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Binding of hexadecyltrimethylammonium bromide to starch polysaccharides. Part II. Calorimetric study

Henrik Lundqvist[a,*], Ann-Charlotte Eliasson[a], Gerd Olofsson[b]

[a]*Department of Food Technology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[b]*Department of Physical Chemistry 1, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*

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Abstract

Isothermal titration calorimetry was used to study the interaction between hexadecyltrimethylammonium bromide, CTAB, and three starch polysaccharides, amylose from potato, amylopectin from potato and amylopectin from barley. The enthalpy change for consecutive additions of CTAB to starch polysaccharide solutions were measured at 27°C. The starch-CTAB interaction enthalpies, ΔH_t, were calculated by subtracting the enthalpy of micelle dissociation and dilution from the observed heat and relating the interaction enthalpy to the amount of interacting CTAB. The interaction was studied at three polysaccharide concentrations, 0.1, 0.25 and 0.5% w/w. The exothermic interaction enthalpy was constant and quite large in the main part of the concentration range studied for all three-starch polysaccharides. Amylose had an interaction enthalpy of −55 kJ/mol CTAB while the amylopectin samples had an interaction enthalpy of −40 kJ/mol CTAB. Amylopectin and amylose seemed to have a similar interaction with CTAB, the small differences were probably due to the differences in structure between the polysaccharides.

Keywords: Amylose; Amylopectin; Isothermal titration calorimetry and surfactant

1. Introduction

The interaction between polar lipids and the starch polysaccharides amylose and amylopectin plays an important role in many food applications. One example is the addition of monoglycerides or other polar lipids to dough as a method to decrease the staling rate of bread (Krog & Jensen, 1970; Rogers, Zeleznak, Lai & Hoseney, 1988; Zobel, 1973). The staling of bread is due to the recrystallisation of amylopectin (Schoch & French, 1947) and there are indications that ligands, such as iodine and polar lipids, form a complex with amylopectin similar to the amylose and lipid inclusion complex (Banks, Greenwood & Khan, 1971; Bhide, Karve & Kale, 1981). Such a complex-formation may be the reason for the reduced recrystallisation of amylopectin. The complex between amylose and polar lipids has been characterised with a number of methods including differential scanning calorimetry (Kugimiya, Donovan & Wong, 1980) and wide angle X-ray scattering (Zobel, 1988). The structure of the amylose–lipid inclusion complex has been determined to be the polysaccharide wound three times around the hydrophobic part of the polar lipid with six to seven glucose units in each turn (French & Murphy, 1977; Zobel, French & Hinkle, 1967). However, an inclusion complex between amylopectin and lipids has not been unambiguously characterised.

The interaction between surfactants and polymers such as polyethylenoxide, PEO, or ethyl(hydroxyethyl)cellulose, EHEC, has previously been studied by isothermal titration calorimetry (Olofsson & Wang, 1994; Olofsson & Wang, 1998) and other methods (Carlsson, Karlström & Lindman, 1989; Olofsson & Wang, 1994; Stam, Almgren & Lindblad, 1991). The interaction can be characterized with three parameters, the critical association concentration, cac, (Jones, 1967; Lindman & Thalberg, 1993), the saturation concentration, C_2, (Jones, 1967) and the observed enthalpy, ΔH_{obs}.

The cac is the surfactant threshold concentration necessary for interaction between the surfactant and the polymer to occur. It is a measure of the strength of the polymer–surfactant interaction, and is more or less independent of the polymer concentration (Goddard, 1986). Factors affecting the cac are salt concentration, ionic groups on the polymer and the polymer hydrophobicity. Since the interaction between nonionic polymers and surfactants is dominated by hydrophobic interactions (Lindman & Thalberg, 1993) the cac is a measure of the polymer hydrophobicity. In the case of ionic polymers electrostatic interaction has great

* Corresponding author. Fax: +46-46-222-95-17.
E-mail address: henrik.lundqvist@livsteki.lth.se (H. Lundqvist).

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Nomenclature

α	fraction of the added surfactant that will be bind to the polysaccharide
β_{Syr}	fraction of the surfactant in the syringe that are in the form of monomers
cac	critical association concentration
cmc	critical micelle concentration
C_2	saturation concetration
C_{mon}	monomer CTAB concentration in the sample cell
C_i	total CTAB concentration in the sample cell after the *i*th addition of CTAB
C^o	total CTAB concentration in the syringe
CTAB(mon)	CTAB in the form of monomers
CTAB(mic)	CTAB in the form of micelles
ΔH_{mic}	enthalpy of CTAB micelle formation
ΔH^i_{dil}	enthalpy of dilution of CTAB at the *i*th addition of CTAB
ΔH^i_r	interaction enthalpy at the *i*th addition of CTAB
ΔH^i_{obs}	enthalpy observed on the *i*th addition of CTAB
ΔH_{int}	total enthalpy change based on polysaccharide dry weight for the binding of CTAB
ϵ	calibration constant
δn	Number of moles of CTAB in one addition
P	power
q^i_{demic}	heat of dissolution of micelles at the *i*th addition
q^i_{dil}	heat of dilution of amphiphile at the *i*th addition
q^i_{obs}	observed heat at the *i*th addition
t	time
τ	time constant
U	voltage

influence on the interaction and might also contribute to the overall interaction energy.

C_2 is the surfactant concentration when micelles start to form in the solution. At C_2, it is more favourable for the surfactant to form micelles than to bind to the polymer. At C_2, the polymer–surfactant interaction ends and further additions of surfactant will behave as an addition of surfactant to a solution without the polymer present. C_2 is a measure of the maximum surfactant binding capacity of the polymer and is linearly dependent on the polymer concentration. The maximum amount of surfactant bound to the polymer can be calculated as the difference between C_2 and the critical micelle concentration, cmc.

ΔH_{obs} is the heat produced when the surfactant is added to the polymer solution. The heat measured includes all processes occurring in the sample cell when the surfactant is added to the starch solution: interaction between starch and surfactant, dissociation of micelles and dilution of surfactants plus any other process that might occur. The interaction enthalpy, ΔH_r, was calculated by combining the results of the calorimetric measurements with the determination of the free surfactant concentration in the starch solution from surface tension measurements.

