Supramolecular catalysis induced by polysaccharides. Homogeneous hydrolysis of *p*-nitrobenzyl amylose xanthate[†]

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ABSTRACT: *p*-Nitrobenzyl amylose xanthate (AmXNB) was synthesized and characterized by ^{13}C NMR spectroscopy in solution and the solid state. The degree of substitution (DS), calculated from the sulfur content, was 7.0, and this value was similar to that obtained from solid-state ^{13}C NMR using the signal of C-1 as internal standard. The hydrolysis of AmXNB was studied in 10% (v/v) DMSO with $\mu = 0.5$ (KCl) at 25 °C. The basic hydrolysis was pseudo-first order, but the water-catalyzed hydrolysis in the pH range 7–9 showed a biphasic plot of ln (ΔAbsorbance) vs time, as has been observed for cellulose xanthate esters, occurring through two parallel reactions with rate constants k'_{H_2O} (fast) = 5.3×10^{-5} s^{-1} and k''_{H_2O} (slow) = 3.3×10^{-6} s^{-1}. The fast hydrolysis was more than three orders of magnitude faster than that of the *O*-ethyl analog. The activation parameters were $\Delta H^{\neq} = 20.5$ kcal mol^{-1} and $\Delta S^{\neq} = +10$ cal K^{-1} mol^{-1}. They showed that the acceleration of the fast hydrolysis of AmXNB and cellulose analogs is due to an entropy of activation effect. There is a linear increase of $\log k'_{H_2O}$ (fast) with increase in the concentration of the small Li^{+} ion that produces an increase of the 3-D hydrogen-bond network of water while the large singly charged iodide ion has a considerable inverse effect. These results are strongly consistent with the theory that the supramolecular catalysis induced by modified polysaccharide esters is due to the 3-D hydrogen-bond network of the water in the solvation shell. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: *p*-nitrobenzyl amylose xanthate; supramolecular catalysis; polysaccharide xanthate esters; water-catalyzed hydrolysis

INTRODUCTION

Mono- and polysaccharides are strongly hydrated in water and this interaction imposes a structure pattern on the water of the cybotactic region, the volume around the molecule in which the ordering of solvent molecules has been influenced by the solute. This solvent structuring can explain the anomalous properties of water solutions and the related entropy.[1]

Monosaccharides, depending on their stereochemistry, inhibit specifically the water-catalyzed reactions of small molecules and the effect increases with the compatibility of the monosaccharide with the three-dimensional hydrogen-bond structure of water.[2] For *β*-D-glucopyranose, the most favorable interaction with liquid water

occurs when the hydroxyl groups are arrayed in a pattern almost the same as the basal plane of the ice I_h lattice (with the distances slightly expanded).[3,4] Liquid water retains some of the character of this extended hexagonal symmetry. This model might not be completely correct, but no better general model has been advanced for sugar hydration. Exocyclic hydroxymethyl groups of hexoses perturb the solvent structuring by torsional angle rotations, and their reactivity is different from that of the cyclic hydroxyl groups.[5,6]
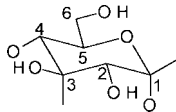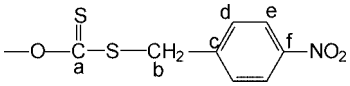
Although hydration of carbohydrates is now better understood, a detailed description of the cybotactic region is not available because of the experimental difficulties. However, some insight can be obtained by comparing the reactivities of mono- and polysaccharide derivatives with small molecules with the same functionalities. It has been observed that the water-catalyzed reaction of cellulose xanthate esters is about three orders of magnitude faster than that of the small analog molecule, and it has been proposed that the acceleration is a consequence of this highly ordered cybotactic region.[7] The fast hydrolysis occurs with a rate-determining nucleophilic attack of a water molecule,[8] catalyzed

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Table 1. [13]C chemical shifts of *p*-nitrobenzyl amylose xanthate

	δ (ppm)	
Carbon	Solution[a]	Solid state[b]

C-1	99.9	103.1
C-4	78.6	82.3
C-2	73.1	72.8
C-3,5	71.4	72.8
C-6	60.3	60.8

C-a	212.1	213.1
C-b	40.4	41.0
C-c	144.1	147.0
C-d	130.1	132.0
C-e	123.5	124.8
C-f	146.6	147.0

[a] In DMSO-*d*.
[b] CP/MAS TOSS.

by a second water molecule that acts as a general base.[5] The water catalysis is not due to a neighboring OH effect[6] and the effect is mainly entropic, since the entropy of activation is nearly zero.

