A CALORIMETRIC STUDY OF THERMAL DENATURATION OF RECOMBINANT HUMAN LACTOFERRIN FROM RICE

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ABSTRACT AND KEYWORDS

Abstract

Thermal denaturation of recombinant human lactoferrin from transgenic rice with different degrees of iron saturation has been studied by differential scanning calorimetry (DSC). The maximum temperature, enthalpy change and activation energy of denaturation were higher when recombinant lactoferrin was more saturated with iron, indicating an increase in the stability of the protein structure. Maximum temperature and activation energy values for apo and holo-lactoferrin were practically identical to those reported for the same forms of lactoferrin from human milk, which indicates a similar thermal stability. However, the value of enthalpy change for denaturation of recombinant lactoferrin was 2.5 to 3-fold lower than that found for the human milk protein. This finding may reflect the influence that the different glycosylation pattern may have in the relationship between lactoferrin domains. Denaturation of recombinant lactoferrin in milk was compared with denaturation in phosphate buffer, and results indicated that the protein was more heat-sensitive when treated in milk than in buffer.

Keywords: recombinant human lactoferrin, heat denaturation, calorimetry

INTRODUCTION

Lactoferrin is a glycoprotein which belongs to the family of iron-binding proteins that also includes transferrin, ovotransferrin and melanotransferrin (*1*). It has a molecular weight of 80 kDa and its three-dimensional structure has been determined by crystallographic analysis (*2*). It consists of a polypeptide chain which forms two globular lobes with two domains each. There is one iron-binding site located in each lobe and the iron atoms bound are coordinated by four protein ligands: two tyrosines, one hystidine, and one aspartate. In the binding site, a CO_3^{-2} or HCO_3^- ion adjacent to an arginine side chain is also participating (*2*).

The ability to bind iron gives lactoferrin several biological activities as protection from pathogens by reducing the availability of iron to microorganisms, regulation of iron absorption in the intestine or inhibition of oxidative reactions (*3*). On the other hand, there are other functions attributed to lactoferrin for which the level of iron bound is not essential, such as the bactericidal activity of lactoferrin by interacting with the bacterial surface (*4*), the modulation of some functions of the immune system (*5*), the promoting activity on cellular growth (*6*) or the antitumoral activity (*7*).

Human milk is particularly rich in lactoferrin; however, bovine milk contains very low levels of this protein (*3*). Since milk products, based on bovine milk, are practically devoid of lactoferrin, supplementation with this protein would be interesting in order to make infant formula more similar to human milk. Up to now, bovine lactoferrin obtained from whey produced in the cheese-making process has been used as a supplement in special products. However, it is still not clear whether the activity of bovine lactoferrin is the same as that of human lactoferrin for all the proposed functions. Therefore, it would be interesting to study the possibility of using human lactoferrin in products for human consumption. Taking into account the difficulty in obtaining human

lactoferrin in high amounts, some systems have been developed to produce recombinant human lactoferrin (rhLF), such as fungi cultures (*8*), transgenic cows (*9*), or plants as tobacco (*10*), maize (*11*) and rice (*12*). The possibility of using the transgenic rice directly, without isolating lactoferrin, in some special products is very interesting because rice is a normal component of children's diet and besides it is a hypoallergenic food (*12*).

It is important to determine the thermal stability of rhLF in order to design treatments which ensure that the structure and biological activity of the protein are maintained. Although it has been reported that rhLF from rice possesses similar biological activities and a similar structure to human milk lactoferrin (*12, 13*), there are differences in the glycosylation pattern which could be important in the behaviour of the protein (*14*). There have been also reported differences in the glycans of the different transferrins (*15*) and also in the lactoferrin isolated from leucocytes compared with that from milk (*16*). Furthermore, recombinant lactoferrin from *Aspergillus awamori* also has different glycan structures from those of lactoferrin from human milk, (*8*). It has been reported that the unglycosylated lactoferrin is much more susceptible to degradation (*17*) and some studies have revealed that the susceptibility of lactoferrin to tryptic proteolysis depend on the type of glycans bound (*18*). Actually, human lactoferrin is more resistant to proteolysis than bovine lactoferrin due to differences in the glycan composition (*19*).

Thermal stability of lactoferrin has been studied in several works (*20-24*). The thermal parameters found for denaturation of bovine lactoferrin studied by calorimetry were lower (*22*) than those obtained for human lactoferrin (*25*), which indicates that the bovine protein is not as stable as the human one. We have reported in a previous work (*25*) that the behaviour of recombinant human lactoferrin from *Aspergillus awamori* is

similar to lactoferrin from human milk when subjected to calorimetry, which reflects the high degree of structural similarity between the two proteins. Several works have demonstrated that the iron bound to lactoferrin confers more resistance to thermal denaturation (*20, 22*) and proteolytic digestion (*26*) to the protein structure.

