})	ΔG^0/(kJ · mole^{-1})	ΔG^0_t/(kJ · mole^{-1})	ΔG^0_{pe}/(kJ · mole^{-1})
10	4.88±0.06	1.08±0.05	-118.45±0.05	-86.53±0.01	-31.92±0.06	-29.23±0.06	-2.69±0.06
20	4.36±0.06	0.92±0.04	-108.16±0.05	-76.51±0.01	-31.65±0.06	-29.36±0.06	-2.29±0.06
30	3.97±0.05	0.98±0.05	-103.48±0.04	-72.06±0.01	-31.42±0.05	-29.37±0.05	-2.05±0.05
50	3.29±0.05	0.94±0.04	-102.93±0.03	-71.97±0.02	-30.96±0.05	-29.21±0.05	-1.75±0.05

[a]All the data in this table are derived from ITC experiments conducted in citrate-phosphate buffer of different [Na^+], pH 7.4 at 293.15 K and are the average of four determinations. The quantity c(NaCl) is the concentration of NaCl in the solution. K, the equilibrium constant, N, the stoichiometry and ΔH^0, the standard molar enthalpy change were determined from ITC profiles fitting to Origin 7.0 software as described in the text. The values of ΔG^0, standard molar Gibbs energy change and $T\Delta S^0$, the standard molar entropy contribution were determined using the equations $\Delta G^0 = -RT\ln K$, and $T\Delta S^0 = \Delta H^0 - \Delta G^0$. R is the gas constant. ΔG^0_t and ΔG^0_{pe} are the standard molar Gibbs energy contributions from the non-polyelectrolytic and polyelectrolytic forces, respectively. All the ITC profiles were fitted to a model of single binding. The overall uncertainties in K, N, ΔH^0, $T\Delta S^0$, ΔG^0, ΔG^0_t and ΔG^0_{pe} are 5, 4, 4, 1, 5, 5 and 5%, respectively.

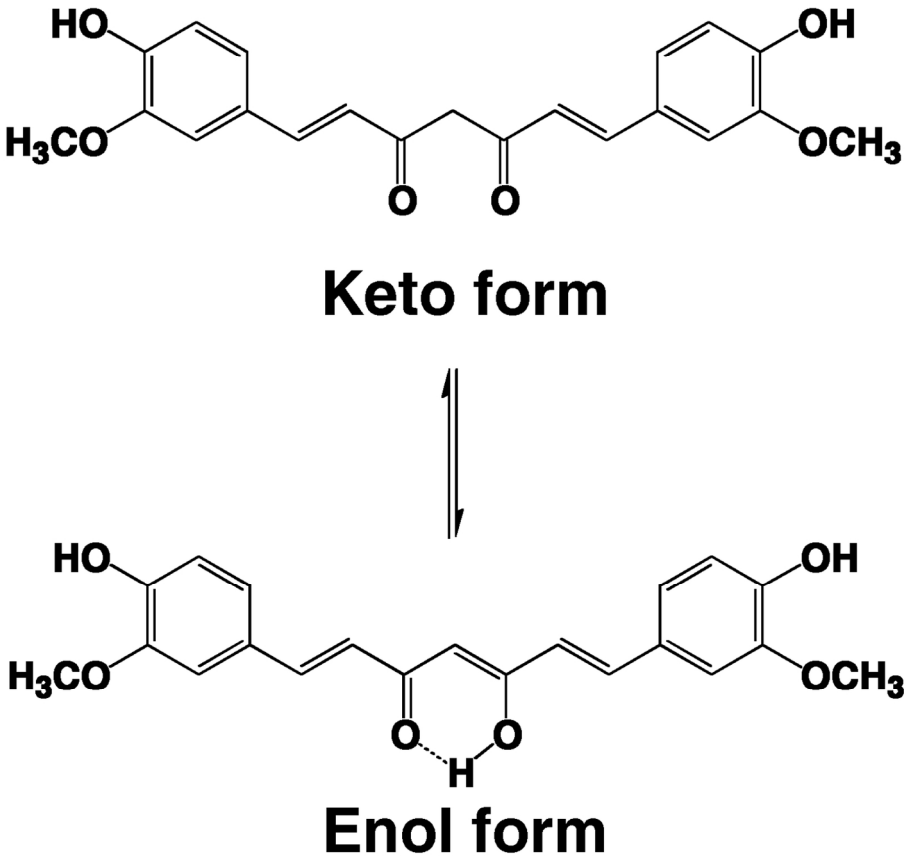

Fig. 1.