In this paper, we compare the results obtained with the hydrolyses of cellulose xanthate esters, under heterogeneous conditions, with *p*-nitrobenzyl amylose xanthate (AmXNB). Cellulose and amylose are related polysaccharides [(1→4)-β-D- and (1→4)-α-D-glucan, respectively], but amylose dissolves well in water and the hydrolysis can be observed under homogeneous conditions, avoiding any possible artifacts.

EXPERIMENTAL

All reagents and solvents were of analytical grade and were used without further purification, except dimethyl sulfoxide (DMSO), which was previously distilled and dried over a molecular sieve. Distilled water was deoxygenated by boiling and cooling under nitrogen.

UV spectra and kinetics were measured using an HP 8452A UV–visible spectrophotometer. Sulfur content was measured on a LECO SC-132 analyzer.

NMR spectra in solution were obtained on a Bruker AC-200 spectrometer. ^{13}C NMR solid-state spectra were recorded at 75.5 MHz on a Bruker MSL300 spectrometer using a standard Bruker magic angle spinning (MAS) probe. The pulse sequence for total suppression of

spinning sidebands (TOSS) combined with the standard [1]H—[13]C cross-polarization (CP) was employed. The contact time used was 2 ms and high-power ^{1}H decoupling was applied during acquisition. All the ^{13}C chemical shifts are given relative to tetramethylsilane.

p-Nitrobenzyl amylose xanthate (AmXNB). Amylose (2 g) was dissolved in 100 ml of 2 M NaOH by magnetic stirring for 1 h and then 1.6 ml (26.5 mmol) of carbon disulfide was added and left to react for 5 h under mechanical shaking. The solution was neutralized with 2 M HCl, the excess of CS_2 was extracted with carbon tetrachloride, and then it was treated with activated carbon and filtered. The filtrate was lyophilized and the solid product was dissolved in 100 ml of dried DMSO. A solution of 1.9 g (0.88 mmol) of *p*-nitrobenzyl bromide in 40 ml of dried DMSO was added dropwise and stirred at room temperature in a stoppered Erlenmeyer flask for 13 h. The AmXNB product was crystallized by adding cold ethanol and subsequently filtered and washed in the filter successively with water, 0.1 M HCl (to eliminate the unreacted xanthate groups), water, ethanol and diethyl ether. The product and the solvents were kept cold during this procedure. AmXNB was finally dried under vacuum at room temperature over P_2O_5; λ_{max} (DMSO) 282 nm; ^{13}C NMR in solution (DMSO-*d*), 200 MHz (Table 1); solid state ^{13}C NMR, 75.5 MHz, CP/MAS TOSS (Table 1).
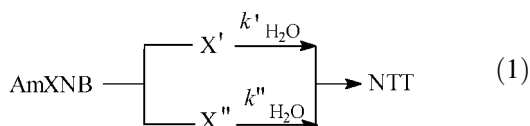
The degree of substitution, DS (average number of xanthate ester groups per 100 glucoanhydropyranose units), was 7.0, calculated from the sulfur content (2.76%). It was similar to that obtained from solid-state ^{13}C NMR using the signal of C-1 as the internal standard.[5]