The characteristics for the binding of ligands to amylose have previously been determined by Yamamoto, Sano, Harada and Yasunaga (1983) and Yamamoto, Sano and Yasunaga (1982) who estimated the binding enthalpy from amperometric titration measurements. However, the characteristics for the binding of ligands to amylopectin have not previously been determined. Moreover, the dependence of the surfactant concentration on the binding enthalpy has not been determined previously either for amylose or amylopectin.

Since it has been clearly established that hexadecyltrimethylammonium bromide, CTAB, is able form inclusion complexes with amylose and also influences the retrogradation of amylopectin in the same way as polar lipids such as monoglycerides (Eliasson, 1988), CTAB was used as a model substance in the present study. Isothermal titration calorimetry was used to study the binding of CTAB to three starch polysaccharides, amylose from potato, amylopectin from potato and amylopectin from barley. The interaction between CTAB and the starch polysaccharides was studied by measuring the enthalpy changes when adding a CTAB solution to a gelatinised starch polymer solution. The interaction was monitored over a broad concentration range by increasing the surfactant concentration through consecutive additions.

2. Experimental

2.1. Materials

The polysaccharide solutions used were prepared from Amylose type III from potato, ‘amylose’, (Sigma, St.

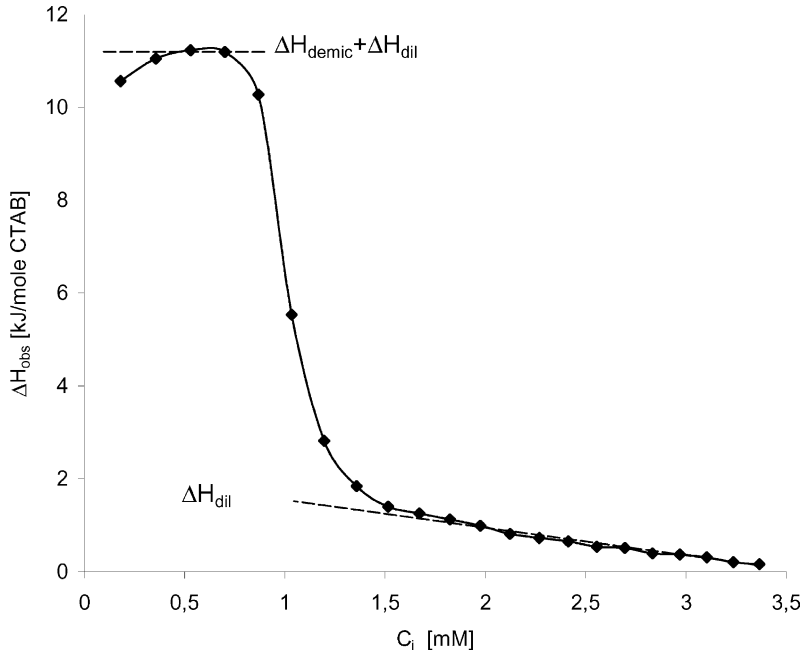

Fig. 1. Enthalpic titration curve from the addition of 22.0 mM CTAB solution to distilled water at 27°C. Abscissa, C_i (total CTAB concentration in units of mM); ordinate, ΔH_{obs} (observed enthalpy in units of kJ/mol CTAB).

Louis USA lot. no. 91H3841), amylopectin from potato, 'PAP', (Lyckeby Stärkelsen, Sweden ref. no. 3292) and amylopectin from barley, 'BAP', (ref. no. 999 from the University of Saskatchewan, Canada) (Bhatty and Rossnagel, 1997). Amylose was dissolved in distilled water by heating the mixture at 120°C for 20 min. Amylopectin samples were dissolved in distilled water by heating the mixture on a heating block at 100°C while mixing until a clear solution was obtained. The polymer samples were freshly prepared for each experiment in order to prevent change in the sample due to retrogradation. While amylopectin from barley was defatted by extraction with isopropanol:water according to Morrison and Coventry (1985), amylose and amylopectin from potato were used as received since they contain no naturally occurring lipids.

Hexadecyltrimethylammonium bromide, CTAB, from Sigma, (St. Louis USA H-5882 Lot. 68F-0283), was used as received.

2.2. Isothermal titration calorimerty

The isothermal titration calorimetry experiments were made using the 2277 Thermal Activity Monitor System (Thermometric AB, Järfälla, Sweden) with a 1 ml sample cell and a gold propeller stirrer at 100 rpm. The experimental set-up is described elsewhere by Suurkuusk and Wadsö (1982). All experiments were run at 27°C, which is above the Kraft temperature of CTAB. One experiment consists of a series of consecutive additions (5 or 7 μl) of CTAB solutions from a gas tight Hamilton syringe kept at 27°C. Two concentrations of CTAB in the syringe were used, 22.0 and 4.1 mM. The experiments were made in the fast titration mode (Bastos, Hägg, Lönnbro & Wadsö, 1991) with 10 min between each injection. All polysaccharides were examined at three concentrations, 0.1, 0.25 and 0.5% w/w, the dry mass was determined by drying the polymer solution at 130°C for at least 2 h. Experiments were made in duplicates and the results are presented as mean of duplicates.

2.3. Evaluation of isothermal titration calorimetry experiments

The experimental results were deconvoluted using the simple Tian Eq. (1) (Bastos et al., 1991).

$$P = \epsilon\left(U + \frac{\mathrm{d}U}{\mathrm{d}t}\right) \tag{1}$$

The instrument was calibrated by introducing electrical heat into the sample cell and the time constant, τ, and the calibration constant of the instrument, ϵ, were calculated according to Randzio and Suurkuusk (1980). The results, q_{obs}, obtained after deconvoluting and integrating each peak includes heats from all the reactions that occurred in the sample cell when the surfactant solution was added, this includes heats from polysaccharide–surfactant interaction, dilution and micelle dissociation. By relating the heat produced in each addition to the added amount of surfactant, the observed enthalpy, ΔH_{obs}, was obtained. By subtracting the contribution from the other known reactions, the enthalpy changes from polymer–surfactant interaction were calculated.