Molecular structure of curcumin.
152x160mm (300 x 300 DPI)

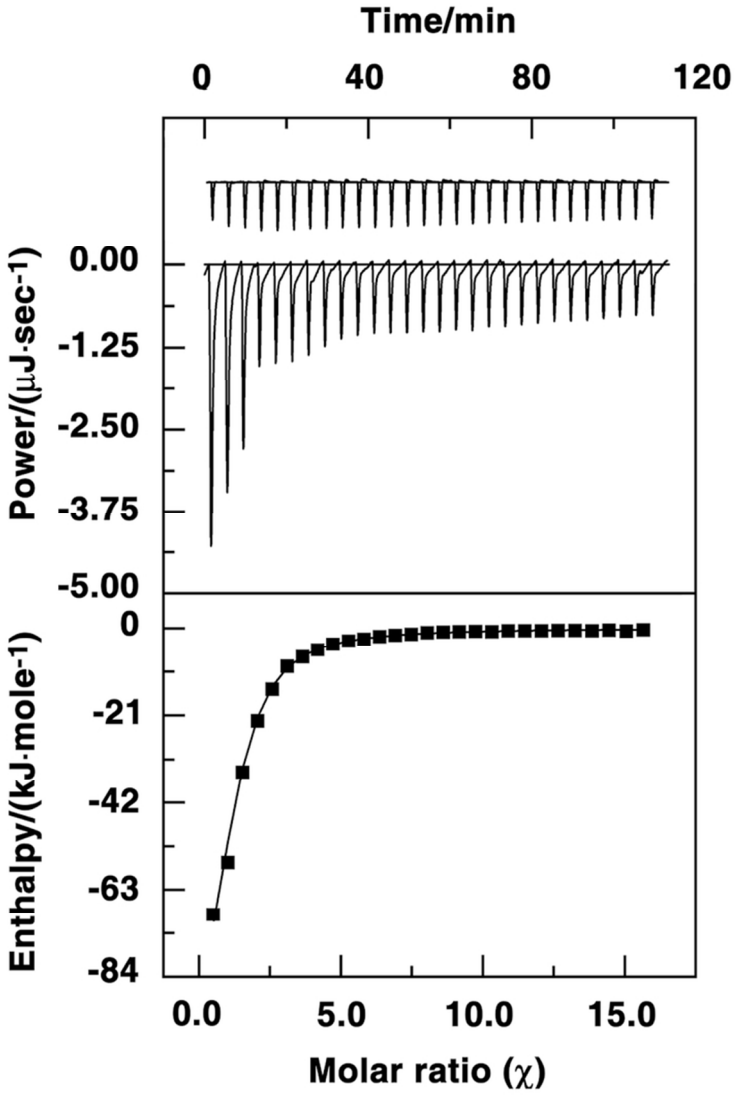

Fig. 2.

Representative ITC profile for the sequential titration of successive aliquots of curcumin to Hb (curve at the bottom), along with the dilution profiles (curves on the top offset for clarity). The top panel represents the raw data and the bottom panel shows the integrated heat data after correction of the heat of dilution. The symbols (■) represent the data points that were fitted to a one-site model and the solid line represent the best-fit data.
64x98mm (300 x 300 DPI)