The *n*-butylaminolysis of AmXNB (Scheme 1)
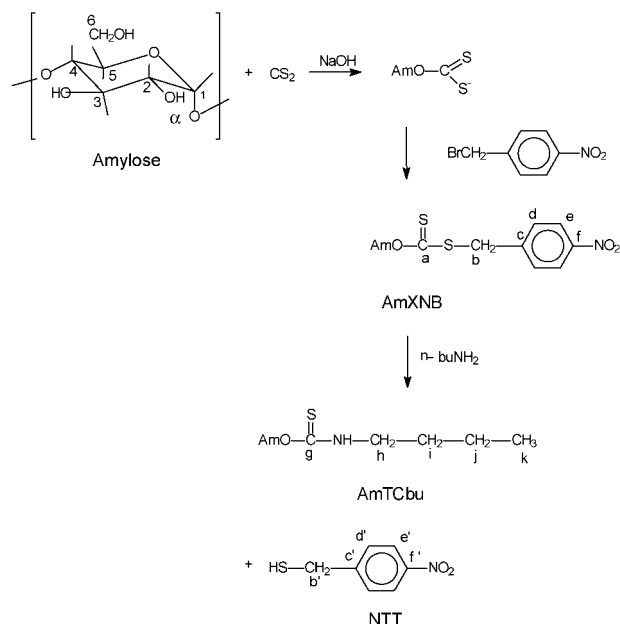
Scheme 1

produced *n*-butyl amylose thioncarbamate [AmTCbu: ^{13}C NMR, 75.5 MHz, CP/MAS TOSS, δ 189.9 (C-g), 102.4 (C-1), 81.8 (C-4), 72.4 (C-2, -3, -5), 61.0 (C-6), 45.7 (C-h), 30.9 (C-i), 20.7 (C-j), 14.4 (C-k)] and *p*-nitrotoluenethiol [NTT: ^{13}C NMR, 75.5 MHz, CP/MAS TOSS, δ 141.9 (C-c', f'), 130 (C-d'), 122.2 (C-e'), 36.7 (C-b')].

Kinetics. The hydrolysis of AmXNB was followed by the disappearance of the xanthate at 282 nm. A saturated solution in DMSO was used as a stock solution. The basic hydrolysis of AmXNB was pseudo-first order, but the spontaneous hydrolysis in the pH range 7–9 showed a biphasic plot of ln (ΔA) vs time, as has been observed for *p*-nitrobenzyl cellulose xanthate (CelXNB)[5] and 2,4-dinitrophenyl cellulose xanthate (CelXDNP),[6] because the hydrolysis occurs through two parallel water-catalyzed reactions that produce the same product (NTT) as observed from the UV spectrum. The kinetics were analyzed according to

$$\text{AmXNB} \longrightarrow \begin{array}{c} X' \xrightarrow{k'_{H_2O}} \\ X'' \xrightarrow{k''_{H_2O}} \end{array} \longrightarrow \text{NTT} \qquad (1)$$

where X' and X'' are two xanthate groups with different reactivities, with rate constants k'_{H_2O} (fast) and k''_{H_2O} (slow).

The rate constant k''_{H_2O} of the slow hydrolysis was calculated from the total absorbance A_t vs time trace (Fig. 1), without considering the segment before 200 min, by an iterative program where the absorbance at any given time was $A'' = A''_\infty - (A''_0 - A''_\infty)\exp(-k''_{H_2O}t)$, as shown in the inset of Fig. 1. Alternatively, the rate constant k''_{H_2O} was calculated from the ln (ΔA_t) vs time plot, as has been done for cellulose xanthate esters.[5,6] Both methods produced similar values that were averaged out. The total absorbance A_t is given by

$$A_t = A'_\infty + (A'_0 - A'_\infty)\exp(-k'_{H_2O}t) + A''_\infty$$

$$+ (A''_o - A''_\infty)\exp(-k''_{H_2O}t) \qquad (2)$$

and in order to obtain the rate constant k'_{H_2O}, A_t was corrected by the term $[A' + A''_\infty + (A''_\infty - A''_\infty)\exp(-k''_{H_2O}t)]$. A plot of ln $(A_t - \Delta)$ vs time produced k'_{H_2O} (Fig. 2).

