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Carbohydrate Polymers 49 (2002) 43–55

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Binding of hexadecyltrimethylammonium bromide to starch polysaccharides. Part I. Surface tension measurements

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Received 19 March 2001; accepted 4 July 2001

Abstract

Surface tension measurements were used to characterise the binding of the cationic surfactant hexadecyltrimethylammonium bromide, CTAB, to three starch polysaccharides, amylose from potato, amylopectin from potato and amylopectin from barley. Surface tension measurements were used according to the axisymmetric drop shape analysis, ADSA, to determine the concentration of free CTAB monomers during consecutive addition of CTAB to the polysaccharide solutions. The interaction between the starch polysaccharides and CTAB was studied at three starch concentrations, 0.1, 0.25 and 0.5% (w/w). Binding isotherms were derived from the concentration of free CTAB and the type of binding was determined with Scatchard plots. The binding capacities of CTAB correlated linearly with the polysaccharide concentration but were independent of the type of starch polysaccharide. All three starch polysaccharides were able to bind 33 mmol CTAB per mole glucose units. The binding of CTAB to amylose was cooperative while the binding of CTAB to amylopectin was of Langmuir type.

Keywords: Amylose; Amylopectin; Surfactant and surface tension

1. Introduction

The effect of adding lipids or surfactants to starch has been extensively studied in recent years. Lipids and surfactants have been shown to change the properties of ageing both in model systems (Eliasson & Ljunger, 1988) as well as in food applications (Rogers, Zeleznak, Lai & Hoseny, 1988; Zobel, 1988). Addition of lipids or surfactants to starch decreases the rate of retrogradation of both amylose and amylopectin (Silverio, Svensson, Eliasson & Olofsson, 1996). This decrease is connected to the rate of ageing in starch based food products. While the retrogradation of amylose is rapid, taking only a couple of hours (Miles, Morris & Ring, 1985; Silverio et al., 1996), the retrogradation of amylopectin is a long-term process that is not complete until after several weeks (Schoch & French, 1947).

The interaction between amylose and ligands, such as iodine, lipids or surfactants, involves inclusion complex formation. The complex formation between amylose and iodine has been studied with spectroscopy (Cronan & Schneider, 1969) and amperometric titration (Yamamoto, Sano & Yasunaga, 1982). The interaction of amylose with sodium dodecyl sulphate, SDS, (Yamamoto, Sano, Harada & Yasunaga, 1983) and sodium myristate (Bulpin, Cutler & Lips, 1987) were investigated with potentiometric titration and surface tension measurements, respectively. The structure of the inclusion complex between amylose and polar lipids or iodine has been determined and characterised by a number of methods including differential scanning calorimetry (Kugimiya, Donovan & Wong, 1980) and wide angle X-ray scattering (Zobel, 1988). In the complex the polysaccharide winds 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.

Surface tension measurements have earlier been used to study the interaction between surfactants and polymers. Jones (1967) studied the interaction between SDS and polyethylene oxide, PEO, and Svensson, Gudmunsson and Eliasson (1996) the interaction between SDS and amylose and amylopectin from potato. From the surface tension measurements the saturation concentrations, C_2, were determined and the total amount of surfactant bound to the polymer was calculated.

It has been possible to determine the characteristics of

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0144-8617/02/$ - see front matter
PII: S0144-8617(01)00299-5

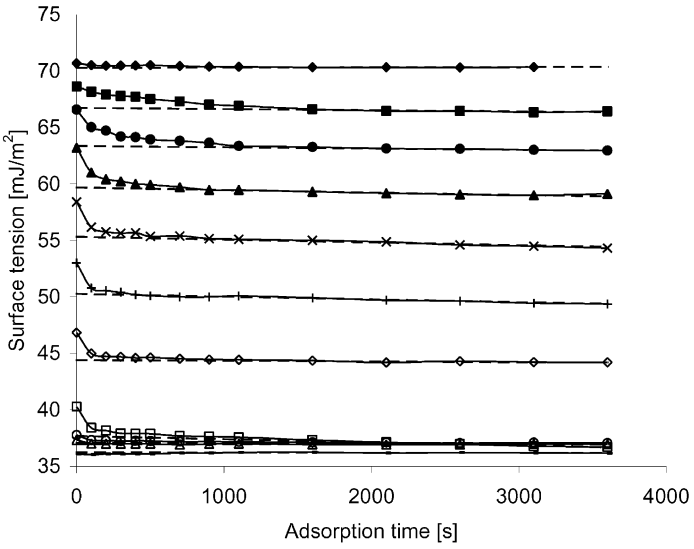


Fig. 1. Dynamic surface tension traces of CTAB solutions at 27°C: (◆) pure water, (■) 96.4 μM, (●) 0.143 mM, (▲) 0.207 mM, (×) 0.295 mM, (+) 0.422 mM, (◇) 0.611 mM, (□) 0.891 mM, (○) 1.288 mM, (△) 2.754 mM and (−) 7.244 mM.

ligands binding to starch polysaccharides. Yamamoto et al. (1983) studied the binding of iodine and SDS to amylose of different lengths (DP 32-1100) and analysed the experimental data with binding models based on protein binding to DNA (McGhee & Hippel, 1974). Long amylose chains ($DP > 76$) have a co-operative binding while shorter amylose chains have a Langmuir type of binding. However, no study has previously been carried out on the formation of amylopectin–surfactant complexes, as pure amylopectin samples have not been available until recent years, when breeding and genetic engineering have produced granular pure amylopectin (Jacobsen, Krijgheld, Hovenkamp-Hermelink, Ponstein, Witholt & Feenstra, 1990).

It has been clearly demonstrated that ligands such as hexadecyltrimethylammonium bromide, CTAB, influences the ageing properties of starch in a fashion similar as polar lipids (Eliasson, 1988). In the present study CTAB was used as a model substance for a polar lipid. The interaction with CTAB was compared for three starch samples: amylose from potato, amylose, amylopectin from potato, PAP, and amylopectin from barley, BAP. The interaction between CTAB and the starch polysaccharides was studied using consecutive additions of CTAB solution to a polysaccharide solution and measuring the surface tension at each concentration. The interactions were analysed with binding models in order to study the mechanism of interaction.

2. Materials and methods

2.1. Starch polysaccharides

The starch polysaccharides used in this study were amylopectin from potato, PAP, Lyckeby Stärkelsen Kristianstad, Sweden, ref. no. 3292, amylopectin from barley, BAP, from the University of Saskatchewan, Canada (Bhatty & Rossnagel, 1997), Canada, ref. no. 999 and amylose from potato type III, amylose, Sigma, St. Louis, USA, lot. no. 91H3841. Polar lipids present in amylopectin from barley were removed by extraction with *n*-propanol/water according to Morrison and Coventry (1985). Amylopectin and amylose from potato are naturally free from lipids and were used as received.

