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Effect of hydrocolloids on the thermal denaturation of proteins

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Abstract

The thermal denaturation of bovine serum albumin (BSA), lysozyme and whey protein isolate (WPI) in the presence of hydrocolloids (pectin, guar gum, ι-carrageenan) was investigated. A decrease in the thermal stability of lysozyme was observed in the mixture of protein with ι-carrageenan. The increase in the enthalpy of denaturation (ΔH) of BSA and lysozyme in the presence of hydrocolloids was attributed to the protection of globular proteins against aggregation through blockage of their hydrophobic binding sites by the bulky polysaccharide moeity. Biopolymers had a stabilizing effect on WPI. The thermal stability was the highest in the presence of pectin, whereas the lowest transition temperature was observed in the presence of guar gum. A single transition peak was observed for pure WPI. However, WPI exhibited two transition temperatures when together with pectin and *i*-carrageenan. WPI was stable against heat denaturation at acidic pH values (pH 4.0), while it was denatured at a low temperature at an alkaline pH (pH 9.0) in the presence of pectin. This was attributed to the formation of extra hydrogen bonding. The increase in the concentration of pectin has little affect on the heat stability of WPI; however, it reduces the cooperativity of transition.

Keywords: DSC; Denaturation; BSA; Lysozyme; Whey protein isolate; Hydrocolloids

1. Introduction

Proteins are functional ingredients of many colloidal food products that are recognised for conferring stability through the formation of interfacial films. Hydrocolloid gums are mostly hydrophilic in nature and they do not exhibit significant surface activity (Dickinson & Stainsby, 1988). However, there are carbohydrate polymers that exhibit sufficient hydrophobic character to adsorb strongly at the oil–water interface, thereby having the capacity for making and stabilizing oil-in-water emulsions. Some naturally occuring galactomannan hydrocolloids, e.g., guar, fenugreek gums (Garti, Madar, Aserin, & Sternheim, 1997) and gum arabic (Dickinson, Elverson, & Murray, 1989), which is a commercially important emulsifying agent for flavour oils, are examples falling in this category.

Heat treatment has a pronounced effect on the structures and functional properties of proteins (de Witt, 1981). Heat denaturation of globular proteins is associated with the destruction of some of the forces that stabilise native conformations, such as hydrogen bonds and hydrophobic interactions. The disruption of electrostatic and van der Waals interactions are observed to a lesser extent (Relkin, 1994). Upon heating, thiol/disulphide interchange reactions are enhanced in β-lactoglobulin leading to conformation change and subsequent high molecular weight polymer formation (Relkin, 1998). However, heat treatment is of great importance in the production, processing and concentration of proteins. Thermal denaturation of globular proteins has been extensively studied and thermodynamic parameters have been determined (Baeza & Pilosof, 2002; Galani & Owusu Apenten, 2000; Khechinashvili, 1990; Lapanje & Poklar, 1989; Li, Klimov, & Thirumalai, 2004; Sreerama, Venyaminov, & Woody, 2000). Differential scanning calorimetry (DSC) has been established as a sensitive technique for studying thermal denaturation and conformational transitions of proteins (Arntfield, Ismond, & Murray, 1990; Boye & Alli, 2000; Hendrix, Griko, & Privalov, 2000; Paulsson, Hegg, & Castberg, 1985; Relkin, 1994) providing qualitative and quantitative information as to the thermodynamic