2.4. CTAB in water

The reactions that occurred when a surfactant solution

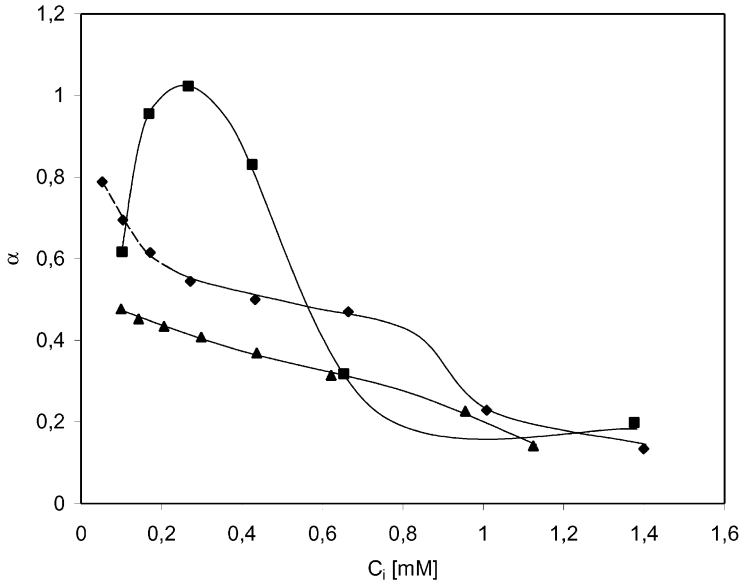

Fig. 2. Fraction of the added CTAB (α) that in each addition binds to the starch polysaccharide at 27°C as a function of total CTAB concentration (C_i), determined by surface tension measurements (Lundqvist et al., 2002): (■) amylose, (◆) amylopectin from potato and (▲) amylopectin from barley. Precipitation was detected in the concentration interval marked with dotted line.

was added to pure water were described in the following way. The micellar solution in the syringe was characterised by the factor β_{syr}, defined as the fraction of surfactant that was in the form of monomers. C^o is the total CTAB concentration in the syringe.

$$\beta_{syr} = \frac{\text{cmc}}{\text{C}^o} \tag{2}$$

When the surfactant concentration in the sample cell was below the cmc, the reaction that occurred when an aliquot (δn) of the surfactant solution in the syringe was added to the sample cell was described by the following reaction, see Nomenclature:

$$\delta n\,(1-\beta_{syr})\text{CTAB(mic)} + \delta n\,\beta_{syr}\,\text{CTAB(mon)} + \text{aq} \overset{q_{obs}}{\rightarrow} \delta n\,\text{CTAB(mon)} \tag{3}$$

The heat observed q_{obs} in each addition has contributions from the individual processes:

$$\begin{aligned} \text{q}^i_{obs} &= \text{q}^i_{dil} + \text{q}^i_{demic} \\ &= \delta n[\Delta H_{dil}(\text{C}^o \rightarrow \text{cmc}) - (1-\beta_{syr})\Delta H_{mic} \end{aligned} \tag{4}$$

The term for the dilution of the surfactant from the cmc to C_i was ignored since the cmc for CTAB is low (0.9 mM). The last term in relation (4) represents the enthalpy of dissociation of micelles.

From Eq. (4) the enthalpy of micelle formation can be calculated. The enthalpy of micelle dilution and the observed enthalpy were obtained from experiments where CTAB was added to distilled water (see Fig. 1) and β_{syr} was calculated according to Eq. (2). The enthalpy of micelle dissociation is the negative value of the enthalpy of micelle formation, which is the reverse reaction.

The process when the concentration of surfactant in the sample cell was above the cmc was described according to the following reaction:

$$\delta n\ \text{CTAB}(\text{C}^o) \overset{q_{obs}}{\rightarrow} \delta n\ \text{CTAB}(\text{C}_i) \tag{5}$$

The only process observed was the dilution process of the micelles.

2.5. CTAB in polymer solution

If the sample cell is filled with a polymer solution the process that occurs when a surfactant solution is added becomes more complex and thus also the model describing it. The process was divided into three surfactant concentration ranges. In the initial range the monomer concentration in the sample cell increased from zero to cac. The second range was the interaction range where the total CTAB concentration in the sample cell increased from cac to C_2 and the free monomer concentration increased from the cac to the cmc. In the final range, at concentrations higher than C_2, no interaction between surfactant and polymer occurred.

The reaction in the initial and second range was described according to Eq. (6), where α was defined as the fraction of the added surfactant in each addition that will bind to the polymer. Values of α were obtained from surface tension measurements where the concentration of free monomeric surfactant in the sample cell was determined (Lundqvist, Eliasson & Olofsson, 2002). The derived values of α for each consecutive injection in the calorimetric titration experiments are plotted against

Table 1
Saturation concentration, C_2, and average interaction enthalpy, $\overline{\Delta Hr}$, calculated according to Eq. (7) from experimental data obtained from isothermal titration calorimetry measurements and surface tension measurements (Lundqvist et al., 2002) at 27°C, for the binding of CTAB to different concentrations of starch polysaccharides

Polymer concentration (%(w/w))	Amylose		Amylopectin from potato		Amylopectin from barley	
	C_2 (mM)	ΔH_r (kJ/mol CTAB)	C_2 (mM)	ΔH_r (kJ/mol CTAB)	C_2 (mM)	ΔH_r (kJ/mol CTAB)
0.1	1.2	− 55	1.5	− 40	1.3	[a]
0.25	1.7	− 60	2.0	− 37	1.9	− 43
0.5	2.0	− 58	2.4	− 40	2.1	− 38

[a] The average interaction enthalpy could not be calculated due to a hazy, light scattering solution in the surface tension measurements.

the total CTAB concentration in Fig. 2.

$$\delta n\,(1-\beta_{syr})\mathrm{CTAB(mic)} + \delta n\,\beta_{syr}\,\mathrm{CTAB(mon)} + P(\mathrm{aq}) \overset{q_{obs}}{\rightarrow} \delta n\,(1-\alpha)\mathrm{CTAB}(C_i) + \delta n\,\alpha P - \mathrm{CTAB}_{aggr} \quad (6)$$

The measured heat can be described in the following way:

$$q^i_{obs} = q^i_{dil} + q^i_{demic} + q^i_r = \delta n[\alpha\,\Delta H_r + \Delta H_{dil}(C^o \rightarrow \mathrm{cmc}) - (1-\beta_{syr})\Delta H_{mic}] \quad (7)$$

The interaction enthalpy, ΔH_r, can be calculated from Eq. (7) since α was known from the measurements of free surfactant concentration (Lundqvist et al., 2002) (see Fig. 2), q_{obs} was obtained from the calorimetric experiments where CTAB was added to the polymer solution and q_{demic} and q_{dil} were obtained from the calorimetric experiments where CTAB was added to water at the same monomer concentration of CTAB in solution.