The starch polysaccharide solutions were prepared by heating the starch/water mixture at 140°C for 20 min in an oil bath. Three polysaccharide concentrations were studied, 0.1, 0.25 and 0.5% (w/w). The solutions were cooled to room temperature and weighed into the sample cell. The dry mass of the polysaccharide solution was determined

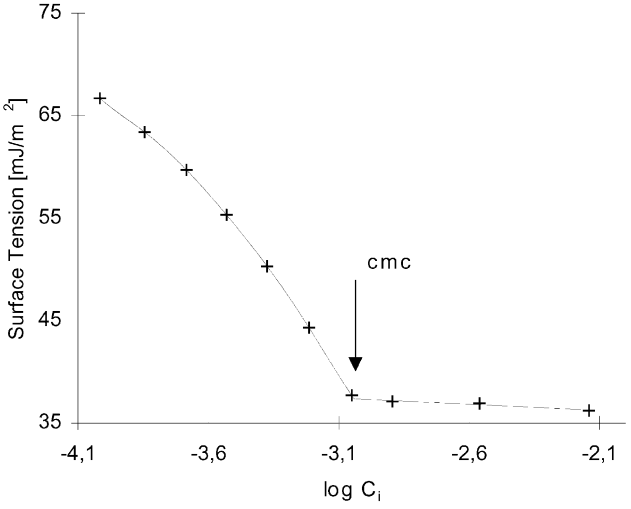


Fig. 2. Equilibrium surface tension of CTAB solutions at 27°C. Created from Fig. 1. Abscissa, log C_i (where C_i is the total CTAB concentration in units of mol dm^{-3}); ordinate, surface tension in units of J/m^2.

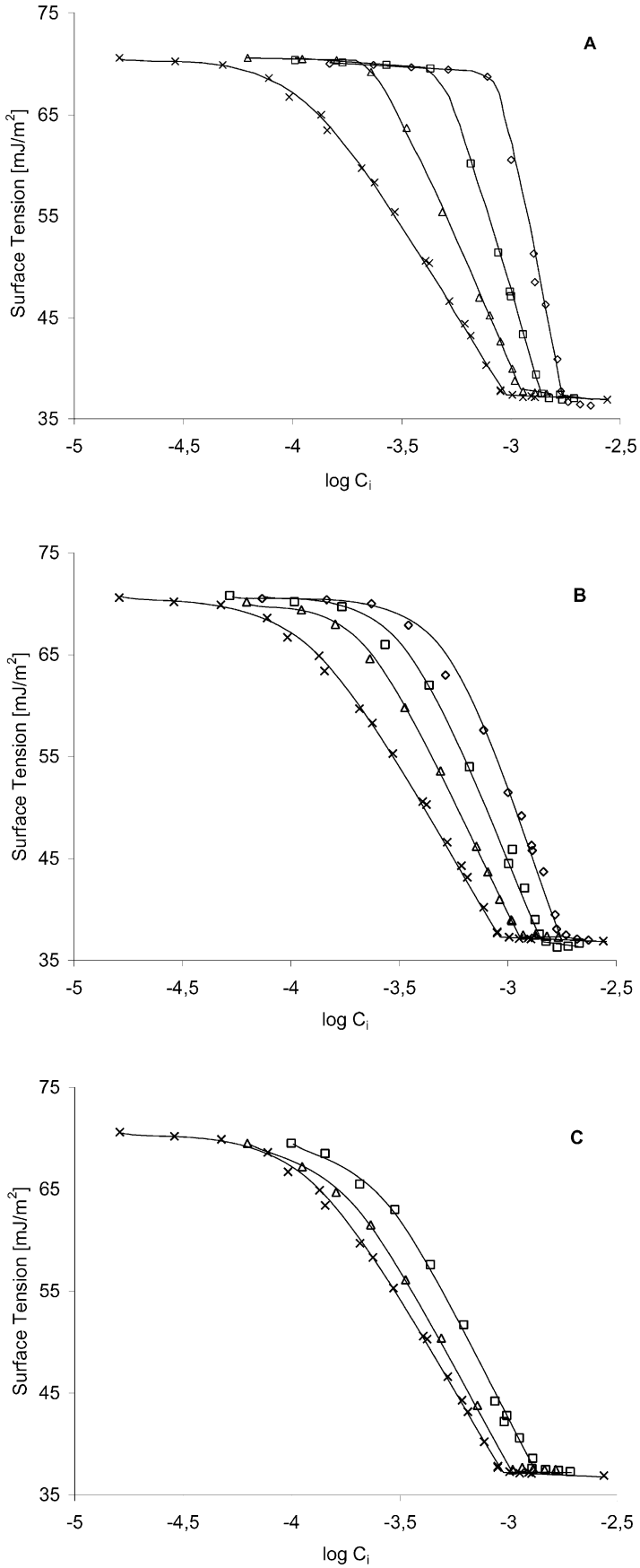


Fig. 3. Equilibrium surface tension of CTAB–starch polysaccharide solutions at 27°C: (◇) 0.5% (w/w), (□) 0.25% (w/w), (△) 0.1% (w/w) and (×) water. (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley. Abscissa, log C_i (where C_i is the total CTAB concentration in units of mol dm^{-3}); ordinate, surface tension in units of J/m^2.

Table 1
Saturation concentration and binding capacity for the binding of CTAB to starch polysaccharides at 27°C

	Polymer concentration (%)	C_2 (mmol dm^{-3})	Binding capacity (mmol CTAB/ mol GU)
Amylose	0.50	1.82 ± 0.01	34
	0.25	1.43 ± 0.03	36
	0.10	1.13 ± 0.05	36
PAP	0.50	1.80 ± 0.01	35
	0.25	1.43 ± 0.03	36
	0.10	1.12 ± 0.05	31
BAP	0.50	1.79 ± 0.01	29
	0.25	1.38 ± 0.03	34
	0.10	1.07 ± 0.05	27

by drying a part of the solution at 130°C for two hours in a heating cabinet. All glassware used in the experiments were washed in a 1:1 mixture of sulphuric acid (95–97% pro analysis, Merck, Darmstadt, Germany) and nitric acid (fuming 100%, pro analysis, Merck, Darmstadt, Germany) and rinsed in mQ-water before use.

2.2. Surfactant

The cationic surfactant used, hexadecyltrimethylammonium bromide, CTAB, from Sigma, St. Louis USA H-5882 Lot. 68F-0283, was purified by recrystallisation eight times in mQ-water (Arnebrant, Bäckström, Jönsson & Nylander, 1989).

2.3. Surface tension measurements

Since the surface tension is a measure of the amount of monomer surfactant in solution, the more the monomer CTAB in the solution, the more the surface tension will decrease before micelles of CTAB start to form. Thus, the concentration of monomer CTAB in the starch polysaccharide–CTAB solutions was assumed to be the same as in water with the same surface tension as the polysaccharide–CTAB solution. The amount of CTAB bound to the polysaccharide was then calculated as the difference between the total amount of CTAB added and the amount of free CTAB.

The surfactant was added as a micellar solution to the polysaccharide solution in the sample cell in consecutive additions. The total surfactant concentration in the sample cell was calculated, taking into account the increase in the volume of the sample. A syringe with a u-shaped needle was lowered into the sample cell and an air bubble was produced from the syringe. The dynamic surface tension was measured by filming the rising bubble and analysing the contour of the bubble according to axisymmetric drop shape analysis, ADSA, (Rotenberg, Boruvka & Neumann, 1983) with the Tracker instrument, IT Concept, Longessan, France. The experiments were preformed at 27°C, which was above the Kraft temperature of CTAB, with temperature control of the sample solution and the syringe. The sample cell and syringe were cleaned with a 10% decon 90 solution, Decon laboratories ltd., Hove UK.