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properties of proteins. Many colloidal foods contain hydrocolloids added for control of stability and rheological properties together with proteins (Dickinson & Euston, 1991). Physicochemical characteristics of proteins and gums determine the type of interaction between the polymers which, in turn, may affect the stability of the colloidal system together with their relative proportions and solution conditions. An attractive electrostatic interaction has been reported between bovine serum albumin (BSA) and carrageenans at pH 7.0, which becomes much stronger at acidic pH values (Dickinson & Pawlowsky, 1998). It has been revealed that in the presence of glucose and sucrose there was no aggregation of α-lactalbumin and denaturation on heating was reversible (Boye, Alli, & Ismail, 1997). Harwalkar and Ma (1992) reported similar results for β-lactoglobulin. However, it has also been indicated that the complexation with a polysaccharide may perturb protein structure and decrease the thermal stability of a protein (Imeson, Ledward, & Mitchell, 1977). Following heat denaturation, much stronger interactions are formed giving rise to stable high molecular weight complexes which inhibit the protein–protein aggregation and therefore increase the solubility of the protein (Kato, Sato, & Kobayashi, 1989). This is very important in commercial applications such that protein–polysaccharide complexes can be used as an emulsifier after sterilisation. There is relatively limited information on the thermal denaturation characteristics of proteins in the presence of hydrocolloids. They have the ability to interact with proteins electrostatically and may behave as spacers to inhibit coagulation of proteins.

The purpose of this work is to examine the thermal denaturation characteristics of the globular proteins (WPI, BSA and lysozyme) in the presence of neutral and charged hydrocolloids (pectin, ι-carrageenan and guar gum) by DSC.

2. Materials and methods

2.1. Materials

WPI was obtained from Davisco Foods International (Le Sueur, MN, USA). BSA (B-4287 and A-6793), lysozyme (L-6876), low methoxy pectin (P-9135), guar gum (G-4129) and ι-carrageenan (C-1138) were purchased from Sigma Chemicals. Buffer salts were purchased from BDH Chemicals.

2.2. Sample preparation

Protein solutions were prepared at room temperature by dissolving proteins (BSA, WPI and lysozyme) in phosphate buffer (ionic strength of 0.05 M, pH 7.0) at a concentration of 40% (w/v). Hydrocolloids were dissolved at a concentration of 2% (w/v) in buffer. Protein–hydrocolloid mixtures were prepared by mixing 1 ml of each solution. An effective concentration of 20% (w/v) was suggested for each protein for satisfactory DSC sensitivity (Boye & Alli, 2000). In the mixtures, the final protein to hydrocolloid weight ratio was 20:1. A mixture of WPI and pectin (protein to hydrocolloid weight ratio of 20:5) was prepared to investigate the effect of a high concentration of hydrocolloid on the thermal behaviour of protein. To study the effect of pH on the WPI/pectin (weight ratio of 20:1), the mixtures were prepared in phosphate buffer at pH 4.0, 7.0 and 9.0.

2.3. Differential scanning calorimetry (DSC)

Aliquots (50 μl) of the mixtures were placed in preweighed DSC pans and the pan was hermetically sealed and weighed accurately. The samples were placed in the Perkin–Elmer Pyris 6 DSC and scanned from 15 to 100 °C at a programmed heating rate of 5 °C/min. The DSC was calibrated by use of indium standards. For each run, an empty sample pan was used as reference. The heat of transition (ΔH) and peak temperature of denaturation (T_d) were computed from each thermal curve. ΔH values were given as corrected per unit weight of protein. All DSC measurements were done in, at least, duplicate.

3. Results and discussion

Fig. 1 shows the DSC thermograms of BSA, whey protein and lysozyme. BSA was observed to give a single transition at the peak temperature (T_d) 87.1 °C. However, the peak temperature of BSA has been observed to be 63.4 °C at pH 7.0 (Boye, Alli, & Ismail, 1996). Fig. 2 represents the DSC thermograms of two different BSA samples from Sigma Chemicals giving transition peaks at 63.5 °C (B-4287) and at 87.1 °C (A-6793). This might arise from the differentiation of the purification methods for the two BSA samples. Since we intended to do further experiments with the sample giving a transition peak at 87.1 °C, the rest of the work has been carried out with this sample.