RESULTS AND DISCUSSION

Hydroxide- and water-catalyzed hydrolysis

The hydrolyses of xanthic esters R_1O—$C(S)$—SR_2 produce initially a thioncarbonate that decomposes rapidly to R_1OH, R_2SH and COS, and it was found that
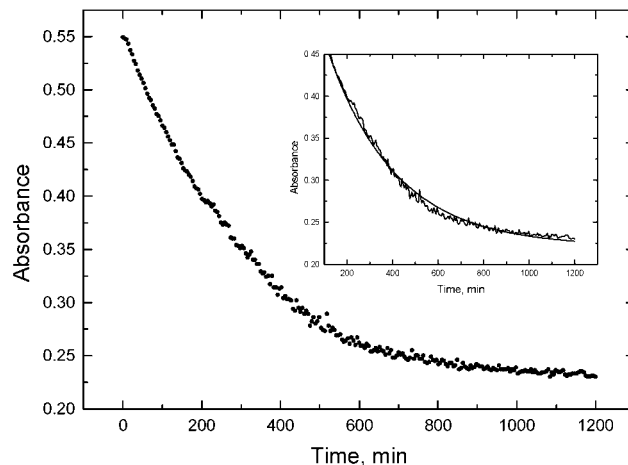

Figure 1. Hydrolysis of *p*-nitrobenzyl amylose xanthate at 25°C, in 10% (v/v) DMSO, pH 9.0 (0.05 M carbonate), $\mu = 0.5$ (KCl), followed by the disappearance of the ester at 282 nm. Inset: calculation of k''_{H_2O}

the thiol appears at the same rate of the disappearance of the xanthic ester.[8] The pH–rate profiles follow the equation

$$k_{obs} = k_{H_2O} + k_{OH}[OH^-] \qquad (3)$$

where k_{obs} is the rate constant extrapolated to zero buffer concentration, k_{H_2O} is the rate constant of the water-catalyzed reaction in the pH-independent region and k_{OH} is the second-order rate constant of the hydroxide-catalyzed reaction.[5,6,8] The basic hydrolysis followed simple first-order kinetics, as has been observed for the reaction of other cellulose xanthate esters with external nucleophiles.[5,6]

Considering the Brønsted parameters, it was concluded that the hydroxide-catalyzed reaction of xanthate esters occurs by a fast tetrahedral intermediate formation
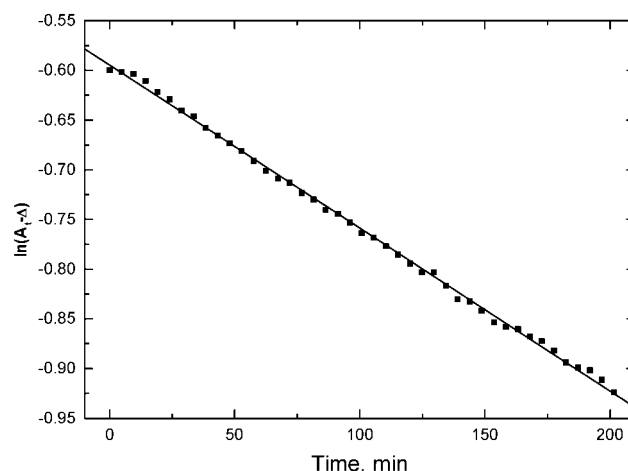

Figure 2. Hydrolysis of *p*-nitrobenzyl amylose xanthate at 25°C, in 10% (v/v) DMSO, pH 9.0 (0.05 M carbonate), $\mu = 0.5$ (KCl). Calculation of k'_{H_2O}

Table 2. Rate constants for the water- and hydroxide ion-catalyzed hydrolysis of some polysaccharide xanthate esters and their analogs in water at 25 °C

Ester	k'_{H_2O} (s^{-1})	k''_{H_2O} (s^{-1})	k_{OH} (l mol^{-1} s^{-1})[b]	Ref.
AmXNB	$(5.3 \pm 0.2) \times 10^{-5a,b}$	$(3.3 \pm 0.2) \times 10^{-6a,b}$	$(3.71 \pm 0.42) \times 10^{-3c,d}$	This work
CelXNB	$8.2 \times 10^{-4d,e}$	$1.4 \times 10^{-5d,e}$	$8.44 \times 10^{-3d,f}$	8
EXNB		$3.49 \times 10^{-8g,h}$	$4.61 \times 10^{-2f,g}$	8
MG-6[i]		1.01×10^{-7g}	$2.05^{f,g}$	6
MG-3[j]		$1.87 \times 10^{-5f,g}$	$43.10^{d,f}$	6