The objective of this work was to determine the thermal stability of recombinant human lactoferrin from rice by differential scanning calorimetry (DSC) in different conditions, in order to compare with that of lactoferrins from other origins and to facilitate the design of heat treatments which maintain its integrity and biological activity.

MATERIALS AND METHODS

Recombinant human lactoferrin (rhLF) isolated from rice was kindly provided by Ventria Bioscience (Sacramento, California, USA). It was supplied in three forms, apo (0.05 mg Fe/g LF); holo (1.3 mg Fe/g LF) and as isolated from rice (0.98 mg Fe/g LF). These proteins were analysed by SDS-PAGE showing a main band which corresponded to a protein with a molecular weight of about 80 kDa; therefore, they were used in the calorimetric experiments without further purification.

Solutions of the different forms of recombinant human lactoferrin described above were prepared in a buffer containing 15 mM potassium phosphate, 150 mM NaCl, pH 7.4 (PBS), at a protein concentration of 100 mg/mL. Samples and references (10 μL) were introduced into aluminium pans (TA Instruments, New Castle, USA) and sealed for analysis. The references consisted of pans containing the same volume of PBS or bovine skimmed milk.

Differential scanning calorimetry (DSC) of proteins was performed in a DuPont thermal analyzer (model DSC 10, Nemours, Germany), using a thermal analyst 2000

125 system. DSC scans were programmed in the temperature range of 35-110 ºC and at
126 heating rates of 2, 3, 4, 5, 7, 10 and 20 ºC/min. Samples were analysed at least by
127 triplicate. After treatment of proteins, denatured samples were left to cool at room
128 temperature and rescanned in the same conditions to evaluate if there was renaturation
129 of the proteins analysed.

130 From the transition peak obtained by DSC it is possible to obtain several
131 thermodynamic parameters. The enthalpy change of denaturation was calculated by
132 integrating the peak area using a straight baseline drawn from the onset to the end of
133 thermal transition. Values of temperature of maximum heat absorption (T_{max}), onset
134 temperature (T_s), and enthalpy change (ΔH_{cal}) of denaturation were plotted as a function
135 of heating rate, and the same parameters were estimated by extrapolation to 0 ºC. The
136 measurement of the endothermic peak width at its half height ($\Delta T_{1/2}$) was used to
137 calculate the van't Hoff enthalpy (*27*):

138 $$\Delta H_{VH} = 4RT_{max}^2/\Delta T_{1/2} \quad (1)$$

139 where R is the universal gas constant and T_{max} is the maximum peak temperature. In
140 order to check the irreversibility of the denaturation process, the value of the ratio
141 $\Delta H_{VH}/\Delta H_{cal}$ was determined (*28*).

142 The kinetic parameters for denaturation were calculated by the Kissinger's
143 method (*29*). This method is based on the relationship between the variation of the
144 maximum heat temperature and the heating rate according to this expression:

145 $$(\beta/T_{max}^2) = (AE_a/R)e^{-Ea/RTmax} \quad (2)$$

146 where β is the scanning rate, T_{max} is the maximum peak temperature, E_a is the activation
147 energy, A is the preexponential factor, and R is the universal gas constant. E_a/R was
148 determined from the slope of $\ln(\beta/T_{max}^2)$ vs $1/T_{max}$ without assuming any order of
149 reaction.

Data obtained were statistically evaluated by t-test using the SPSS 13.0 package for Windows.

RESULTS

Thermal denaturation of recombinant human lactoferrin was studied by DSC using different heating rates. The thermograms obtained for lactoferrin with different degrees of iron-saturation at a heating rate of 10 ºC/min are shown in **Figure 1**. The comparison between the three forms of lactoferrin is made considering the main peak of each form as the most representative. The apo and holo forms of recombinant human lactoferrin show one main denaturation peak with differences in position and shape due to the different conformation that present the two forms of lactoferrin. The thermogram obtained for apo-lactoferrin present a maximum temperature of denaturation at 72 ºC and, only at the heating rate of 10 ºC/min a minor peak appears with a maximum temperature at 85 ºC. Denaturation thermogram of lactoferrin as isolated, with 60 % iron-saturation, shows a main peak coincident with that of holo-lactoferrin and a minor peak with the same maximum temperature as the main peak of apo-lactoferrin. Temperatures of maximum heat absorption and enthalpy change are higher when lactoferrin is more saturated with iron as it is shown in **Table 1**. However, the half-peak height of the thermogram of apo-lactoferrin is wider than that of the iron-saturated forms. This fact could indicate that the apo form presents lower cooperativity in the denaturation process. The van't Hoff enthalpy was calculated considering the maximum peak temperature and width at half-peak height, as it has been described previously in Materials and Methods. The ratio between the calorimetric enthalpy change and the van't Hoff enthalpy (ΔH_{cal}:ΔH_{VH}) was also calculated. When the ratio ΔH_{cal}:ΔH_{VH} is around 1, it means that the calorimetric enthalpy change is equal to the van't Hoff