In the final stage where the surfactant concentration was above C_2 the monomer concentration was cmc. The only reaction that occurred was the dilution process equal to the dilution process in the case where no polymer was present in the sample cell, see Eq. (5).

The concentration C_2 was determined from a graph showing dq_{obs}/dc plotted vs. CTAB concentration. C_2 is defined as the concentration when an addition of surfactant to the polymer solution gives the same result as when added to the aqueous solution, i.e. when the derivative dq/dc was zero.

3. Results and discussion

The observed enthalpy, ΔH_{obs}, when CTAB was added to water is shown as a function of total CTAB concentration in Fig. 1. The cmc and the enthalpy of micelle formation ΔH_{mic} for CTAB were determined from Fig. 1 according to Eq. (4). The cmc was determined to 0.93 mM at 27°C as the inflection point of the slope, which can be compared to the literature values of 0.8–1.0 mM at 25°C (Bashford & Woolley, 1985; Sepúlveda & Cortés, 1985; Wang & Olofsson, 1995). The ΔH_{mic} was determined to −9.5 kJ/mol CTAB at 27°C, which agrees well with the literature values between −9.5 and −10.9 kJ/mol CTAB at 25°C (Bashford & Woolley, 1985; Paredes, Tribout & Sepúlveda, 1984; Wang & Olofsson, 1995).

C_2 and cac were determined for the starch polymers from a plot of the incremental enthalpy changes against surfactant concentration. A cac was not detected for any of the starch polymers in the concentration range examined as the interaction between CTAB and starch starts below the concentration range examined in the present measurements. Thus cac was below 0.03 mM for all three polysaccharides. The presence of phosphate groups in the PAP might shift cac to lower concentrations since the electrostatic interaction between the phosphate groups and the cationic headgroup of the surfactant will dominate the interaction. However, the hydrophobic interaction alone was very strong, since BAP and amylose, which are nonionic, also had a low cac. Addition of even small amounts of ligands to starch will influence the properties since they will bind to starch already at low concentrations.

The C_2 for the different polysaccharides and polysaccharide concentrations are listed in Table 1. The differences in C_2 between the polymers were small and within the experimental error of the method. The C_2 increased with the concentration of the polymer, which agrees well with Goddard (1986) who states that there is a linear relationship between polymer concentration and C_2. However, the C_2 was more or less independent of the nature of the starch polysaccharide. Even though amylopectin is a highly branched polymer, it was evidently able to bind the same amount of CTAB as amylose, which is a linear polymer.

A number of studies have determined the amount of ligands that were able to bind to amylose respectively amylopectin (Bulpin, Cutler & Lips, 1987; Hahn & Hood, 1987; Lagendijk & Pennings, 1970; Rutschmann & Solms, 1990; Solms, Osman-Ismail & Beyeler, 1973; Svensson, Gudmunsson & Eliasson, 1996) but the amount of ligands reported to bind to starch varies. Amylose was reported to be able to bind several times more ligand than amylopectin (Svensson et al., 1996). The choice of ligand can to some degree explain the difference between the results. Rutschmann et al. (1990) showed how the type of ligand influenced the number of ligands bound to starch. The size and the charge of the ligand influence the binding, a cationic

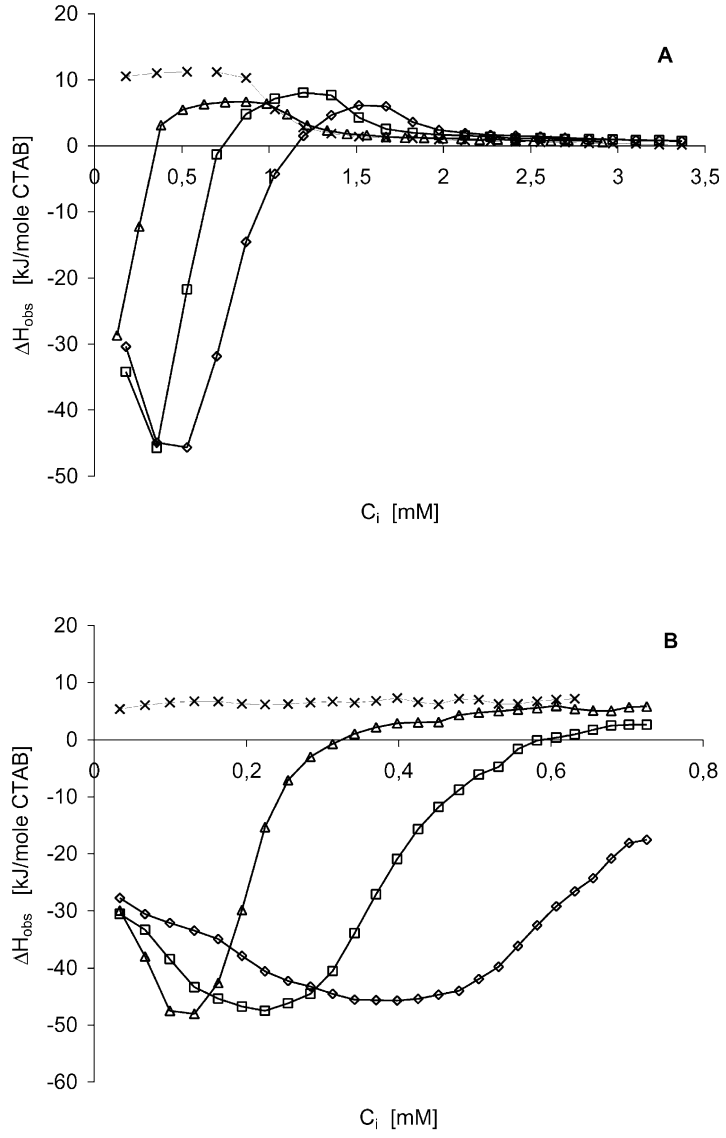

Fig. 3. Enthalpic titration curves from the addition of (A) 22.0 mM CTAB and (B) 4.1 mM CTAB to solutions of amylose (◇) 0.5% w/w, (□) 0.25% w/w, (△) 0.1% w/w and (×) water at 27°C. Abscissa, C_i (where C_i is the total CTAB concentration in units of mM); ordinate, ΔH_{obs} (observed enthalpy in units of kJ/mol CTAB).

surfactant will have attractive electrostatic interaction while an anionic surfactant will give a repulsive interaction. Differences in the amylopectin polysaccharide, such as average chain length and fragmentation, might also explain the differences in the results. It is not until recent years that granular pure amylopectin has become available through breeding and genetic engineering (Jacobsen, Krijgheld, Hovenkamp-Hermelink, Ponstein, Witholt & Feenstra, 1990). Earlier amylopectin has been purified from starch, which might have influenced the polysaccharide (Young, 1984). For nonionic polysaccharides the hydrophobic interaction will dominate and the ligand charge is unimportant.