[a] 10% (v/v) DMSO.
[b] $\mu = 0.5$ (KCl).
[c] $\mu = 1.0$ (NaOH).
[d] 10% (v/v) EtOH.
[e] $\mu = 0.6$ (KCl).
[f] $\mu = 1.0$ (KCl).
[g] 20% (v/v) EtOH.
[h] $\mu = 0.1$ (KCl).
[i] Methyl α-D-glucopyranoside 6-(*S-p*-nitrobenzyl xanthate).
[j] Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 3-(*S-p*-nitrobenzyl xanthate).

followed by a slower expulsion of the thiolate moiety.[8] The second-order rate constants k_{OH} of polysaccharides are similar to those for the *O*-alkyl xanthate analogs, whereas the simple sugar xanthates hydrolyze more than three orders of magnitude faster (Table 2), possibly because of the micelle catalysis.[8]

For *S-p*-nitrobenzyl xanthate esters, the pH-independent plateau starts at pH <10,[5,8] and consistently the rate constants of the hydrolysis of AmXNB do not change in the pH range 7–9. The values of k'_{H_2O} (fast) and k''_{H_2O} (slow) shown in Table 2 were obtained at pH 9.0. The water-catalyzed hydrolysis of AmXNB is slower than that for CelXNB but the fast hydrolysis is still more than three orders of magnitude faster than for the *O*-ethyl analog (EXNB). It was concluded that the rate acceleration of CelXNB was not due to an anchimeric effect of the neighboring OH group.[6]

At low level of substitution, the glucopyranose ring is substituted on positions 2, 3 and 6 during the xanthation reaction and the C-6 isomer predominates over the C-2 + C-3 isomers.[5,9,10] Sugar xanthates such as methyl α-D-glucopyranoside 6-(*S-p*-nitrobenzyl xanthate) (MG-6) and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 3-(*S-p*-nitrobenzyl-xanthate) (MG-3) also hydrolyze faster

than EXNB, perhaps owing, to the structure of the hydration shell of the sugar moiety. In particular, the rate constant of MG-3 is ca 300 times larger than for the 6-substituted analog and similar to the fast hydrolysis of AmXNB.

Activation parameters

The activation parameters for the hydrolyses that are given in Table 3 show that the $\Delta H^{\neq}$ values are lower for small hydrophilic molecules such as MG-3, but the entropy of activation is highly negative, as has been found for all the basic hydrolyses of xanthate esters.[5,6] On the other hand, for the fast water-catalyzed hydrolysis of polysaccharides such as AmXNB and cellulose analogs, $\Delta G^{\neq}$ is favorable owing to the difference in $\Delta S^{\neq}$. It is important to note that only the fast water-catalyzed hydrolyses of polysaccharide xanthates present a $\Delta S^{\neq}$ value near zero, or slightly positive as in the case of AmXNB.

The Brønsted-type plot of the water-catalyzed hydrolyses of the *S*-substituted *O*-ethylxanthate series supports the conclusion that the rate-determining step is the

Table 3. Activation parameters for the hydrolysis of some polysaccharides and sugar xanthates in water

Ester	Nucleophile	$\Delta H^{\neq}$ (kcal mol^{-1})	$\Delta S^{\neq}$ (cal K^{-1} mol^{-1})[a]	Ref.
AmXNB	H$_2$O (fast)[b]	20.5 ± 0.2	10 ± 1	This work
CelXNB	H$_2$O (fast)[c]	22.7 ± 0.2	-4 ± 1	5
	OH^{-d}	11.8 ± 0.7	-33 ± 3	5
CelXDNP	H$_2$O (fast)[d]	20.9 ± 0.4	-1 ± 1	6
	OH^{-d}	7.5 ± 0.2	-37 ± 1	6
MG-3	H$_2$O[d]	11.9 ± 0.1	-41 ± 1	6
	OH^{-d}	4.4 ± 0.2	-46 ± 1	6