Table 1 represents the peak temperatures and the enthalpy changes for heat denaturation of pure proteins and protein + hydrocolloid mixtures. The peak temperatures for BSA and BSA + hydrocolloids were observed to change within the experimental error range. Therefore, the presence of these biopolymers does not affect the thermal stability of the protein. However, enthalpy values for heat denaturation increased from 1.51 to 1.66, 1.77, and 1.84 J/g for mixtures of protein with guar gum, ι-carrageenan, and pectin, respectively. Aggregation and disruption of hydrophobic interactions have been reported as exothermic reactions, which lower the observed

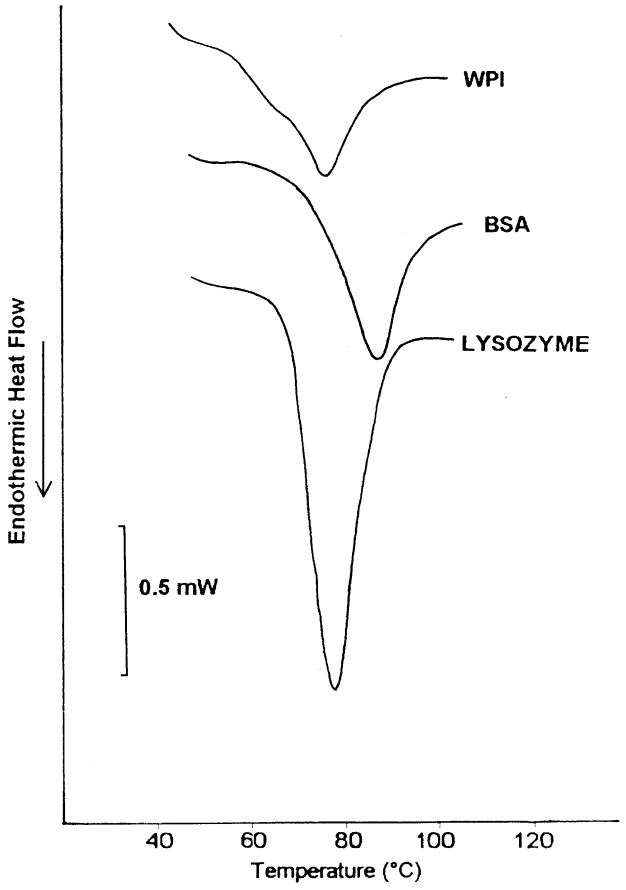

Fig. 1. DSC thermograms of BSA, lysozyme and WPI (20% w/v) solutions in phosphate buffer (0.05 M, pH 7.0).