175 enthalpy, which occurs in small single-domain globular proteins (*28*). However, in
176 proteins in which the conformation is stabilized by interactions among several domains
177 the ratio ΔH_{cal}:ΔH_{VH} is greater than 1. Thus, we have found a ratio ΔH_{cal}:ΔH_{VH} above 1
178 for all the forms of recombinant lactoferrin, being for the iron-saturated protein very
179 close to 2.

180 As it is shown in **Figures 2** and **3**, the maximum peak temperature and enthalpy
181 change of denaturation are different depending on the heating rate suggesting that the
182 denaturation process is kinetically determined. The data were fitted to one line with
183 correlation coefficients from 0.92 to 0.94 for the maximum peak temperature and below
184 0.37 for the calorimetric enthalpy change. For recombinant human lactoferrin as
185 isolated, values of maximum peak temperature and enthalpy change of denaturation at a
186 heating rate of 2 ºC/min have not been considered because it was not possible to
187 determine the baseline properly in any of the replicates. The maximum peak
188 temperature parameters clearly increase with the heating rate; however, for the enthalpy
189 change, the increase with the heating rate was slight for the apo form, and this
190 parameter even decreased for lactoferrin as isolated. The values of maximum
191 temperature of denaturation, denaturation enthalpy change and onset temperature
192 obtained by extrapolation to 0 ºC/min are shown in **Table 2**. These parameters increased
193 with the iron-saturation degree of lactoferrin, mainly for the parameter of the
194 denaturation enthalpy change which is 2.5 times higher for holo-lactoferrin than for the
195 apo form.

196 The denaturation of recombinant human lactoferrin in bovine skimmed milk has
197 also been studied and the results obtained are shown in **Table 3**, together with those
198 obtained in PBS. The values of the maximum peak temperature, denaturation enthalpy
199 change and onset temperature of lactoferrin heated in bovine skimmed milk were

200 significantly lower for the majority of parameters than those obtained when heated in
201 PBS.
202 The Kissinger method was used to calculate the activation energy for thermal
203 denaturation of recombinant human lactoferrin. The Kissinger plots for the three forms
204 of lactoferrin adjust very well to straight lines, with correlation coefficients from 0.98 to
205 0.99 (**Figure 4**). The activation energy values were calculated from the slopes of those
206 straight lines. It has been observed that the activation energy increases with the iron-
207 saturation degree of lactoferrin, being the values obtained of 240.0, 318.6 and 387.4
208 KJ/mol for the apo, as isolated and holo forms, respectively.
209
210 **DISCUSSION**
211 In this work, we have studied the thermal behaviour of recombinant human
212 lactoferrin from rice by differential scanning calorimetry (DSC). The thermograms
213 obtained showed values of maximum peak temperature and enthalpy change higher
214 when lactoferrin was saturated with iron than when it was in its apo form. These results
215 indicate an increase in the protein stability with the increase in the degree of iron
216 saturation that has been also found for human milk lactoferrin and recombinant human
217 lactoferrin from *Aspergillus awamori* (*25*), and for bovine lactoferrin (*20, 22*).
218 Denaturation of the holo recombinant human lactoferrin from rice gave a single peak
219 which indicates that the two lobes of the protein have the same thermosensitivity and
220 denature simultaneously. The endotherm obtained for denaturation of the apo form
221 presented a single peak ~~for~~ at all heating rates, except for the 10 ºC/min heating rate, in
222 which a second small peak appeared at a maximum temperature around 85 ºC, peak
223 which might correspond to aggregated molecules. For lactoferrin as isolated (60% iron
224 saturation) an endotherm with two peaks has been found, a main peak coincident with

225 that of holo-rhLF and a minor peak coincident with the main peak of apo-rhLF. The
226 finding of a double peak in the endotherm had been previously reported for the 30%
227 iron-saturated bovine lactoferrin and for the holo-bovine lactoferrin (*22*). The presence
228 of these two peaks has been attributed to differences in the thermostability of the two
229 lobes of lactoferrin (*30*), since the C-lobe appears more compact than the N-lobe in the
230 iron-saturated protein (*31*), and it has been also explained by the presence of monoferric
231 species (*20*).