[a] 1 M, 25 °C.
[b] 10% (v/v) DMSO, $\mu = 0.5$ (KCl).
[c] 10% EtOH, $\mu = 0.6$ (KCl).
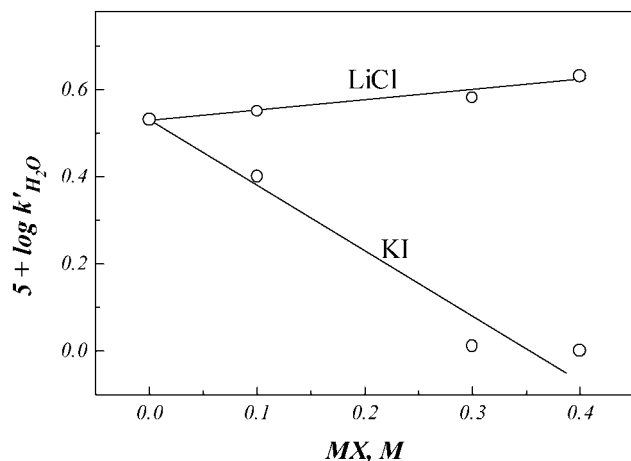[d] 10% EtOH, $\mu = 1.0$ (KCl).

Figure 3. Salt effect on the fast water-catalyzed hydrolysis of p-nitrobenzyl amylose xanthate at 25 °C, in 10% (v/v) DMSO, pH 9.0 (0.1 M carbonate), $\mu = 0.5$ (MX, M = K$^+$, Li$^+$; X = Cl$^-$, I$^-$)

nucleophilic attack of the water molecule.[8] For CelXNB it was shown that a second molecule acts as a general base catalyst for the deprotonation of the tetrahedral intermediate.[5,7] The reacting water molecule that acts as a nucleophile is inserted in the highly ordered solvation shell of the polysaccharide.[5–8,11,12] The second water molecule that catalyzes the reaction is part of this supermolecular structure and the efficiency of the catalysis is a consequence of this 3-D hydrogen-bond network. For this reason, this catalysis has been called supramolecular catalysis.[8] The rate acceleration observed with respect to small hydrophobic and hydrophilic molecules occurs mainly because the entropy of activation for the polysaccharide derivatives is nearly zero due to the well oriented water molecules involved in the transition state.

The solubility of AmXNB permitted the hydrolysis to be carried out in a homogeneous solution, showing that the results obtained in the heterogeneous cellulose system were not due to artifacts.

Salt effects

The cybotactic region includes the first solvation shell and a transition region that may extend well beyond.[13,14] Some recent MD simulations of the solvation of pentose sugars[15] and a disaccharide[16] show that these molecules do indeed impose a strong anisotropic 3-D structure on the surrounding associated water molecules that depends on the molecular topology of the substrate. The structuring is stereospecific and is most favorable when the hydration requirements of the functional groups of the solute molecules are mutually compatible. The first and second solvation shells are well defined around the sugar molecule, with specific locations determined by the arrangements of the functional groups of the solute.

In the case of polysaccharides, the structuring of water appears to be stronger than that of the monosaccharides considering the differences in $\Delta S^{\neq}$, and the effect of salts on the rate constant of the fast hydrolysis of AmXNB could give a further insight into the properties of the cybotactic region.

The effect of Li$^+$ and I$^-$ ions on k'_{H_2O} is shown in Fig. 3. There is a linear increase in log k'_{H_2O} with the concentration of Li$^+$ ions, while I$^-$ produces a rapid decrease.

The properties of cations and anions have been studied with respect to their effect on the structure of water. Measurements of viscosity, heat capacity and dielectric relaxation,[17] and also the entropy of hydration,[18] indicate that small ions such as Li$^+$ present strong positive hydration, producing an increase in the 3-D hydrogen-bond network of water; the solvation shell is looser for bulkier ions such as K$^+$, and they present a negative hydration.[19] Large, singly charged ions (I$^-$, Cs$^+$) produce a considerable decrease in the H-bond network, following the order Cl$^-$ < I$^-$. Therefore, substitution of K$^+$ by Li$^+$ in a solution of KCl should increase the 3-D H-bond network of water. Alternatively, substituing Cl$^-$ by I$^-$ should decrease the H-bond network.

The results presented in Fig. 3 are highly consistent with the theory that the supramolecular catalysis induced by modified polysaccharide esters is due to the 3-D H-bond network of water in the solvation shell. When the concentration of Li$^+$ increases, the H-bond network becomes stronger, favoring the water catalysis. The opposite affect was observed when the concentration of I$^-$ was increased because of the decrease in the H-bonding between the water molecules.