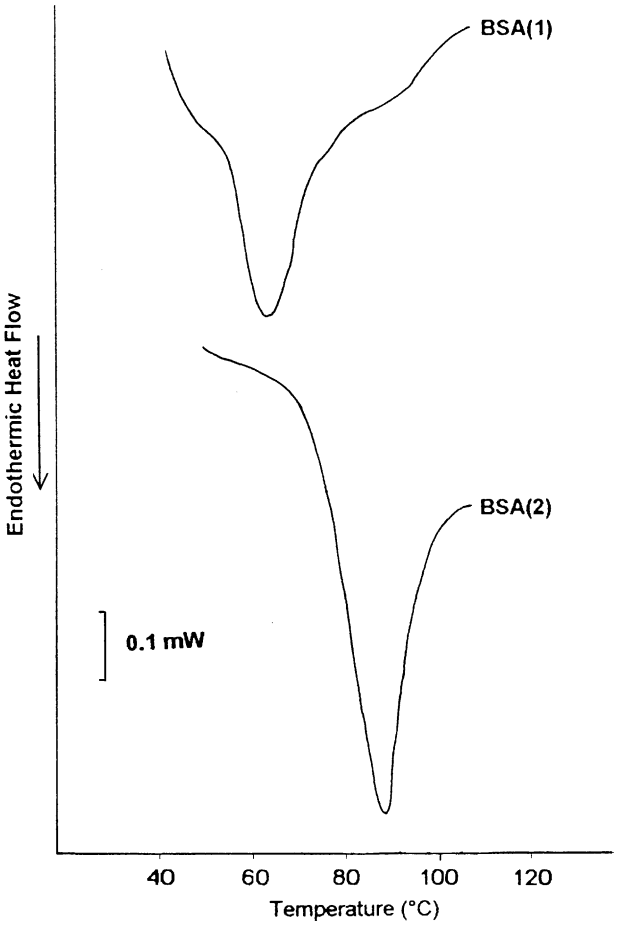

Fig. 2. DSC thermograms BSA (BSA1: Sigma Chemicals, B-4287 and BSA2: A-6793) (20% w/v) solutions in phosphate buffer (0.05 M, pH 7.0).

enthalpy (Boye et al., 1996). A slight increase in the enthalpy change might arise from reduction in the aggregation, especially in the case of neutral polysaccharide guar gum, which is not capable of interacting electrostatically with the protein molecules. Therefore, the hydrocolloid molecules may behave as steric spacers between the protein molecules, protecting the globular protein against aggregation by blocking the hydrophobic binding sites on the surface. Unlike the non-interacting guar gum, ι-carrageenan and pectin have high negative charge density. When both protein and hydrocolloid have a net negative charge, the attraction of positively charged local patches on the protein and the negative charges on the polysaccharide result in the formation of soluble protein–polysaccharide complexes (Park, Muhoberac, Dubin, & Xia, 1992). An electrostatic complexation of BSA with ι-carrageenan has been demonstrated at low ionic strength (Galazka, Smith, Ledward, & Dickinson, 1999). Substantially, the complex formation before heating protects against the loss of solubility resulting from protein aggregation (Ledward, 1979). While describing the relative effects of carboxylated hydrocolloids (e.g., pectin) and sulfated hydrocolloids (e.g., carrageenan) in protein–polysaccharide systems, it has

Table 1
The peak temperatures (T_d) and the enthalpy changes (ΔH) for heat denaturation of pure proteins and protein + hydrocolloid mixtures

	Pure protein		Guar gum		ι-Carrageenan		Pectin	
	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)	T_d (°C)	ΔH (J/g)
Lysozyme	76.8	2.88	77.3	3.46	74.2	3.17	77.5	3.29
BSA	87.1	1.51	87.5	1.66	87.4	1.77	87.9	1.84
WPI	74.6	1.34	77.1	0.62	78.5	0.60	80.1	0.65
(pH 4.0)							80.8	0.63
(pH 9.0)							77.5	0.98
(5% pectin)							79.4	0.65

been stated (Dickinson, 2003) that the positively charged groups on a protein ($-NH_3^+$) attract $-OSO_3^-$ groups more strongly compared with $-CO_2^-$ groups. Therefore, the increased net negative charge contributes to the enhancement of protein–protein electrostatic repulsion at low ionic strengths for carboxylated polysaccharides, and the protein–polysaccharide complexation becomes very weak or non-existent at pH > pI. However, the protein is capable of forming fairly strong complexes with sulfated hydrocolloids, even at neutral or alkaline pH.

The reason why we observed fairly high enthalpy values in BSA + pectin mixtures (Table 1) may result from a strong repulsion in the network of negatively charged molecules that reduce the extent of aggregation. Similarly, lysozyme in the presence of those three biopolymers needed more energy for thermal denaturation (Table 1) compared with pure lysozyme. The highest energy was consumed by the lysozyme + guar gum mixture. Thus, the best protection against aggregation was achieved by the neutral hydrocolloid. Lysozyme carries a net positive charge at neutral pH and is capable of interacting with negatively charged biopolymers molecules. The presence of ι-carrageenan reduces the transition temperature of lysozyme from 76.8 to 74.2 °C, so the thermal stability of the lysozyme might be reduced upon complex formation. However, the change in the peak temperature of the protein in the presence of guar gum and pectin (77.3 and 77.5 °C, respectively) are slightly above the experimental error range. There were very little changes observed in the peak width at half height of the DSC thermograms of BSA and lysozyme in the presence of those three hydrocolloids, which may suggest that the biopolymers do not interfere with the cooperativity of unfolding.