The enthalpy measured when CTAB was added to the polysaccharide solutions, ΔH_{obs}, plotted as a function of total concentration of CTAB can be seen in Fig. 3 for amylose, Fig. 4 for PAP and Fig. 5 for BAP. Each dot represents one discreet addition of CTAB solution. In the case of amylose the initial ΔH_{obs} was exothermic and independent of the polysaccharide concentrations. When the CTAB concentration increased the enthalpy decreased to its maximum exothermic value, maximum ΔH_{obs}, which was reached at $C_i = 0.1$, 0.22 and 0.4 mM for the 0.1, 0.25 and 0.5% w/w amylose solution respectively. The level of the maximum ΔH_{obs} was about -48 kJ/mol CTAB, independent of amylose concentrations. However, the maximum ΔH_{obs} was reached at surfactant concentrations that were linearly dependent on the polysaccharide concentration. At even higher CTAB concentrations the enthalpy increased and became endothermic at $C_i = 0.3$, 0.7 and 1.2 mM, for the 0.1, 0.25 and 0.5% w/w amylose solution respectively, after which it decreased to zero. The observed enthalpy obtained when adding CTAB to PAP was

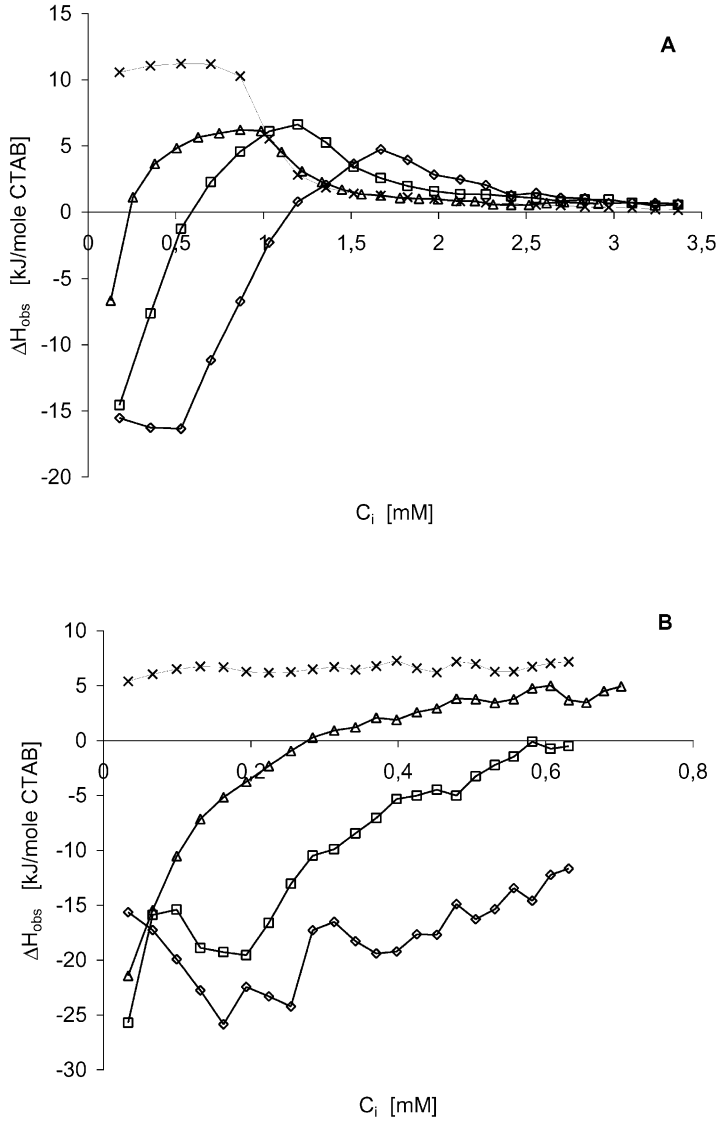

Fig. 4. Enthalpic titration curves from the addition of (A) 22.0 mM CTAB and (B) 4.1 mM CTAB to solutions of amylopectin from potato (◇) 0.5% w/w, (□) 0.25% w/w, (△) 0.1% w/w and (×) water at 27°C. Abscissa, C_i (where C_i is the total CTAB concentration in units of mM); ordinate, ΔH_{obs} (observed enthalpy in units of kJ/mol CTAB).

similar to adding CTAB to amylose (Fig. 4). As in the case of amylose, the initial value of ΔH_{obs} was independent of the polysaccharide concentrations. The largest enthalpy for the 0.1% PAP solution was obtained at so low CTAB concentration that it could only be seen in the detailed study at low CTAB concentrations (Fig. 4B). At higher CTAB concentrations the ΔH_{obs} increases to a maximum endothermic ΔH_{obs}. The difference between the initial and maximum enthalpy was not as big for amylopectin as for amylose. BAP showed similar behaviour as PAP (Fig. 5), the main difference is the initial ΔH_{obs}, which was lower for amylopectin from barley. The initial ΔH_{obs} for BAP was also the maximal exothermic value, the higher CTAB concentration the lower the ΔH_{obs}.

The shift from exothermic to endothermic observed enthalpy values at concentrations close to C_2 was due to micelle dissociation as only a small part of the added CTAB was bound to the polysaccharide. This was clearly seen in the plot of ΔH_{obs} vs. C_{mon}, in Fig. 6. The interaction between amylose and CTAB was independent of the polysaccharide concentration since the results for the different polysaccharide concentrations overlap. However, ΔH_{obs} for the two amylopectin samples did not overlap, which means that the polysaccharide concentration influenced the interaction.