232 The maximum temperatures of the endotherm obtained for recombinant human
233 lactoferrin from rice in the different forms, are very similar to the values we obtained in
234 previous studies for human milk lactoferrin and for the recombinant protein from
235 *Aspergillus awamori* (*25*), which means that all the proteins present similar thermal
236 stability. However, the value of enthalpy change for denaturation of recombinant human
237 lactoferrin from rice is about 2.5 or 3 times lower, depending on the degree of iron
238 saturation, than that obtained for human milk lactoferrin or for the protein produced in
239 *Aspergillus awamori*. This fact could be due to some differences in the structure of the
240 protein. It has been reported that the molecular weight of the recombinant lactoferrin
241 from rice is lower than that of lactoferrin from human milk, 78.5 and 80.6 kDa,
242 respectively (*12*). This difference is likely the result of a lower degree of glycosylation
243 for the recombinant protein, with 2.9% of glycans, compared with the milk protein ~~from~~
244 in which glycans represent 5.5% (*12, 13*). There are also differences in the type of
245 glycans due to the different mechanisms of glycosylation ~~of~~ that the vegetable cells
246 have compared to those of the mammary-gland epithelial cells. Thus, lactoferrin from
247 human milk has the typical glycans of mammals, such as α2-6-linked neuraminic acid,
248 β1-4- linked galactose and α1-6-linked fucose (*32*); and lactoferrin from rice has the
249 typical glycans of vegetables such as α1-3-linked fucose and β1-2-linked xilose (*14*).

250 Differences in glycosylation have been also found in other recombinant human
251 lactoferrins like those produced in *Aspergillus awamori* (*8*), in the milk of transgenic
252 cows (*9*) or in the milk of transgenic mice (*33*).

253 Glycans may be involved in intermolecular carbohydrate-carbohydrate or
254 carbohydrate-protein interactions and thus they can have an influence on the
255 relationship between protein domains (*34*). It is known that in the process of protein
256 denaturation there are many endothermic reactions involved (break-up of electrostatic
257 and van der Waals' interactions, and hydrogen bonds) and also exothermic reactions
258 (break-up of hydrophobic interactions) which can lower the overall observed enthalpy
259 (*35*). Furthermore, in the case of large globular proteins, like lactoferrin, the
260 denaturation state is irreversible and aggregation processes, which are generally
261 exothermic, can take place especially at high concentrations of protein, as those used in
262 DSC technique. For these reasons, the enthalpy change (ΔH) observed by DSC can vary
263 depending on the denaturation temperature and on hydrophobicity and aggregation
264 processes (*35*). Interactions among hydrophobic regions, could be more strengthened in
265 recombinant human lactoferrin from rice than in human milk lactoferrin due to
266 differences in glycosylation and, consequently, their break-up with thermal denaturation
267 could decrease the enthalpy change value.

268 The analysis of the crystallographic structure of human lactoferrin revealed that
269 the sites of glycan attachment are situated on the surface of the molecule (*2*).
270 Furthermore, it has been reported that human lactoferrin bears three potential sites for
271 N-glycosylation at Asn^{138} in the N-lobe, and Asn^{479} and Ans^{624} in the C-lobe (*17*); a
272 study with glycosylation-site mutants (*18*) has shown that Asn^{138} and Asn^{479} are the
273 preferential glycosylation sites. Although it is well known that glycosylation affects the
274 three-dimensional structure and the dynamics of a protein (*34*), any study has shown

how the variations in position and type of glycans attached may influence the structure of lactoferrin.

A work carried out by van Berkel et al., (*17*) showed that unglycosylated rhLF from human kidney 293(S) cells was much more susceptible to degradation by trypsin than the glycosylated form. However, another study published by the same group revealed that the susceptibility of the protein to proteolysis was more affected by an altered conformation of glycans rather than by absence of glycosylation (*18*). It has been reported that the oligosaccharides bound to proteins confer resistance to proteolysis; either because they cause an increase of the protein rigidity or because glycans sterically protect the susceptible sites for proteolytic enzymes (*34*).

In the studies reported until now, no significant differences in the biological activities have been found between recombinant lactoferrin from rice and lactoferrin from human milk. These proteins bind the same amount of iron at various pHs, they have the same pH dependency in iron release and they both inhibit the growth of human pathogens (*12, 13*). Moreover, hLF and rhLF from rice bind to the human colon carcinoma cell line Caco-2, being the binding constant similar for both proteins, though the number of binding sites reported was slightly higher for rhLF, difference that has been attributed to differences in glycosylation (*12*).