Fig. 3 shows the DSC thermograms of WPI and WPI + hydrocolloid mixtures. Pure WPI solution showed a single thermal transition at 74.6 °C. A single peak for the transition of whey protein concentrate has been reported by Boye, Alli, Ismail, Gibbs, and Konishi (1995). From the thermogram of WPI + pectin mixture, two transitions were observed at 69.2 and 80.1 °C. For the WPI + ι-carrageenan mixture, the thermogram also shows two transition peaks at 52 and 78.5 °C. However, the first transition observed in the WPI + pectin mixture appeared as a shoulder in the presence of carrageenan. The second big transition peak in both thermograms presumably represents the denaturation of β-lactoglobulin considering the high percentage of this protein in the WPI (de Witt & Swinkel, 1980). The presence of guar gum together with WPI led to a single transition peak at 77.1 °C with two shoulders at approximately the same temperatures corresponding to the first transition peaks observed in the presence of pectin and *i*-carrageenan. The presence of hydrocolloid biopolymers increased the thermal stability of the WPI. The highest thermal stability was observed in the presence of pectin

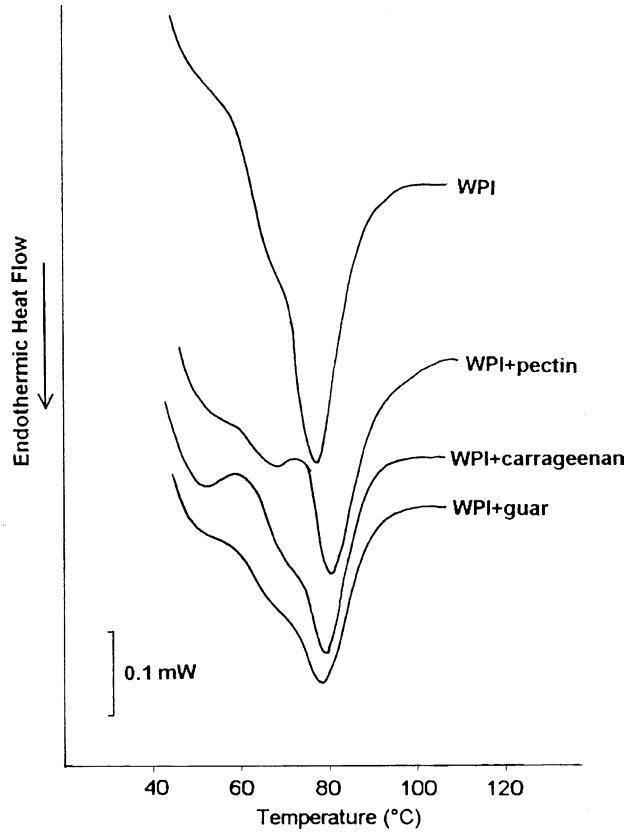

Fig. 3. DSC thermograms of WPI + hydrocolloids solutions (20% w/v protein, 1% w/v hydrocolloid) in phosphate buffer (0.05 M, pH 7.0).

(80.1 °C), whereas the lowest transition temperature was observed in the presence of guar gum (77.1 °C). The peak width at half height showed that WPI exhibited a transition with a great cooperativity. The decrease in cooperativity was observed in the presence of hydrocolloid molecules. The least ccoperative transition was exhibited by WPI + guar gum mixture.