The features of the calorimetric titration curves in Figs. 4–6 differ considerably from curves obtained when adding ionic surfactants to aqueous solutions of linear, non-ionic polymers. Studies of systems such as PEO–SDS and EHEC–SDS (Olofsson & Wang, 1994; Olofsson & Wang, 1998) show the initial interactions to be moderately endothermic, the maximal ΔH_{obs} was less than 20 kJ/mol SDS, compared to −45 kJ/mol CTAB for amylose.

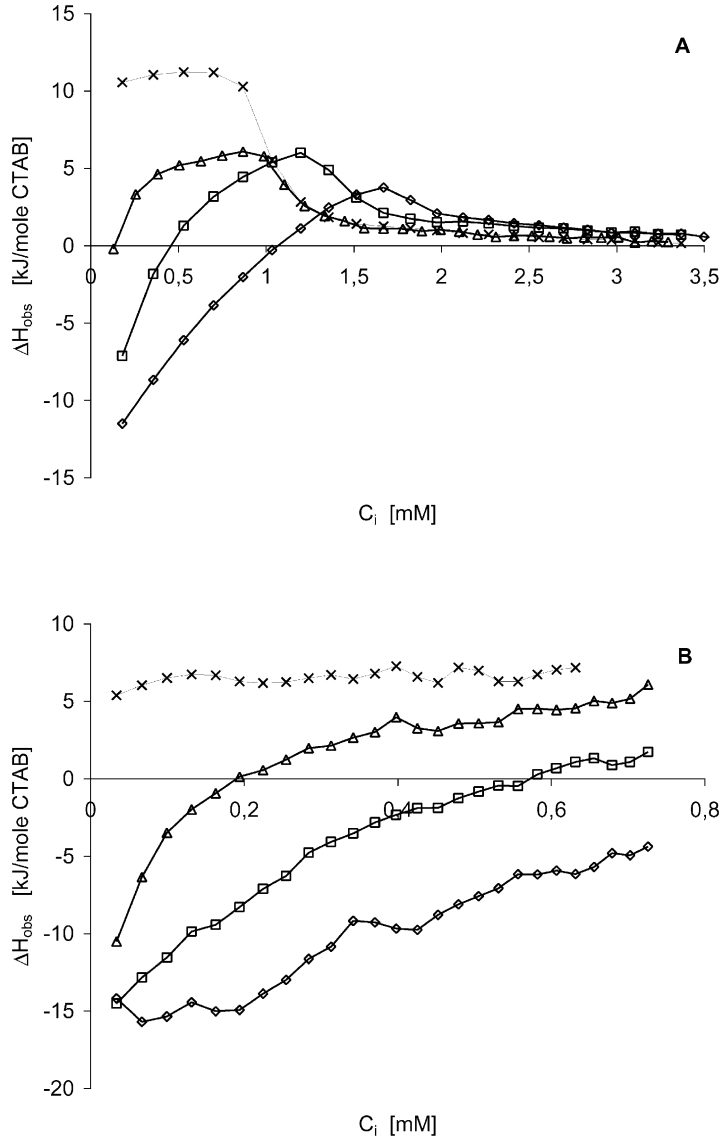

Fig. 5. Enthalpic titration curves from the addition of (A) 22.0 mM CTAB and (B) 4.1 mM CTAB to solutions of amylopectin from barley (◇) 0.5% w/w, (□) 0.25% w/w, (△) 0.1% w/w and (×) water at 27°C. Abscissa, C_i (where C_i is the total CTAB concentration in units of mM); ordinate, ΔH_{obs} (observed enthalpy in units of kJ/mol CTAB).

The interaction enthalpies, ΔH_r, for binding CTAB to starch polysaccharides were calculated according to Eq. (7) and can be seen in Fig. 7 as a function of total CTAB concentration. The average interaction enthalpy, $\overline{\Delta H}r$, which is the average value of the interaction enthalpy in the constant region, see Fig. 7, for the different samples can be found in Table 1. The interaction enthalpies were exothermic and differed between the starch polysaccharides, but the ΔH_r was independent of the polymer concentration. The interaction enthalpy for amylose–CTAB determined in this study, −60 kJ/mol CTAB was surprisingly close to the enthalpy estimated by Yamamoto et al. (1983) for the amylose–SDS interaction, −40 kJ/mol SDS despite SDS being an anionic surfactant and CTAB a cationic surfactant. The ΔH_r for binding CTAB to amylose was constant at low CTAB concentrations, however, it became less exothermic when the CTAB concentration was close to the saturation concentration. Most of the interaction took place in the region where the interaction enthalpy was constant but a substantial amount of the surfactant was bound to the polymer after the enthalpy had started to increase. The ΔH_r for the binding of CTAB to the amylopectin samples was also exothermic but lower than for amylose. The two amylopectin samples yielded the same ΔH_r. There were no differences in the interaction enthalpy between the different concentrations of polysaccharide. The ΔH_r for BAP was constant in the whole concentration range examined (Fig. 7C). The ΔH_r for PAP on the other hand has a deviation from the constant value at low CTAB concentrations (Fig. 7B). The deviation was probably due to precipitation of PAP that occurred as a consequence of the electrostatic interaction between the phosphate groups of amylopectin from