The surface tension was determined for the pure polysaccharide solution and after each addition of surfactant. The dynamic surface tension was monitored for one hour in each measurement, the surface tension was determined as the value when the dynamic surface tension reached a stable level as can be seen in Fig. 1. Each measurement was performed in duplicates.

The surfactant concentration where micelles start to form, i.e. the critical micelle concentration, cmc, or in the case of polymer solutions, the saturation concentration, C_2, was determined by plotting the surface tension against the total concentration of CTAB. Both occur at the concentration where there is a breakpoint in the surface tension at 37 mJ/m^2 (Fig. 2). The cmc was determined to be 0.93 mM at 27°C, see Fig. 2. This value agrees well with the literature value of 0.8–1.0 mM at 25°C (Paredes, Tribout & Sepúlveda, 1984; Sepúlveda & Cortés, 1985; Wang & Olofsson, 1995). This method is possible because the breakpoint indicates the formation of micelles. At C_2 in a polymer–surfactant solution, micelles start to form because the free energy of the surfactant in micelles is less than in the surfactant polymer aggregates or as free monomer in solution. In some polymer–surfactant systems, for example PEO-SDS (Jones, 1967), two breakpoints can be seen, one at high surface tensions, which represents the critical association concentration (cac), and the other the breakpoint at low surface tension due to micelle formation.

2.4. Data analysis

Scatchard plots and binding isotherms were used to evaluate the interaction between starch polysaccharides and CTAB in order to give a better understanding of the mechanism of binding. The Scatchard plot is based on Langmuir type of binding, which involves binding of ligands to n equivalent binding sites on the polysaccharide that is not influenced by the fraction bound ligand.

The reaction occurring is:

$$\mathrm{BS} + \mathrm{CTAB(mon)} \rightarrow \mathrm{CTAB(bound)} \quad (1)$$

where BS is the binding site on the polysaccharide and CTAB(mon) and CTAB(bound) denote monomer CTAB and bound CTAB, respectively. The Scatchard equation, assuming a Langmuir type of reaction, is given by Eq. (2) where ν is the number of CTAB molecules bound to the starch polysaccharide (mmole CTAB/mole glucose units), n is the number of binding sites, K is the binding constant and C_{mon} the monomer CTAB concentration.

$$\nu = \frac{nKC_{\mathrm{mon}}}{1 + KC_{\mathrm{mon}}} \quad (2)$$

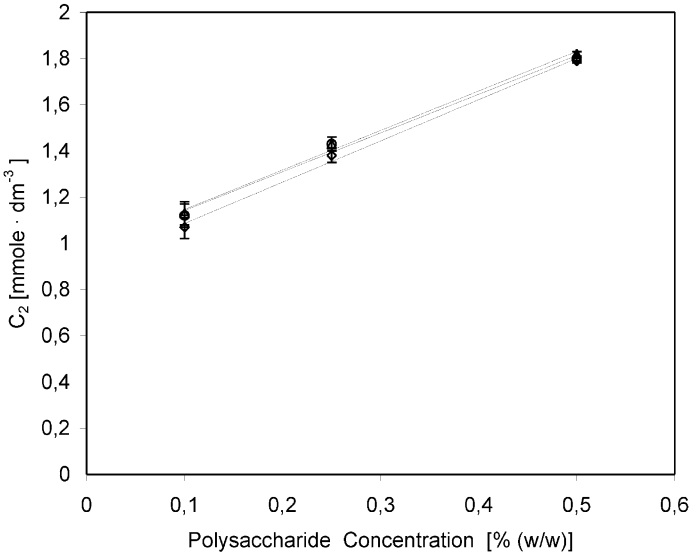


Fig. 4. Saturation concentration, C_2, for the binding of CTAB to starch polysaccharides, determined by surface tension measurements at 27°C, as a function of polysaccharide concentration: (○) amylose, (△) amylopectin from potato and (◇) amylopectin from barley.

or can be rewritten as

$$\frac{\nu}{C_{\text{mon}}} = K(n - \nu) \tag{3}$$

A plot of ν/C_{mon} vs. ν will give a straight line when a Langmuir type of binding occurs, where −K is the slope and n is the intercept of the abscissa. If the conditions postulated by Langmuir, that each binding site has the same binding heat and the binding of one ligand does not influence the binding of the second ligand, are not fulfilled the binding is not of Langmuir type. This will give a curve instead of a straight line when ν/C_{mon} is plotted vs. ν as K will be a function of ν.

3. Results and discussion

Binding isotherms were derived from the surface tension data in the form of plots of ν as a function of the free CTAB concentration. The shape of the binding isotherm gives information about the binding of CTAB to the starch polysaccharides.

Fig. 3 shows the surface tension as a function of total concentration of CTAB (C_i) at three polysaccharide concentrations, 0.1, 0.25, 0.5% (w/w), of amylose, PAP and BAP solutions. The surface tension for amylose, PAP and BAP without any added CTAB was 71.3 mJ/m^2 at 27°C. It was thus the same as for pure water since the starch polysaccharides used were not surface active. As the total CTAB

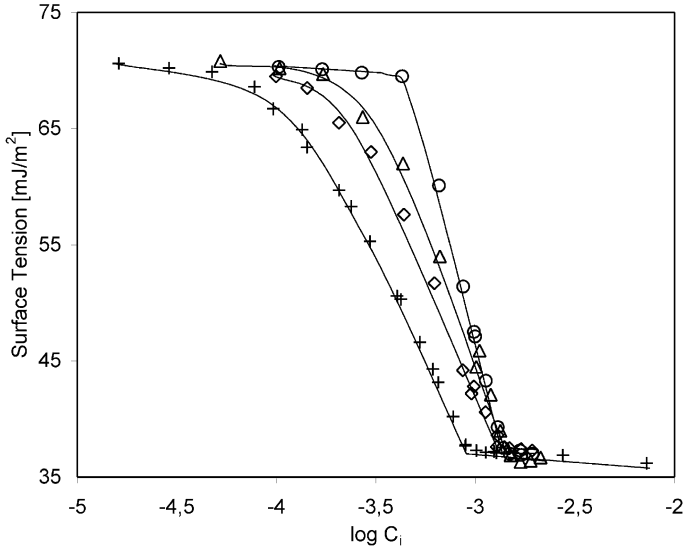
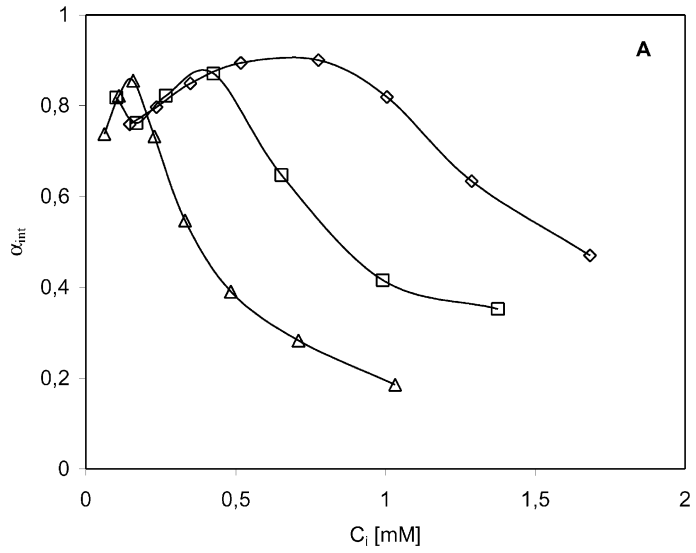