For further information on the effects of the hydrocolloids on the denaturation of WPI, the areas underneath the DSC transition peaks (ΔH) were compared. A decrease in denaturation enthalpy half of the total enthalpy obtained by WPI was observed with WPI + hydrocolloid mixtures (Table 1). The lower values suggest that less energy is required for the denaturation. This may arise either from the partial denaturation of WPI prior to heat treatment due to high charge density of biopolymers or from the aggregation of protein as a result of phase separation. The latter induces a high exothermic enthalpy value (due to the aggregation of protein), which results in a decrease in the total enthalpy observed underneath the transition peak. A phase separation was reported in the solution of milk proteins and polysaccharides (guar gum, locust bean gum or xanthan gum) even at low concentrations (0.36%) (Thaiudom & Goff, 2003).

To investigate the influence of a high hydrocolloid concentration and the effect of pH on the thermal denaturation of WPI in the presence of pectin, the DSC analysis of the WPI + pectin samples was performed. The highest peak temperature (80.8 °C) was observed at pH 4.0, while a relatively low peak temperature (77.5 °C) was obtained at pH 9.0. This coincides with the results of Varunsatian, Watanabe, Hayakawa, and Nakamura (1983) who reported that whey protein concentrate was stable against heat denaturation at acidic pH values (pH < pI), while it was denatured extensively by heating at alkaline pHs (pH > pI). This increase in thermal stability at acidic pH values has been attributed to extra hydrogen bonding (Kella & Kinsella, 1988). The width at half peak height of the DSC thermogram at pH 4.0 is narrow (Fig. 4) showing the cooperativity of the transition. However, the peak at pH 9.0 is relatively broad indicating less cooperativity. The transition enthalpy of the WPI + pectin mixture is higher at pH 9.0 than those at pH 7.0 and pH 4.0 (Table 1). This may indicate a reduction in the extent of aggregation at alkaline pH.

In the sample containing 5% (w/w) pectin three peaks (49.8, 66.8, 79.4 °C) were observed during transition (Fig. 4). The thermal stability is slightly reduced compared with the stability of mixture containing 1% pectin (Table 1). This may arise from the high charge density of molecules resulting in a strong repelling effect which, in turn, promotes the unfolding of molecules.

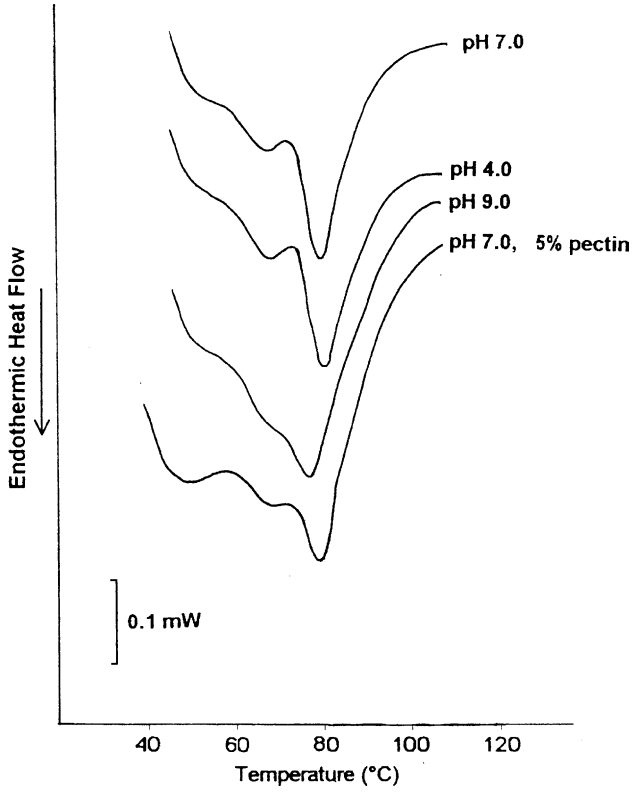

Fig. 4. DSC thermograms of WPI (20% w/v) + pectin solutions (at pH 7.0, with 1% w/v pectin, at pH 4.0, with 1% w/v pectin, at pH 9.0, with 1% w/v pectin, and at pH 7.0, with 5% w/v) pectin in phosphate buffer (0.05 M).

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