The calculation of van't Hoff enthalpy from the width at half-peak height of the transition peak gives information about the complexity of the denaturation process. When this value approaches the actual value of enthalpy obtained by integrating the endotherm, it means that the protein denaturation follows a two-state kinetic model (*28*). However, in the case of rhLF from rice the ratio ΔH_{cal}:ΔH_{VH} is above 1.0 for the three forms of lactoferrin, being for the holo-rhLF almost 2. Those results indicate that partially unfolded intermediates are in equilibrium with the native state during the

thermal denaturation process. This occurs in proteins with a conformation ~~is~~ stabilized by interactions among several domains. The three-dimensional structure of lactoferrin is in agreement with these results, as it consists of two globular lobes each of them organized into two domains with the iron site at the domain interface (*2*). In the case of lactoferrin from human milk, the values obtained for the ratio ΔH_{cal}:ΔH_{VH} were close to 4 (*25*), much higher than those obtained for the recombinant protein from rice which also reflects differences between them in their thermal behaviour.

The activation energy for denaturation of rhLF from rice has been calculated by the method of Kissinger (*29*). It has been observed that the activation energy increases with the iron-saturation degree of lactoferrin that is in agreement with previous results which show higher thermal stability for iron-saturated lactoferrin. Although this method is applied to single denaturation reactions which ~~are~~ is not the case of lactoferrin, we have used it to obtain a kinetic approximation to thermal denaturation of rhLF. The Kissinger plot obtained for rhLF gives a straight line which indicates a good correspondence with single reactions (*36*). The values of activation energy obtained for lactoferrin from rice are very close to those obtained for lactoferrin from human milk and for the recombinant protein from *Aspergillus awamori* (*25*), which indicate a very close similarity in their structure.

In order to determine if the composition of the medium can influence the thermal behaviour of recombinant human lactoferrin from rice, its denaturation has also been studied by DSC in bovine skimmed milk. Milk subjected to DSC without added proteins did not show any endothermic peak. This is due to the low concentration of proteins in bovine whey and also to the absence of signal given by casein when subjected to DSC (*37*). The results obtained for lactoferrin denaturation in milk were compared with those obtained for the protein denatured in PBS. We found that the peak

maximum temperature and the onset temperature were significantly lower when lactoferrin was heated in bovine skimmed milk than in buffer. The denaturation enthalpy was also lower for the three forms of lactoferrin when heated in skimmed bovine milk, although only in the case of apo-rhLF the difference was found to be statistically significant. The lower values of thermal parameters obtained for lactoferrin denatured in bovine milk compared to those in buffer could be due to the pH decrease that milk experiments when is heated and also to changes in the balance of calcium (*22*). Similar results were also found for bovine lactoferrin (*22*), for human milk lactoferrin and for recombinant lactoferrin produced in *Aspergillus awamori* (*25*). Therefore, the composition of the medium should be taken into account to determine the thermostability of lactoferrin in each product.

The results of this work did not show a different thermal stability of human recombinant lactoferrin from rice when compared to human lactoferrin from milk. However, the lower value of the enthalpy change observed for the recombinant protein may suggest the existence of differences in the relationship between the glycans and the domains of lactoferrin. Further experiments are needed to evaluate if those differences may affect to the various biological activities exerted by lactoferrin.

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FIGURE CAPTIONS

Figure 1. Differential scanning calorimetry thermograms of recombinant human lactoferrin from rice as apo form (a), as isolated (b) and as holo form (c). Scanning was performed at a heating rate of 10 ºC/min.

Figure 2. Maximum peak temperature (T_{max}) at different heating rates for denaturation of recombinant human lactoferrin from rice in PBS as apo (■), as isolated (▲) and as holo form (□).

Figure 3. Enthalpy change (ΔHcal) at different heating rates for denaturation of recombinant human lactoferrin from rice in PBS as apo (■), as isolated (▲) and as holo form (□).

Figure 4. Kissinger plot for heat denaturation of recombinant human lactoferrin from rice in PBS as apo (■), as isolated (▲) and as holo form (□). T_{max} is the peak maximum temperature (degrees Kelvin) and β is the scanning rate. E_a = activation energy.

TABLES

Table 1. Thermal parameters for denaturation of recombinant human lactoferrin from rice in PBS at a heating rate of 10 ºC/min. Each value represents the mean ± SD of 3 or 4 replicates.

	T_{max}[a] (ºC)	ΔH_{cal}[b] (KJ/mol)	$\Delta T_{1/2}$[c] (ºC)	ΔH_{VH}[d] (KJ/mol)	ΔH_{cal}:ΔH_{VH}
Apo-rhLF	71.92 ± 0.23	801 ± 55	6.28 ± 0.53	634 ± 58	1.27 ± 0.14
As-isolated-rhLF	93.47 ± 0.09	1041 ± 150	5.35 ± 0.48	840 ± 80	1.26 ± 0.27
Holo-rhLF	93.84 ± 0.26	1549 ± 79	5.58 ± 0.26	804 ± 39	1.93 ± 0.19

[a]Peak maximum temperature. [b]Denaturation enthalpy change. [c]Half-width denaturation peak. [d]Van't Hoff enthalpy of denaturation.