Fig. 5. Equilibrium surface tension of CTAB–0.25% (w/w) polysaccharide solutions at 27°C: (○) amylose, (△) amylopectin from potato, (◇) amylopectin from barley. Abscissa, log C_i (where C_i is the total CTAB concentration in units of mol dm^{-3}); ordinate, surface tension in units of J/m^2.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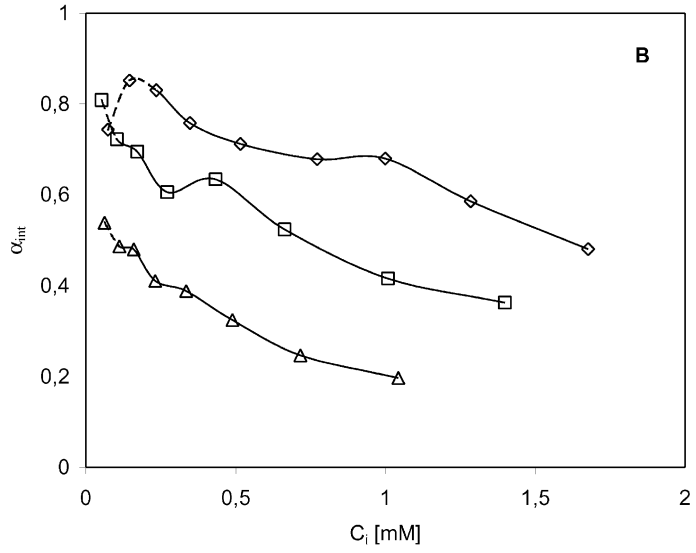
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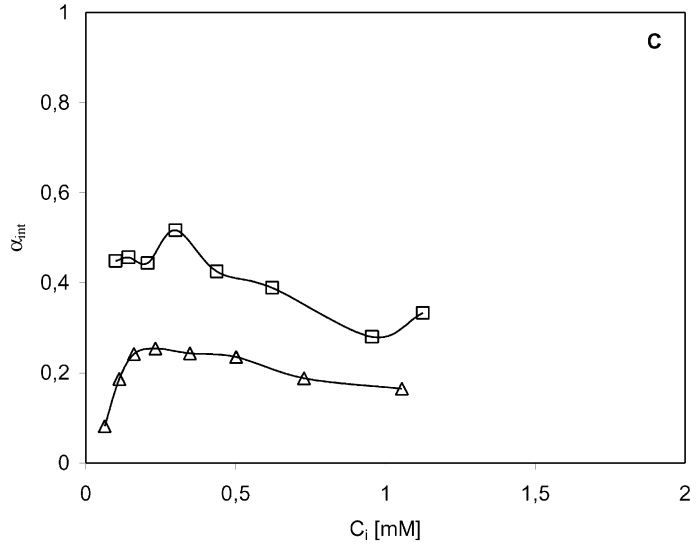
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concentration increased in the polysaccharide solution, the surface tension did not decrease as much as in water. The surface tension at the saturation concentration, C_2, was the same as of the cmc in water, 37 mJ/m^2. The higher the polysaccharide concentration, the more the CTAB had to be added in order to reach C_2. In the case of BAP only two polysaccharide concentrations were examined, 0.1 and 0.25% (w/w). The 0.5% (w/w) BAP solution was a hazy solution that scattered light, which made it difficult to focus the video camera on the air bubble in the sample cell. The saturation concentration for the 0.5% (w/w) BAP concentration was however possible to determine since the haziness decreased when CTAB was added.

A critical association concentration, cac, could not be detected in the concentration range studied for any of the starch polysaccharides. The cac was thus below 0.10 mM for all three polysaccharides used.

The C_2 for the starch samples were determined from the plots in Fig. 3 as the total concentration of CTAB at the breakpoint in the surface tension plots. Values of C_2 for the starch polysaccharides can be found in Table 1. C_2 was dependent on the polysaccharide concentration; however, it was not dependent on the type of starch polysaccharide. When plotting C_2 as a function of polysaccharide concentration, as in Fig. 4, a linear relationship between C_2 and polysaccharide concentration was evident. From the slope of C_2 vs. polysaccharide concentration in Fig. 4, it was possible to calculate the number of CTAB molecules able to bind to the polysaccharides. The maximum binding was the same for the three starch polysaccharides, 30 mmol CTAB per mole glucose units, which agrees with the values in Table 1.

The saturation concentration and the number of bound CTAB molecules show similar behaviour for the different polysaccharides despite the difference in the structure of the polysaccharides. Such behaviour has not been reported previously. The number of monoglycerides able to bind to amylose and amylopectin have previously been determined by Lagendijk and Pennings (1970) and Hahn and Hood (1987). Svensson et al., 1996 examined the binding of SDS to starch. The result in common of those studies was that amylose was able to bind 7–8 times more lipids than amylopectin, which does not agree with this study. One factor contributing to the difference in the results may be the positive electrostatic interaction between the cationic head group of CTAB and the phosphate groups of the amylopectin from potato. However, amylopectin from barley, which contains no phosphate groups, binds as much as amylopectin and amylose from potato.

The shape of the surface tension plots, a measure of the binding of CTAB, indicates that the binding process differs between amylose and amylopectin and also to some degree between the two amylopectin samples. The differences were seen especially at low CTAB concentrations. In the case of amylose, Fig. 3a, the surface tension hardly decreased as the CTAB concentration increased until a certain CTAB concentration was reached after which the surface tension decreased rapidly. This behaviour of the surface tension was the same for all amylose concentrations. In the case of PAP and BAP, Fig. 3b and c, the surface tension decreased gradually over a concentration range. The difference between the polysaccharide concentrations was the point where the surface tension started to decrease.

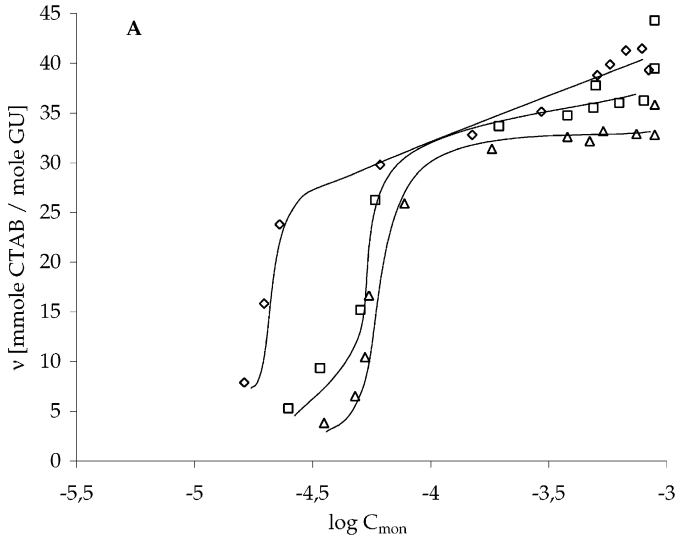
The differences in surface tension between the starch polysaccharides are better seen in Fig. 5, where the surface tension as a function of CTAB concentration is compared for 0.25% (w/w) solutions. The difference in surface tension suggests that the fraction of CTAB bound to the starch polysaccharide in each addition varies for the different polysaccharides. The fraction of the total added surfactant that is bound to the polysaccharide, α_{int}, was calculated according to Eq. (4) where n_{mon} is the amount of monomer CTAB in solution and n_{tot} is the total amount of CTAB.