CONCLUSIONS

The water-catalyzed hydrolysis of AmXNB under homogeneous conditions occurs through two parallel processes with rate constants k'_{H_2O} (fast) and k''_{H_2O} (slow). The fast spontaneous hydrolysis is more than three orders of magnitude faster than that for the O-ethyl analog.

The acceleration of the hydrolysis of AmXNB and cellulose analogs is due to an entropy of activation effect. The fast water-catalyzed hydrolyses of polysaccharide xanthates are unique, since their entropies of activation are either near zero or slightly positive, as in the case of AmXNB, because the strong 3-D H-bond network of water in the solvation shell.

Small ions, such as Li$^+$, which produce an increase of the 3-D H-bond network of water, accelerate the fast hydrolysis further. Large, singly charged spherical ions, such as iodide, known by their effect of decreasing the H-bond network of water, decrease the rate of hydrolysis.

These results support the theory that the supramolecular catalysis induced by modified polysaccharide esters is due to the 3-D H-bond network of water of the solvation shell.

Acknowledgements

REFERENCES

1. (a) Einsenberg D, Kauzmann W. *The Structure and Properties of Water*. Oxford University Press: Oxford, 1969; (b) Stillinger FH. *Science* 1980; **209**: 451–457; (c) Israelachvili J, Wennerstrom H. *Nature (London)* 1996; **379**: 219–225.
2. (a) Galema SA, Blandamer MJ, Engberts JBFN. *J. Am. Chem. Soc.* 1990; **112**: 9665–9666; (b) Galema SA, Blandamer MJ, Engberts JBFN. *J. Org. Chem.* 1992; **57**: 1995–2001; (c) Engberts JBFN, Blandamer MJ. *Chem. Commun.* 2001; 1701–1708.
3. Warner DT. *Nature (London)* 1962; **196**: 1055–1058.
4. (a) Franks F. *Water. A Matrix of Life*. Royal Society of Chemistry: Cambridge, 2000; Chapt. 8; (b) Ha S, Gao J, Tidor B, Brady JW, Karplus M. *J Am. Chem. Soc.* 1991; **113**: 1553–1557.
5. Humeres E, Sequinel LF, Nunes M, Oliveira CMS, Barrie PJ. *J. Phys. Org. Chem.* 1994; **7**: 287–295.
6. Humeres E, Sequinel LF, Nunes M, Oliveira CMS, Barrie PJ. *Can. J. Chem.* 1998; **76**: 960–965.
7. Humeres E, Oliveira CMS, Osellame VT, Souza IG. *J. Phys. Org. Chem.* 1991; **4**: 573–578.
8. Humeres E, Soldi V, Klug M, Nunes M, Oliveira CMS, Barrie PJ. *Can. J. Chem.* 1999; **77**: 1050–1056.
9. (a) Kamide K, Saito M. *Adv. Polym. Sci.* 1987; **83**: 1–56; (b) Kamide K, Kowsaka K, Okajima K. *Polym. J.* 1987; **19**: 231–240.
10. Trimnell D, Doane WM, Russell CR, Rist CE. *Carbohydr. Res.* 1967; **5**: 166–175.
11. Khanagov AA. *Biopolymers* 1971; **10**: 789–794.
12. Fleming WW. *Univ. Microfilms* 1978; **38**: 3256–3257.
13. Stewart GW, Morrow RM. *Proc. Natl. Acad. Sci. USA* 1927; **13**: 222–223.
14. Reichardt C. *Solvents and Solvent Effects in Organic Chemistry* (2nd edn). VCH: Weinheim, 1988; Chapt. 2.
15. Liu Q, Brady JW. *J. Am. Chem. Soc.* 1996; **118**: 12276–12286.
16. Vishnyakov A, Widmalm, G, Laaksonen A. *Angew. Chem., Int. Ed. Engl.* 2000; **39**: 140–142.
17. Frank HS, Wen W-Y. *Discuss. Faraday Soc.* 1957; **24**: 133–140.
18. Frank HS, Evans MW. *J. Chem. Phys.* 1945; **13**: 507–532.
19. Samoilov OYa. *Discuss. Faraday Soc.* 1957; **24**: 141–145.