515 **Table 2**. Thermal parameters for denaturation of recombinant human lactoferrin from
516 rice in PBS, extrapolated to 0 ºC/min.
517

	T_{max}[a] (ºC)	ΔH_{cal}[b] (KJ/mol)	T_s[c] (ºC)
Apo-rhLF	66.47	583	63.10
As-isolated-rhLF	89.36	1361	85.07
Holo-rhLF	89.81	1500	85.45

518 [a]Peak maximum temperature. [b]Denaturation enthalpy. [c]Onset temperature.
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Table 3. Comparison of the thermal parameters for denaturation of recombinant human lactoferrin from rice in PBS and in bovine skimmed milk at a heating rate of 10 ºC/min. Each value represents the mean ± SD of 3 or 4 replicates.

	Apo-rhLF			As-isolated-rhLF			Holo-rhLF		
	T_{max} (ºC)	ΔH_{cal} (KJ/mol)	T_s (ºC)	T_{max} (ºC)	ΔH_{cal} (KJ/mol)	T_s (ºC)	T_{max} (ºC)	ΔH_{cal} (KJ/mol)	T_s (ºC)
PBS	71.92 ± 0.23	801 ± 55	66.00 ± 0.65	93.47 ± 0.09	1041 ± 150	88.32 ± 0.56	93.84 ± 0.26	1549 ± 79	89.10 ± 0.90
Milk	66.50* ± 0.39	602* ± 130	59.62* ± 1.68	91.51* ± 0.07	951 ± 98	87.51* ± 0.21	91.56* ± 0.10	1281 ± 310	87.69* ± 0.17

* Significant difference for $p<0.05$

Figure 1.