$$\alpha_{int} = \frac{n_{tot} - n_{mon}}{n_{tot}} \quad (4)$$

The uncertainty of α_{int} at low CTAB concentrations is rather high since the change in surface tension is low, which gives an uncertainty in the determination of n_{mon}. In Fig. 6, α_{int} is plotted as a function of the total concentration of CTAB. Amylose had an initial α_{int} of 0.7, which increased to a maximum of 0.9, after which it decreased. Amylopectin from potato had an initial α_{int} value of 0.7–0.8, which for the 0.5% (w/w) PAP sample increased in the beginning to 0.9, after which it decreased. The 0.25 and 0.1% (w/w) samples had no maxima; they decreased with the CTAB concentration. In the case of amylopectin from barley the α_{int} values were lower than those for the amylose and PAP sample. The 0.1% (w/w) BAP sample had a linear decrease of α_{int} with the total CTAB concentration above 2 mM. The 0.25% (w/w) BAP sample had a linear decrease in α_{int} with CTAB concentration.

Binding isotherms shown in Fig. 7 were derived from the results of surface tension measurements by plotting the amount of CTAB bound to the starch polysaccharide, ν, vs. the logarithmic monomer concentration of CTAB. The shape of the binding isotherm for amylose was sigmoidal, which is the typical shape for a cooperative binding. The amount CTAB bound to amylose increased rapidly at low monomer concentrations, after which it levelled out at about 33 mmol CTAB/mole glucose units, see Fig. 7a. The binding of CTAB to amylopectin from potato can be seen to start

Fig. 6. Fraction (α_{int}) of the total added CTAB that will bind to starch polysaccharides at 27°C as a function of total CTAB concentration, C_i, determined by surface tension measurements: (◇) 0.5% (w/w), (□) 0.25% (w/w) and (△) 0.1% (w/w). (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley. Precipitate was detected in the concentration interval marked with dotted lines.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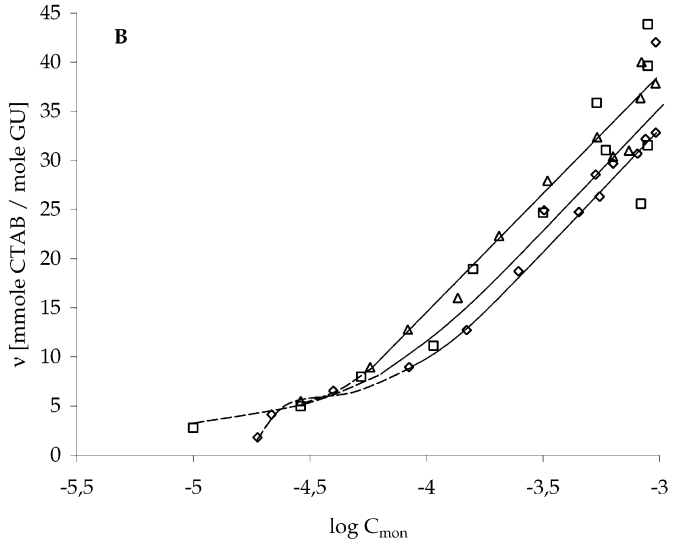
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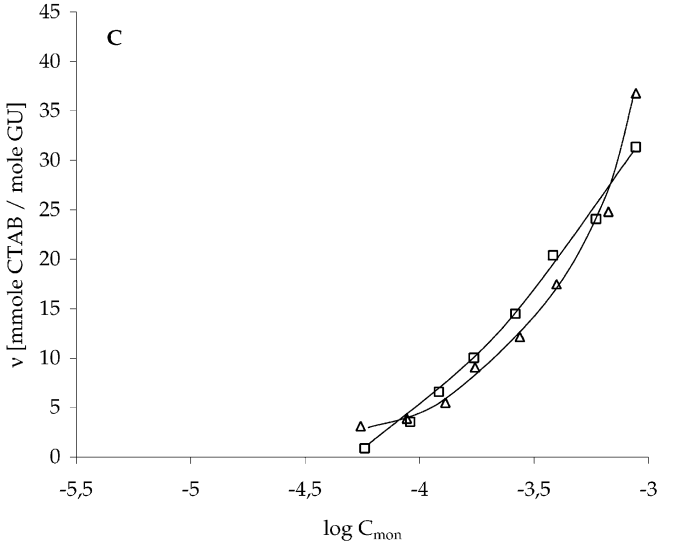
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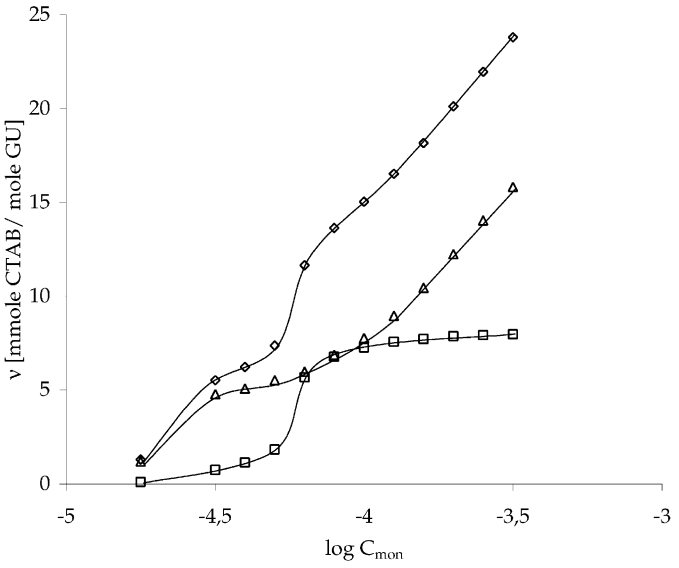


Fig. 8. Theoretical binding isotherm for CTAB binding to potato starch (75% amylopectin and 25% amylose) constructed from the individual binding isotherms for CTAB binding to amylose and amylopectin: (◇) Starch, (△) contribution from amylopectin and (□) contribution from amylose. Abscissa, log C_{mon} (where C_{mon} is the monomer CTAB concentration in units of mol dm^{-3}); ordinate, ν (amount of CTAB bound to the polysaccharide in units of mmol CTAB/mole glucose units).