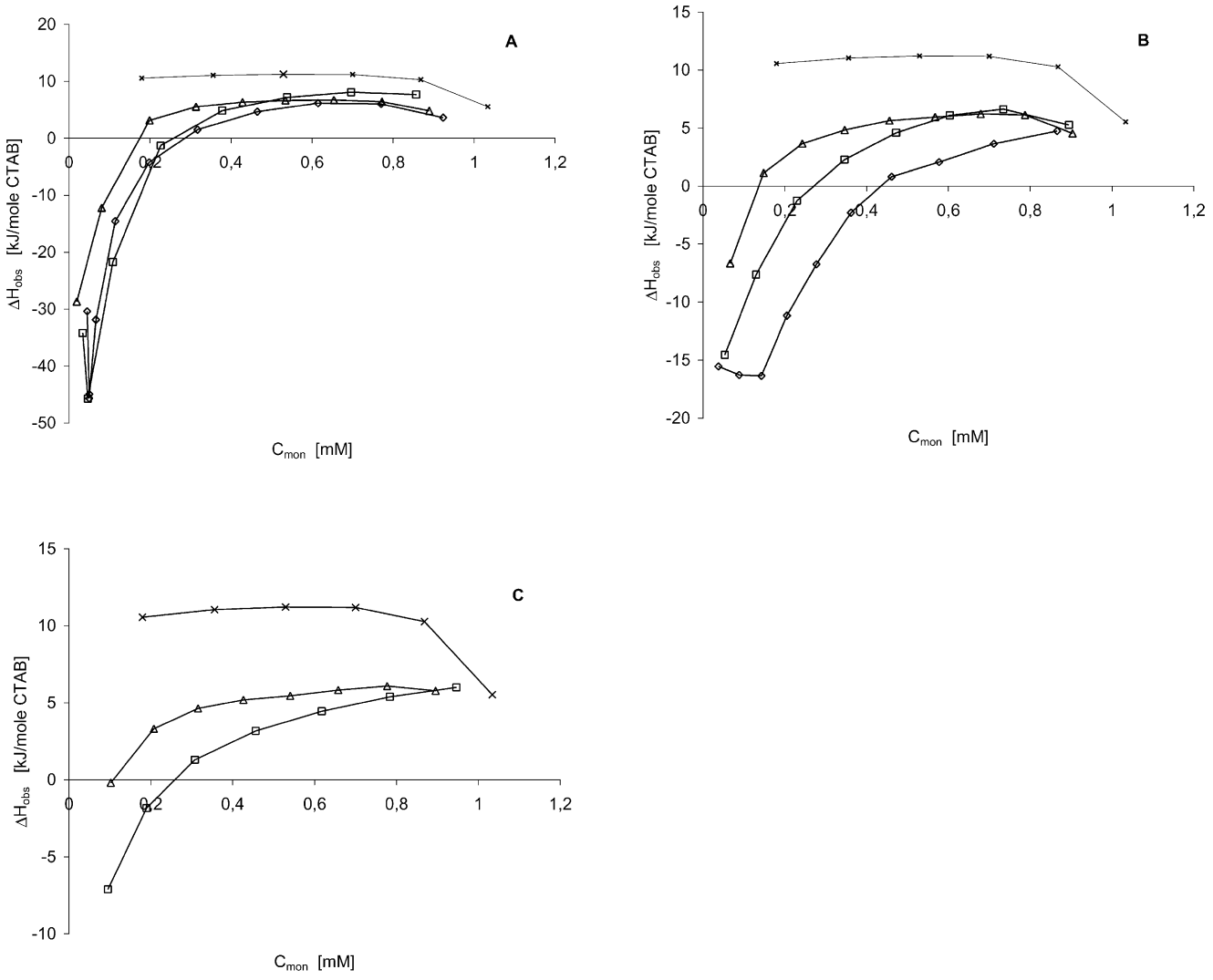

Fig. 6. Observed enthalpy as a function of free CTAB from the addition of 22.0 mM CTAB to solutions of (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley at 27°C, (◇) 0.5% w/w, (□) 0.25% w/w, (△) 0.1% w/w and (×) water. Abscissa, C_{mon} (where C_{mon} is the monomer CTAB concentration in units of mM); ordinate, ΔH_{obs} (observed enthalpy in units of kJ/mol CTAB).

potato and the cationic head group of CTAB (Lundqvist et al., 2002). Amylopectin from potato is known to contain some phosphate groups (Muhrbeck & Tellier, 1991) (about 1 phosphate group per 100–200 glucose units). The precipitation occurred, in the case of PAP, in the region where the ΔH_r deviated from the constant value. However, this precipitate dissolved at higher CTAB concentrations.

It is known that amylose together with CTAB forms an inclusion complex (French & Murphy, 1977; Zobel et al., 1967). A similar inclusion complex might also be formed with amylopectin, especially in the case of amylopectin from potato where the average exterior chain length of amylopectin is 15 glucose units, GU, (Nilsson, Bergquist, Nilsson & Gorton, 1996) and the required length of one inclusion complex is 18 GU (Takeo, Tokumura & Kuge, 1973). Amylopectin from barley has a lower average chain length, which might make it more difficult to form an inclusion complex. However, it seems that the exterior chain length of the amylopectin polysaccharides did not influence the ΔH_r. The first complexes formed would probably be those with the longest amylopectin branches, but if the chain length influenced the binding heat this would have resulted in decreasing ΔH_r. The difference in chain length might instead influence the type of binding, the amylose–CTAB interaction is a cooperative binding while amylopectin–CTAB is a Langmuuir type of binding (Lundqvist et al., 2002).

The enthalpy change of moving CTAB monomers surrounded by water molecules to a micelle where the hydrophobic part of the CTAB is shielded from water is ΔH_{mic} which equals −10 kJ/mol at 27°C. To move CTAB monomers in solution surrounded by water into an amylose helix should not involve a larger enthalpy than the ΔH_{mic} but the observed ΔH_r were five times larger than the ΔH_{mic}. In