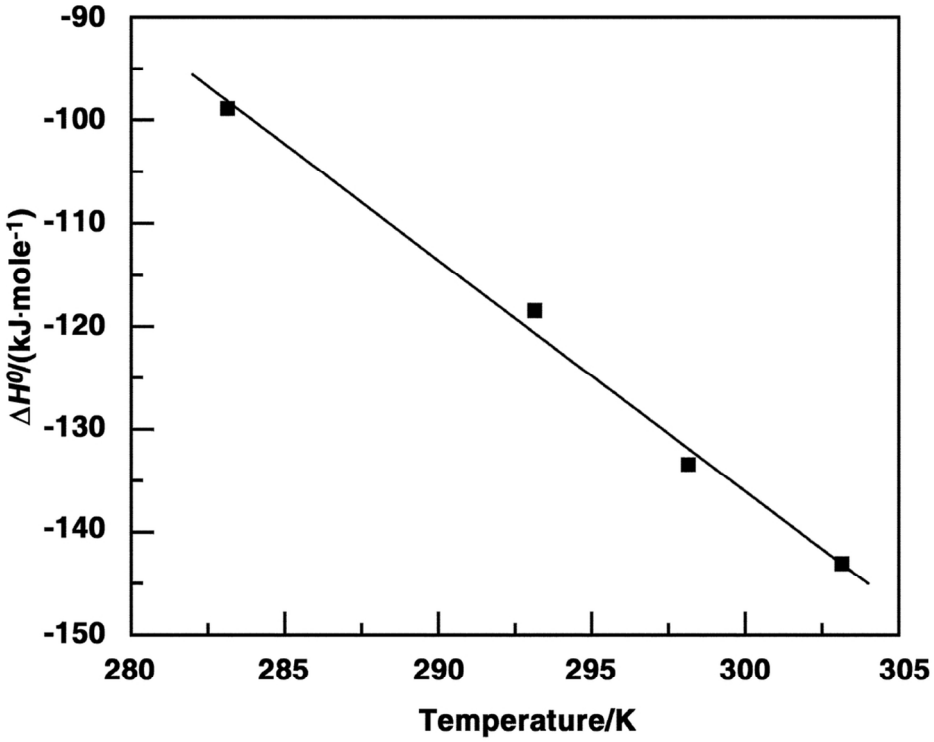

Fig. 3.

Plot of variation of standard molar enthalpy change (ΔH0) with temperature for the complexation of curcumin with Hb.
102x83mm (300 x 300 DPI)

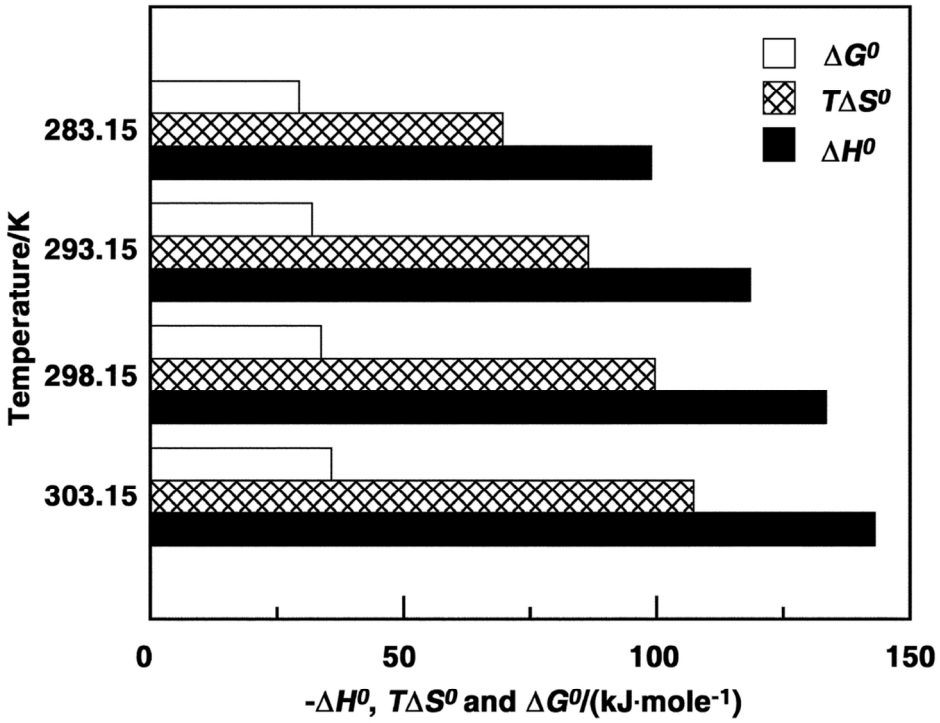

Fig. 4.

Variation of ΔH0 (black), TΔS0 (hatched) and ΔG0 (white) for the complexation of curcumin with Hb as derived from temperature dependent ITC studies.
101x81mm (300 x 300 DPI)