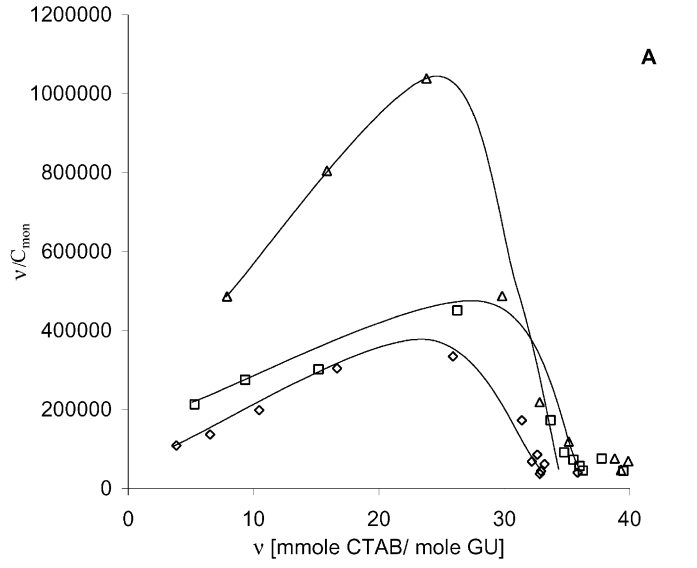
at low monomer CTAB concentrations, see Fig. 7b. After the initial binding it increased almost linearly. Fig. 7c shows the isotherms for CTAB binding to amylopectin from barley. They look similar to the binding isotherms of PAP except that it lacked binding at low monomer concentrations. The different concentrations of polysaccharide solutions overlap, which means that the interaction between CTAB and the different starches were independent of the polysaccharide concentration. The phosphate groups on the PAP did not influence the total amount of CTAB bound to PAP but they probably caused the binding of CTAB molecules already at low monomer concentrations.

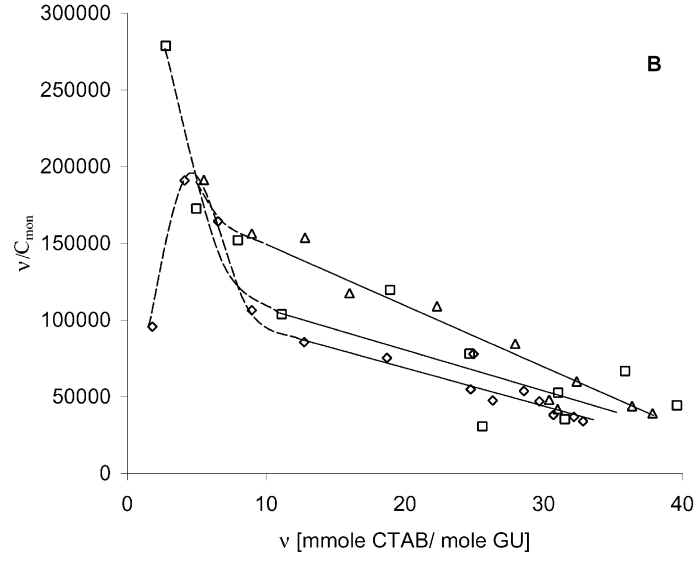
From the individual binding isotherms for amylose and amylopectin, it is possible to calculate a theoretical binding isotherm for starch from potato, consisting of 75% amylopectin and 25% amylose (see Fig. 8). From the theoretical binding isotherm it is possible to predict what happens when adding CTAB to potato starch. The first CTAB added will bind to the amylopectin due to electrostatic interaction. After that CTAB will bind to amylose until the amylose is saturated. When amylose is saturated, CTAB will continue to bind to amylopectin until CTAB micelles start to form in the sample. When adding other nonionic substances known to form inclusion complexes with amylose and amylopectin, e.g. polar lipids, the added polar lipid will first bind and saturate the amylose polysaccharides and thereafter start to bind to the amylopectin polysaccharides. Rutschmann and Solms (1990) examined the binding of a number of ligands to starch from potato. From the experimental data of Rutschmann and Solms (1990) it is possible to see the different regions, which also can be seen in Fig. 8, where the ligand was bound to amylose and amylopectin, respectively. The slope of the last part of the binding isotherm, which is due to binding to amylopectin, depends on the number of ligands that are able to bind to amylopectin.

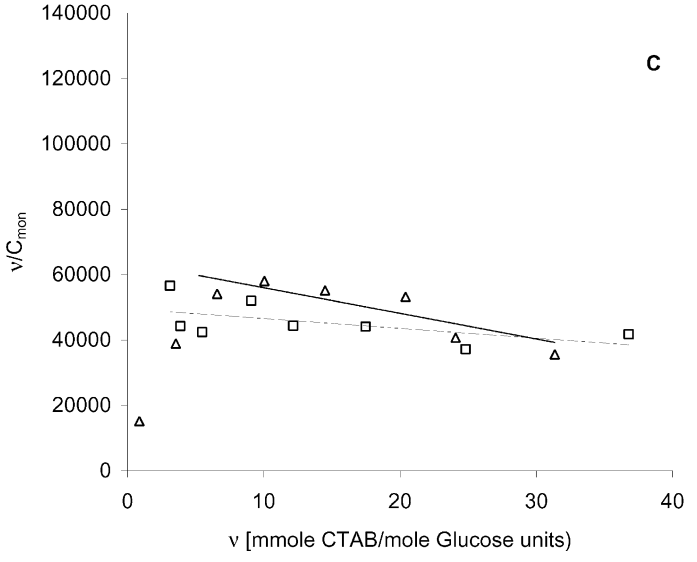
Scatchard plots, ν/C_{mon} plotted vs. ν, were used to characterise the interaction between CTAB and starch polysaccharides, see Fig. 9. The interaction enthalpy, ΔH_r, in Fig. 10, which has been determined by Lundqvist, Eliasson and Olofsson, in press, shows that the first condition postulated by Langmuir, that the binding enthalpy is constant, is fulfilled at least for a part of the binding. With the Scatchard plot, it was possible to confirm that the binding of CTAB to amylose was a cooperative binding process. The Scatchard plot for PAP (Fig. 9b) shows that the binding of CTAB was of Langmuir type for the binding of 9–33 mmol CTAB per mole glucose units. The first bound CTAB deviated from the Langmuir shape, which was probably due to the electrostatic interaction between the cationic head group and the phosphate groups on the PAP chain. The shape of the Scatchard plot for binding CTAB to BAP show that this binding could be of Langmuir type.

The electrostatic interaction between the phosphate groups on amylopectin from potato and the cationic head group of CTAB results in a precipitate of PAP at charge neutrality. This influences the shape of the binding isotherm

Fig. 7. Binding isotherms determined from surface tension measurements of the binding of CTAB to starch polysaccharide solutions at 27°C: (◇) 0.5% (w/w), (□) 0.25% (w/w) and (△) 0.1% (w/w). (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley. Precipitate was detected in the concentration interval marked with dotted lines. Abscissa, log C_{mon} (where C_{mon} is the monomer CTAB concentration in units of mol/dm^3); ordinate, ν (amount of CTAB bound to the polysaccharide in units of mmol CTAB/mole glucose units).