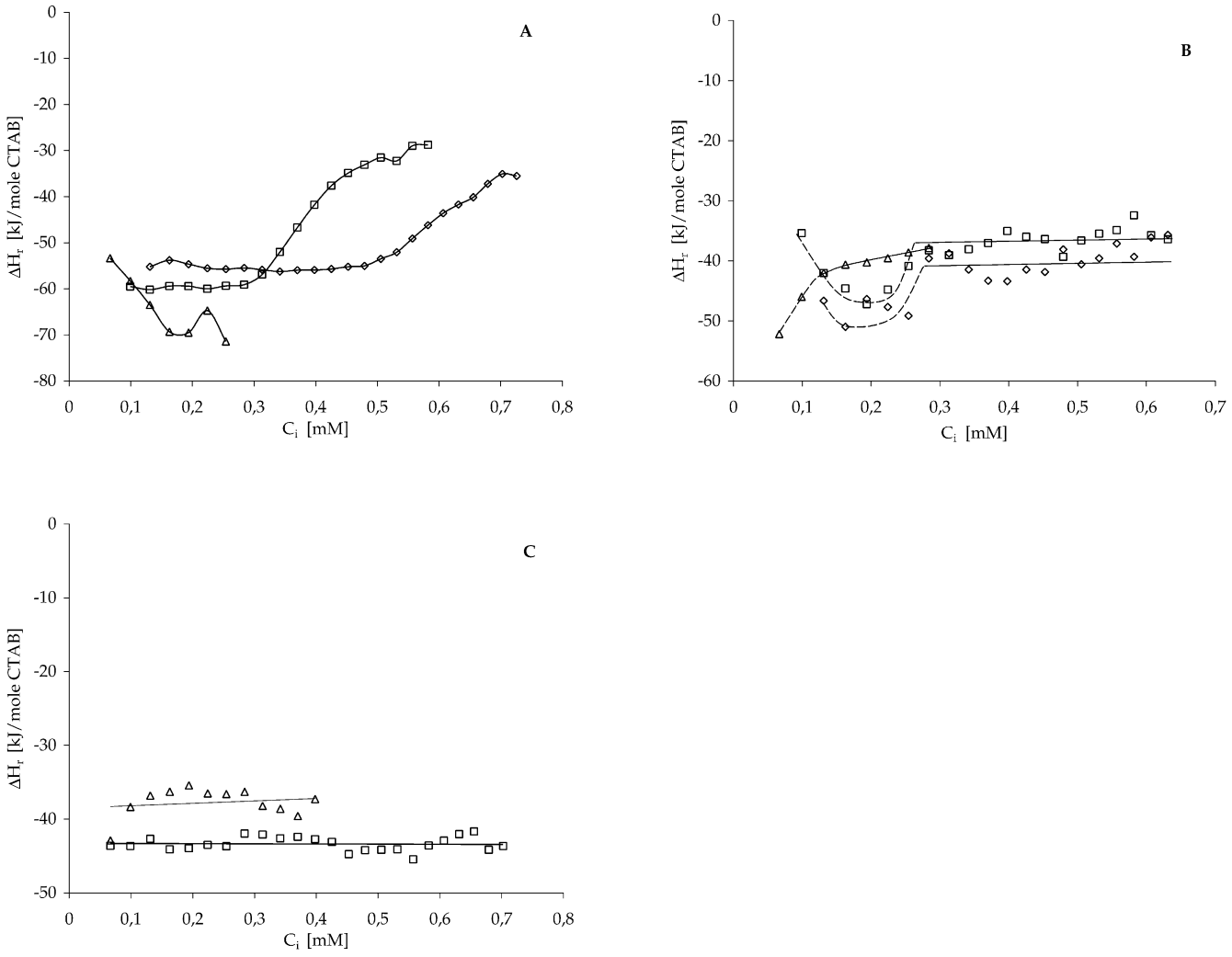

Fig. 7. Interaction enthalpy for the interaction between CTAB and solutions of (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley at 27°C, (◇) 0.5% w/w, (□) 0.25% w/w and (△) 0.1% w/w. Calculated from Eq. (7). Precipitation was detected in the concentration interval marked with dotted lines. Abscissa, C_i (where C_i is the total CTAB concentration in units of mM); ordinate, ΔH_r (interaction enthalpy in units of kJ/mol CTAB).

addition to the dehydration of the alkyl chain configurational changes of the polymer, from random coil to helix structure, when the polymer was wound around the CTAB molecule may contribute to ΔH_r. Formation of hydrogen bonds between hydroxyl groups of adjacent chains can also be a contribution to ΔH_r.

Whether the surfactant binds to the polysaccharide or not depends on what is energetically more favourable, binding to the polysaccharide or stay as a free monomer in solution. When it is more favourable for CTAB to bind to the polysaccharide a higher fraction, α of the added surfactant will bind. The extent of binding in each addition was derived from measurements of the concentration of free CTAB after each addition (Lundqvist et al., 2002) and can be seen in Fig. 2 as a function of total CTAB concentration. In the case of amylose α started at 0.7 and increased to 1 after which it decreased to zero. The two amylopectin samples showed no increase in α, it decreased with CTAB concentration. The reason α differed between the polysaccharides was due to the course of binding, amylose binds CTAB cooperatively while amylopectin has a Langmuir type of binding (Lundqvist et al., 2002). The cooperative effect may arise from a configuration change of amylose from random-coil to helix. When the polysaccharide coils itself around the alkyl chain the outside of the coil has a hydrophilic character while the inside have hydrophobic character (Immel & Lichtenthaler, 2000). The hydrophobic interaction between the alkylchain and the hydrophobic inner surface of the polysaccharide coil might explain the cooperativity, as in the case of double helix formation of DNA where the cooperative process is due to hydrophobic interaction between the base pairs (Evans & Wennerström, 1999). Initiation of the helix in a random coil is the slowest and energetically the most unfavoured step, whereas subsequent growth of the helix nucleus is rapid and relatively favoured (Creighton, 1984). When the configuration changes in the amylose had initiated, by formation of one inclusion complex, the resistance was less for the formation of the second complex, and the ΔH_{obs} decreased

because more of the added CTAB being involved in the interaction. The amylopectin samples could not bind more than one CTAB molecule per branch since the average exterior chain length of amylopectin is 15 glucose units (Richardson, Nilsson, Bergquist, Gorton & Mischnick, 2000) and a cooperative process is thus not possible. However, the total amount of CTAB bound was independent of the type of binding.

The integrated enthalpy, ΔH_{int}, that is the enthalpy calculated by relating the total enthalpy from the polysaccharide–CTAB interaction to the amount of starch, was highest for amylose with −10.9 J/g, PAP had −6.8 J/g and BAP −5.1 J/g. The ΔH_{int} can be compared with DSC measurements of the melting of the amylose–lipid complex that has been determined to 16.8 J/g amylose (Eliasson, 1988). The enthalpies were obtained at different water contents and temperature, which influence the value. Moreover, it is not certain that it is the same reaction that is examined. In the DSC the process studied was melting and dissociation of the amylose–lipid complex. In the experiments in this study the reactions taking place was formation of the complex. Still, the values were of the same order of magnitude.

4. Conclusions

This is the first study where the heat involved in binding CTAB to amylopectin has been determined. The interaction enthalpy of binding CTAB to the different polysaccharide showed similarities. It was exothermic, of the same magnitude and constant for the main part of the binding. The type of binding of CTAB to the amylopectin samples was probably of the same type as the amylose–CTAB inclusion complex. The starch polysaccharides amylose, amylopectin from potato and amylopectin from barley were able to bind the same amount of CTAB despite differences in molecular structure.

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