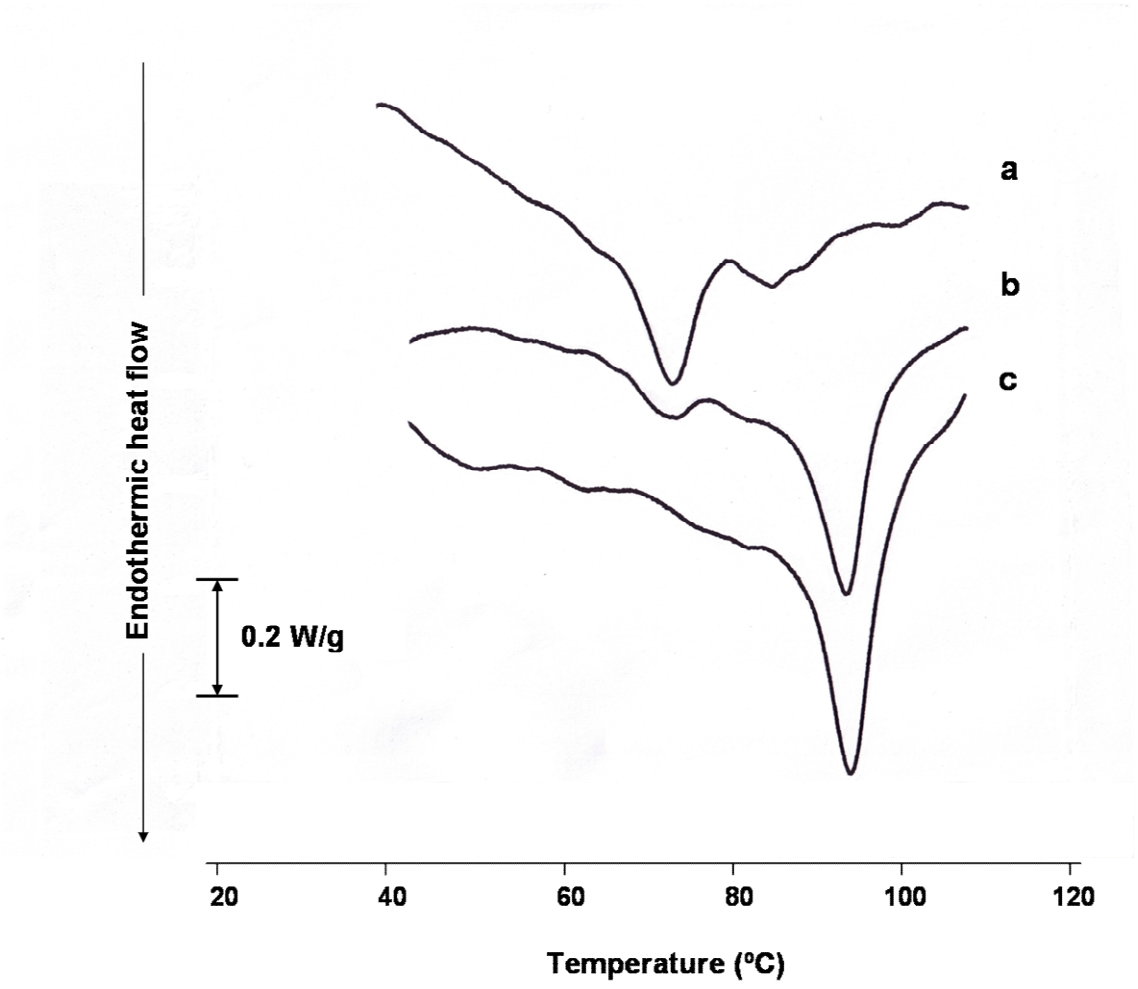

Endothermic heat flow
0.2 W/g
a
b
c
20
40
60
80
100
120
Temperature (°C)

Figure 2.

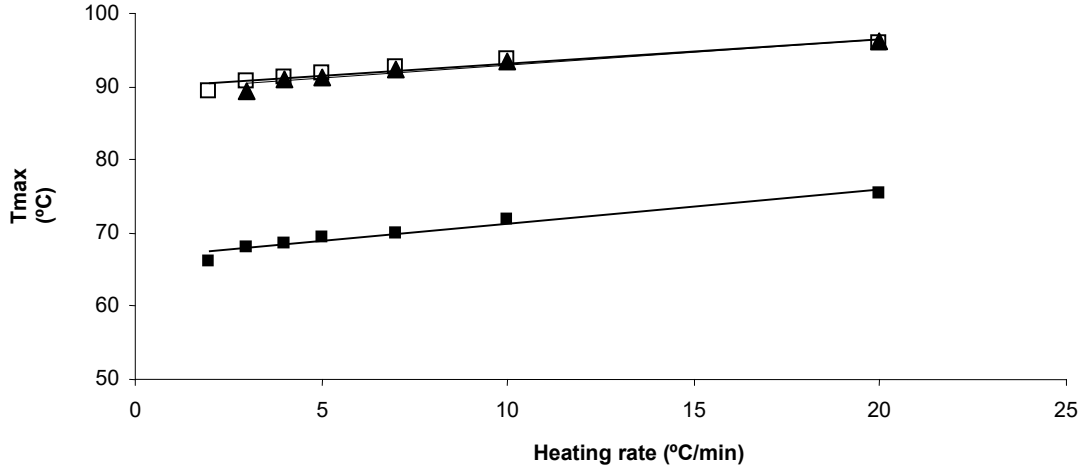


Figure 3.