A
1200000
1000000
800000
600000
400000
200000
0
ν/C_mon
0
10
20
30
40
ν [mmole CTAB/ mole GU]


B
300000
250000
200000
150000
100000
50000
0
ν/C_mon
0
10
20
30
40
ν [mmole CTAB/ mole GU]


C
140000
120000
100000
80000
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ν/C_mon
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ν [mmole CTAB/mole Glucose units)

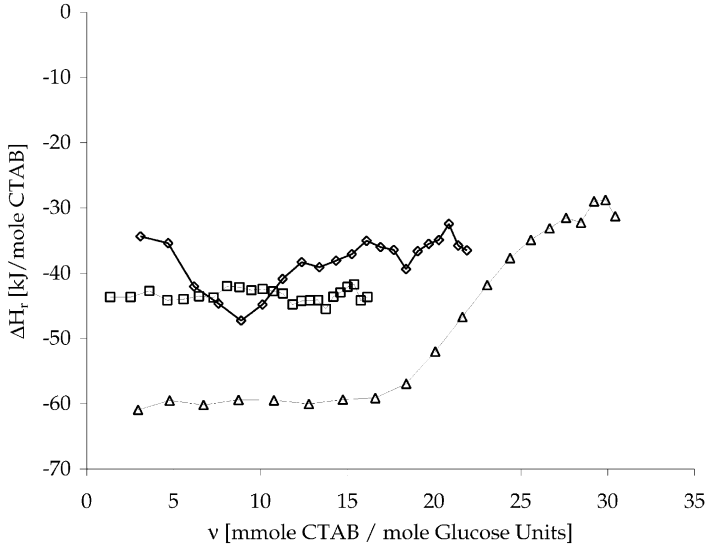

Fig. 10. Binding heat (ΔH_r), determined by isothermal titration calorimetry (Lundqvist et al., to be published) and surface tension measurements at 27°C, for the binding of CTAB to starch polysaccharides as a function of number of bound CTAB per glucose unit (ν): (△) amylose, (◇) amylopectin from potato, (□) amylopectin from barley.

and the Scatchard plot of PAP. The precipitation could visually be seen in the concentration range 0.1–0.2 mM of total CTAB it dissolved when the CTAB concentration increased. In the binding isotherm of Fig. 7 there was a plateau at $\nu = 8$ in the concentration region where there was a precipitation, when the precipitate was dissolved the binding continued and ν increased. The overall shape of the Scatchard plot of PAP was linear except where precipitation occurred, which is the typical shape of a Langmuir type of binding. The reason the precipitation could only be seen for the amylopectin from potato sample is probably that it contains about 1 phosphate group per 100–200 glucose units (Muhrbeck & Tellier, 1991), while amylose and amylopectin from barley can be considered to be non-ionic polysaccharides.

The binding characteristics, K_O, K_C and n, were determined by fitting the experimental data from CTAB binding to amylose in Fig. 9a according to McGhee and Hippel (1974) with non-linear regression. The calculated constants can be found in Table 2. The shape of the Scatchard plot and the calculated constants of the binding of CTAB to amylose agree well with earlier studies of iodine binding to amylose (Yamamoto et al., 1982) and SDS binding to amylose (Yamamoto et al., 1983). The interaction between amylose and CTAB seems to be of the same type as the interaction between amylose-iodine, which is the formation of an inclusion complex.

The binding of CTAB to amylopectin from potato could be divided into two regions: first a binding for the first 8 mmol CTAB per mole GU, which resulted in a precipitation due charge neutrality and then a second binding region after the precipitate had dissolved. The binding constant for binding CTAB to amylopectin was obtained from fitting the linear part $\nu = 9$–33 with Eq. (3), since this is the only part of the plot that fulfils the postulate by Langmuir (i.e. the constant binding enthalpy). The binding constant for the binding of CTAB to 0.5% (w/w) solutions of amylose and amylopectin can be found in Table 2.

The influence of the chain length of amylose on the type of interaction between amylose and SDS was studied by Yamamoto et al. (1983) who showed that amylose chains shorter than 76 glucose units bound SDS with a Langmuir type of binding. Since amylopectin have branches shorter than 76 glucose units this makes it natural that the binding of surfactants to amylopectin ought to be of Langmuir type. The binding seems to be similar to the formation of inclusion complex between short amylose chains and iodine. Amylopectin can be regarded as a collection of independent short amylose chains. The reason that the binding takes place over a broad CTAB concentration interval can be due to that amylopectin consists of a spectrum of chain lengths, and the first CTAB molecules bind to the longest branches. Binding to shorter branches might only take place at higher free CTAB concentrations. BAP have shorter chains than PAP and the binding of CTAB to BAP took place at higher monomer CTAB concentrations than PAP.

Fig. 9. Scatchard plot for the binding of CTAB to starch polysaccharides at 27°C: (◇) 0.5% (w/w), (□) 0.25% (w/w) and (△) 0.1% (w/w). (A) amylose, (B) amylopectin from potato and (C) amylopectin from barley. Precipitate was detected in the concentration interval marked with dotted lines. Abscissa, ν (where ν is the amount of CTAB bound to the polysaccharide in units of mmol CTAB/mole glucose units); ordinate, ν/C_{mon} (where C_{mon} is the monomer CTAB concentration in units of mol dm^{-3}).

Table 2
Binding constants for the binding of ligands to starch polysaccharides

	Temperature (°C)	Cooperative binding		Langmuir binding
		K_O	K_C	K_L
Amylose–CTAB	27	1000 ± 300	18 ± 5	–
Amylose–iodine[a]	30	14 000 ± 2000	85 ± 10	–
Amylose–SDS[b]	30	2000 ± 700	22 ± 2	–
PAP–CTAB	27			2700 ± 200
BAP–CTAB	27			900 ± 250
Short chain amylose (DP 32)–SDS[b]	30			1700 ± 300

[a] Yamamoto et al., 1982.
[b] Yamamoto et al., 1983.

According to Yamamoto et al. (1983) the cooperative effect, when a ligand was bound to amylose, was due to interaction of the outer part of the alkyl chain of SDS bound to the interrupted helix of amylose. However, the hydrophobic interaction alone, due to dehydration of the alkyl chain accompanying aggregation, cannot give rise to such large interaction enthalpies as found in Fig. 9. The hydrophobic interaction give rise to an enthalpy of interaction of less than the micelle formation enthalpy, which equals −9.5 kJ/mole CTAB (Lundqvist et al., in press), whereas the ΔH_r for amylose–CTAB interaction equals −60 kJ/mol CTAB. Starch polysaccharides solutions are known to be unstable and the configuration in solution is not fully understood. Amylose in a diluted water solution is believed to have a random coil configuration with some parts of helix structure locally (Nakata, Kitamura, Takeo & Norisuye, 1994). One contribution to the high ΔH_r can be configuration changes of the amylose chain from random coil to helix. A change of the polysaccharide from random coil to helix might also explain the cooperative effect. The binding of the first CTAB to amylose will induce the configuration change of amylose that will simplify the successive formation of complexes on the amylose chain which might be the reason for the cooperative effect. In the case of amylopectin–CTAB each branch can only form one inclusion complex and a cooperative effect is then not possible since no more complexes can be formed on that branch.

The cooperative binding of CTAB to amylose means that the formation of the individual complexes is coupled to each other. The dissociation of the inclusion complexes is then also coupled to each other. This can be seen in a differential scanning calorimetry (DSC) thermogram as a process taking place in a narrow temperature region. The binding of CTAB to amylopectin, on the other hand, is of Langmuir type and takes place over a wide concentration region which means that the formation/dissociation of the individual amylopectin–CTAB complexes are not coupled to each other. This will probably give process taking place over a wide temperature range in a DSC thermogram and explains why no peak from the dissociation of amylopectin–ligand complex can be seen in a DSC thermogram. The peak will be so broad that it will be lost in the background noise.