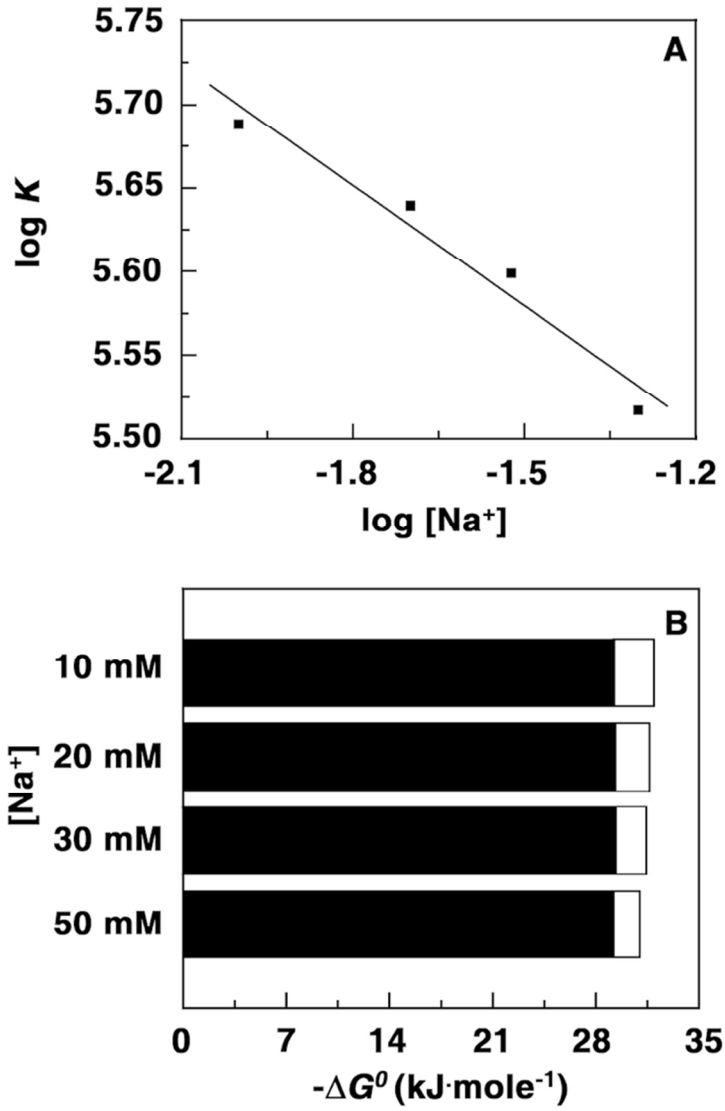

Fig. 5.

(A) The slope of the plot of log K versus log [Na+] for curcumin-Hb complexation. (B) Partitioned polyelectrolytic (ΔG0pe) (white) and non-polyelectrolytic (ΔG0t) (black) contributions to the standard molar Gibbs energy at different salt concentrations.
66x103mm (300 x 300 DPI)

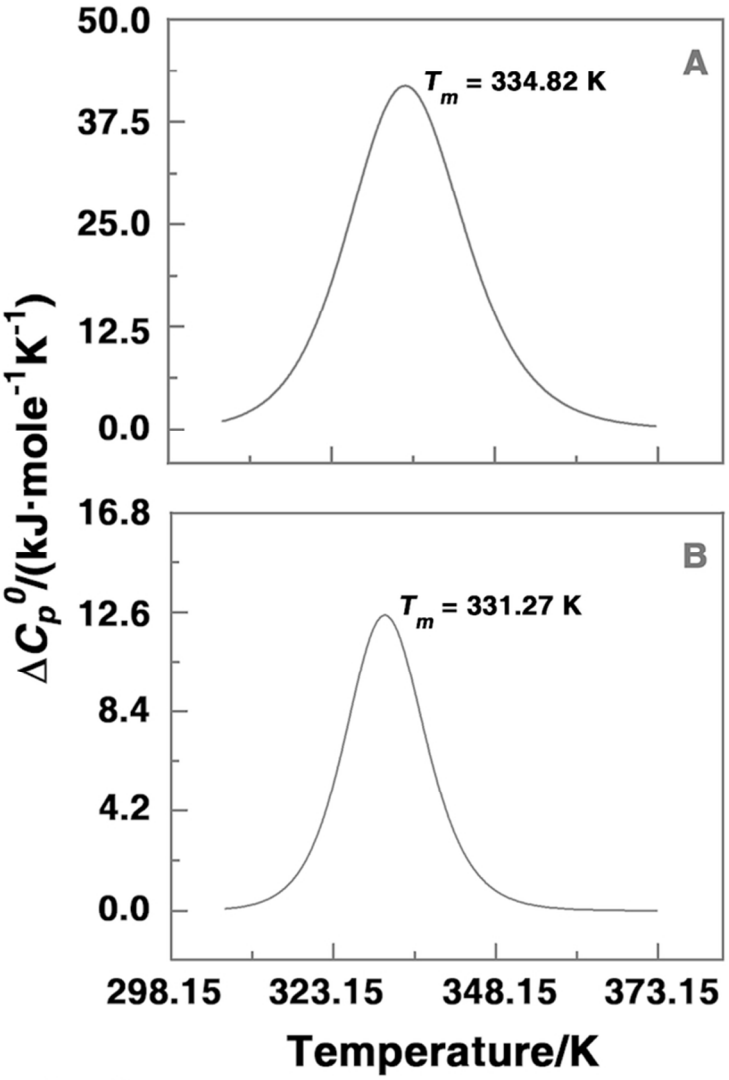

Fig. 6.

DSC thermograms of (A) Hb and (B) curcumin-Hb complex.
62x92mm (300 x 300 DPI)