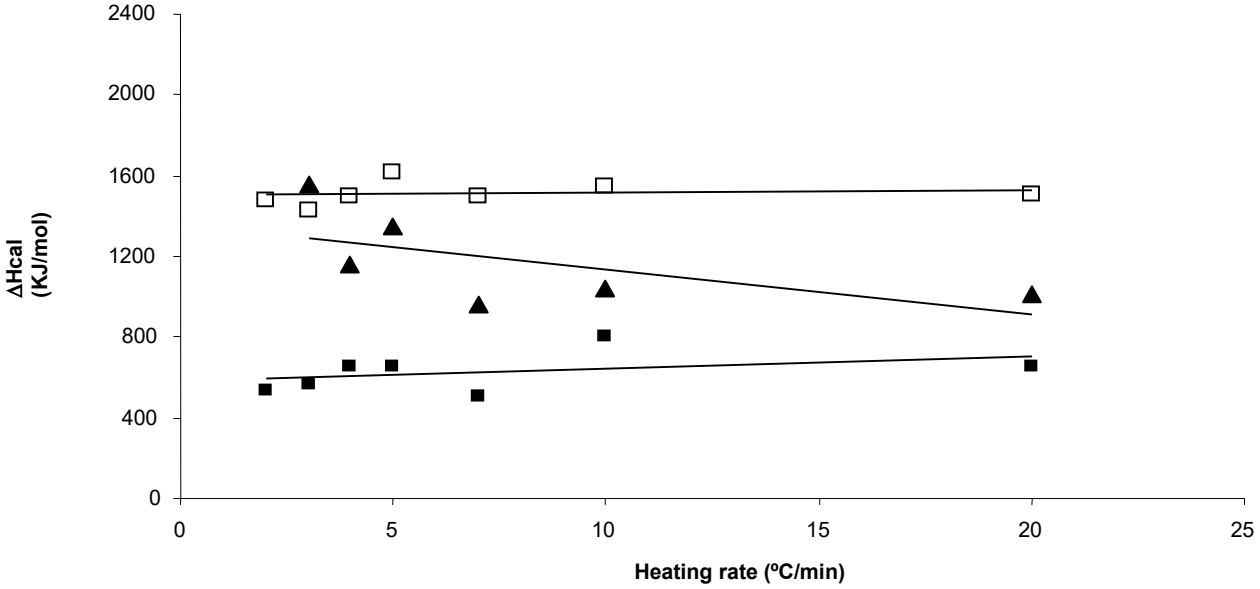
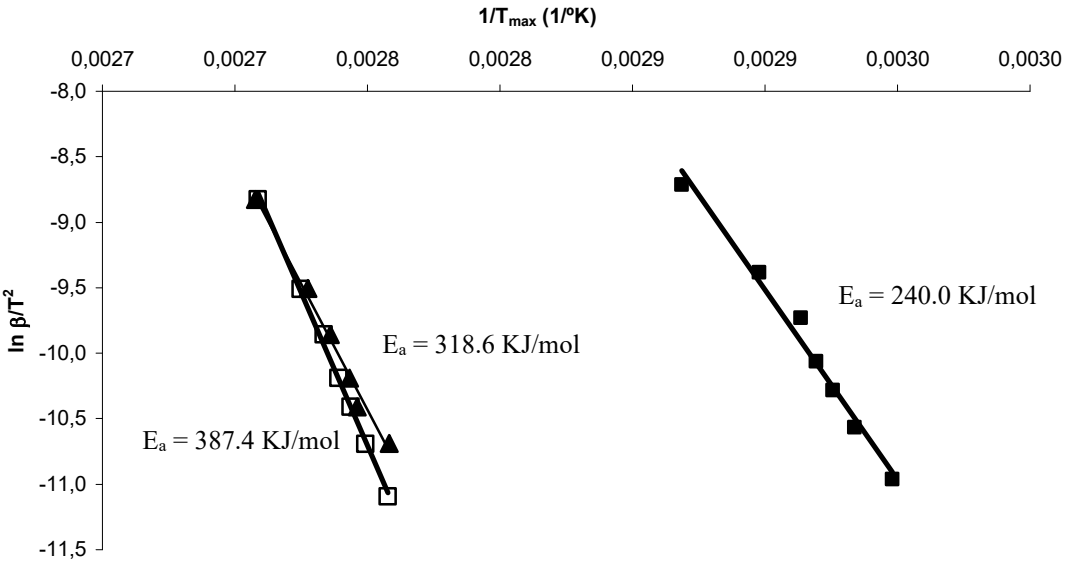
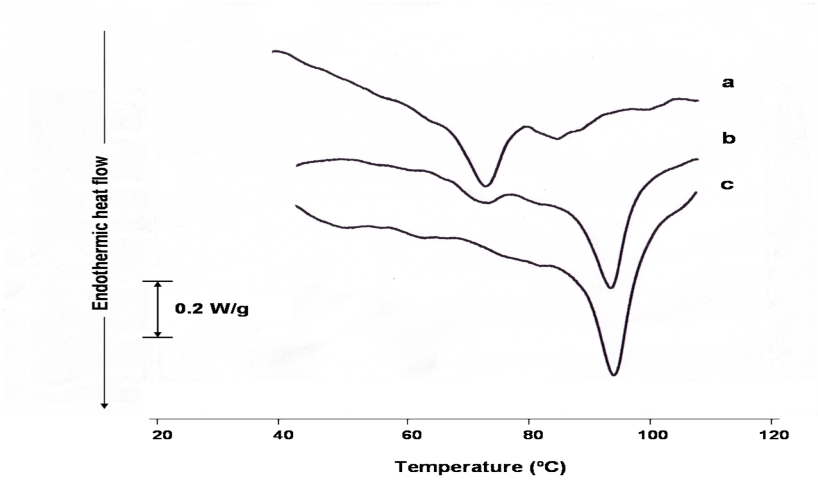

2400
2000
1600
1200
800
400
0
ΔHcal (KJ/mol)
0
5
10
15
20
25
Heating rate (ºC/min)

Figure 4.


1/T_max (1/°K)
0,0027
0,0027
0,0028
0,0028
0,0029
0,0029
0,0030
0,0030
-8,0
-8,5
-9,0
-9,5
-10,0
-10,5
-11,0
-11,5
ln β/T²
E_a = 318.6 KJ/mol
E_a = 387.4 KJ/mol
E_a = 240.0 KJ/mol

Graphic for Table of Contents



Differential scanning calorimetry thermograms of recombinant human lactoferrin from rice as apo form (a), as isolated (b) and as holo form (c).