4. Conclusions

To our knowledge this is the first study of the binding of CTAB to amylopectin. The binding of CTAB to amylose was cooperative, the interaction triggers a configuration change of the amylose chain from random coil to helix structure with an amylose–CTAB complex as a result. The binding of CTAB to amylopectin was of Langmuir type with the formation of inclusion complexes similar to the amylose–iodine inclusion complex. Amylopectin can be regarded as a collection of independent short amylose chains. The binding capacity seem to be independent of the structure of the starch polysaccharide with 33 mmol CTAB/mole glucose units.

Acknowledgements

This work was part of the LiFT programme financed by the Swedish Foundation for Strategic Research. We are greatful to Professor Ron Bhatty from the University of Saskatchewan, Saskatoon, Canada for providing the barley grain. We wish to thank Lyckeby Stärkelsen for providing the amylopectin from potato and for preparing the barley starch samples.

References

Arnebrant, T., Bäckström, K., Jönsson, B., & Nylander, T. (1989). An ellipsometry study of ionic surfactant adsorption on chromium surfaces. *Journal of Colloid and Interface Science*, *128*, 303–312.

Bhatty, R. S., & Rossnagel, B. G. (1997). Zero amylose lines of hull-less barley. *Cereal Chemistry*, *74*, 190–191.

Bulpin, P. V., Cutler, A. N., & Lips, A. (1987). Cooperative binding of sodium myristate to amylose. *Macromolecules*, *20*, 44–49.

Cronan, C. L., & Schneider, F. W. (1969). Cooperativity and composition of the linear amylose–iodine–iodine complex. *Journal of Physical Chemistry*, *73*, 3990–4004.

Eliasson, A. -C. (1988). On the thermal transitions of the amylose–cetyltrimethylammonium bromide complex. *Carbohydrate Research*, *172*, 83–95.

Eliasson, A. -C., & Ljunger, G. (1988). Interactions between amylopectin and lipid additives during retrogradation in a model system. *Journal of the Science of Food and Agriculture*, *44*, 353–361.

French, A. D., & Murphy, V. G. (1977). Computer modelling in the study of starch. *Cereal Foods World*, *22*, 61–70.

Hahn, D. E., & Hood, L. F. (1987). Factors influencing corn starch–lipid complexing. *Cereal Chemistry*, *64*, 81–85.

Jacobsen, E., Krijgheld, H. T., Hovenkamp-Hermelink, J. H. M., Ponstein, A. S., Witholt, B., & Feenstra, W. J. (1990). Revertants of the amylose-free potato clone 86.040 ($2n = 1x = 12$). *Plant Science*, *67*, 177–182.

Jones, M. N. (1967). The Interaction of sodium dodecyl sulfate with polyethylene oxide. *Journal of Colloid and Interface Science*, *23*, 36–42.

Kugimiya, M., Donovan, J. W., & Wong, R. Y. (1980). Phase transitions of amylose–lipid complexes in starches: a calorimetric study. *Starch/Stärke*, *32*, 265–270.

Lagendijk, J., & Pennings, H. J. (1970). Relation between complex formation of starch with monoglycerides and the firmness of bread. *Cereal Science Today*, *15*, 354–356.

Lundqvist, H., Eliasson, A.-C. & Olofsson, G. Binding of hexadecyltrimethylammonium bromide to starch polysaccharides. Part II. Calorimetric study. In press.

McGhee, J. D., & Hippel, P. H. v. (1974). Theoretical aspects of DNA–protein interactions: co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. *Journal of Molecular Biology*, *86*, 469–489.

Miles, M. J., Morris, V. J., & Ring, S. G. (1985). Gelation of amylose. *Carbohydrate Research*, *135*, 257–269.

Morrison, W. R., & Coventry, A. M. (1985). Extraction of lipids from cereal starches with hot aqueous alcohols. *Starch/Stärke*, *37*, 83–87.

Muhrbeck, P., & Tellier, C. (1991). Determination of the phosphorylation of starch from native potato varieties by ^{31}P NMR. *Starch/Stärke*, *43*, 25–27.

Nakata, Y., Kitamura, S., Takeo, K., & Norisuye, T. (1994). Conformation of amylose and excluded-volume effects on its chain dimensions. *Polymer Journal*, *26*, 1085–1089.

Paredes, S., Tribout, M., & Sepúlveda, L. (1984). Enthalpies of micellization of quaternary tetradecyl- and cetyltrimethylammonium. *Journal of Physical Chemistry*, *88*, 1871–1875.

Rogers, D. E., Zeleznak, K. J., Lai, C. S., & Hoseney, R. C. (1988). Effect of native lipids, shortening and bread moisture on bread firming. *Cereal Chemistry*, *65*, 398–401.

Rotenberg, Y., Boruvka, L., & Neumann, A. W. (1983). Determination of surface tension and contact angel from the shapes of axisymmetric fluid interfaces. *Journal of Colloid and Interface Science*, *93*, 169–183.

Rutschmann, M. A., & Solms, J. (1990). Formation of inclusion complexes of starch with different organic compounds. II. Study of ligand binding in binary model systems with decanal, 1-naphthol, monostearate and monopalmitate. *Food Science and Technology*, *23*, 70–79.

Schoch, T. J., & French, D. (1947). Studies on bread staling. I. The role of starch. *Cereal Chemistry*, *24*, 231–249.

Sepúlveda, L., & Cortés, J. (1985). Ionization degrees and critical micelle concentrations of hexadecyltrimethylammonium and tetradecyltrimethylammonium micelles with different counterions. *Journal of Physical Chemistry*, *89*, 5322–5324.

Silverio, J., Svensson, E., Eliasson, A. -C., & Olofsson, G. (1996). Isothermal microcalorimetric studies on starch retrogradation. *Journal of Thermal Analysis*, *47*, 1179–1200.

Svensson, E., Gudmunsson, M., & Eliasson, A. -C. (1996). Binding of sodium dodecylsulphate to starch polysaccharides quantified by surface tension measurements. *Colloids and Surfaces B: Biointerfaces*, *6*, 227–233.

Wang, G., & Olofsson, G. (1995). Ethyl(hydroxyethyl)cellulose and ionic surfactants in dilute solution. Calorimetric and viscosity study of the interaction with SDS and some cationic surfactants. *Journal of Physical Chemistry*, *99*, 5588–5596.

Yamamoto, M., Sano, T., & Yasunaga, T. (1982). Interaction of amylose with iodine. I. Characterisation of cooperative binding isotherms for amyloses. *Bulletin of Chemical Society of Japan*, *55*, 1886–1889.

Yamamoto, M., Sano, T., Harada, S., & Yasunaga, T. (1983). Cooperativity in the binding of sodium dodecyl sulfate to amylose. *Bulletin of Chemical Society of Japan*, *56*, 2643–2646.

Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch/Stärke*, *40*, 1–7.

Zobel, H. F., French, A. D., & Hinkle, M. E. (1967). X-Ray diffraction of oriented amylose fibres. II. Structure of V amyloses. *Biopolymers*, *5*, 837–845.