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INTERACTIONS OF AMYLOSE WITH CERTAIN BASES

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ABSTRACT

Results of the interaction of NaOH, KOH, $Ca(OH)_2$, and NH_4OH with amylose, over a temperature range of 283–313 K, using conductometric, viscometric, and equilibrium-dialysis methods, are presented. The specific conductance of the strong bases decreases in the presence of the biopolymer. Examination of the Walden product at a fixed concentration of a base and various concentrations of the polymer revealed binding of the base with the biopolymer. The results followed the Langmuirian isotherm, indicating weak binding, accompanied by a free-energy change of $\sim$12 kJ/mol. The interaction of amylose and the weak base NH_4OH was conspicuous. The specific conductance showed an increment in the presence of amylose, as well as of D-glucose and poly(ethylene glycol), all of which are polyoxygen-centered non-electrolytes. An increase in the Walden product at a fixed concentration of base and various concentrations of polymer indicated an increase in the degree of dissociation of the weak base over and above its binding with amylose. Evaluation of the degree of dissociation of NH_4OH in the presence of D-glucose and amylose has been attempted.

INTRODUCTION

Amylose, a natural polymer, has divergent physicochemical properties that have long attracted the attention of chemists. Like other factors[1–6], the hydrogen-ion concentration affects the conformation of amylose in solution. At high pH values, the random-coil chain of the polymer is elongated[7–10], and the intrinsic viscosity increases. One reason for the chain elongation in alkaline medium could be through adsorption, or binding, of ions, resulting in formation of a charged polymer that expands by way of segmental repulsion. Such a hypothesis receives support from the binding of bases with simple[11–16] polyhydroxy compounds and reducing sugars (amylose being a polymeric chain of D-glucosyl residues). Binding of metal ions (particularly calcium ion) with carbohydrates that are considered necessary for gastrointestinal absorption of dietary calcium and other alkaline-earth-metal ions[17] may be compared with such a complexation process. Transport of metal ions under physiological conditions is considered to be routed through complexation *via* carbohydrates. This kind of binding may involve hydrogen bonding, and consequently,

be weak. Hence, it may be a physical adsorption or physisorption type of process that is thermodynamically reversible and exothermic.

In connection with our studies on the complexation of simple sugars with salts and bases, and of amylose with iodine, we became interested in studying the interactions of weak and strong bases with amylose. As in our hypothesis of complexation of bases with reducing sugars prior to their degradation, we[11-13] also anticipated binding of bases with the amylose chain, which, after prolonged exposure at relatively high concentrations, can hydrolyze (degrade) the chain into smaller fragments. We now report the results of a study of the interaction of NaOH, KOH, $Ca(OH)_2$, and NH_4OH with an amylose of relatively low molecular weight, at various temperatures in the range of 10–40 °C, by performing viscometric, conductometric, and equilibrium-dialysis measurements.

EXPERIMENTAL

Materials. — The sample of amylose (source unknown, G.R. grade) of low molecular weight was obtained from E. Merck, Darmstadt. The sodium hydroxide, potassium hydroxide, and calcium hydroxide were of A.R. grade, from Sarabhai M. Chemicals, India. The ammonia solution (sp. gravity 0.91), also of A.R. grade, was from Glaxo Laboratories, India. Dextrose (anhydrous, G.R. grade) was also obtained from Sarabhai M. Chemicals, India. Poly(ethylene glycol) 6000 was a product of BDH Chemicals Ltd., Poole, England.

Methods. — Resistance measurements were made with a Philips conductivity bridge PR9500. A maximum error[12] of 2% was observed in the range of 1 to 100 kΩ. The resistances of the solutions were measured by using a dip type of conductivity cell (cell constant, 1.2990 cm^{-1}).

An Ostwald viscometer of flow time 200 s for water at 30° was used for viscosity measurements. Densities were measured in a pycnometer.

The viscosity average molecular weight of the amylose sample was found to be 24,040, determined in 0.5M KOH. The viscosity average molecular weight was supported by the appearance of λ_{max} of the iodine complex[18] of the same amylose sample at 570 nm.

Dialysis bags, obtained from Medicell International, London, were of specification Visking, size 6-27/32".

Preparation of solutions. — All solutions were prepared in conductivity water (specific conductance, $3-5 \times 10^{-6}$ mho.cm^{-1} at 30°). All experimental solutions were thermostated in a thermostatic bath controlled within an uncertainty of ±0.02°. The time required for attainment of equilibrium was tested for each of the experimental sets, and the solutions were allowed to equilibrate accordingly, before final readings were recorded. This equilibrating time varied in the range of 2–10 h, depending on the temperature of the experiment. Atmospheric contamination of the solutions was avoided as far as possible by maintaining a nitrogen atmosphere.

Conductometric experiments. — Conductometric studies were performed at

temperatures of 10, 20, 30, and 40°. The final, ionic strength of the strong bases in solution lay between 0.001 and 0.020. This low ionic strength in effect lessened the contribution of interionic attraction to the conductance. For the weak base NH_4OH, the range of concentration used was 0.01 to 0.10M. In conductometric experiments, the final concentration of amylose was kept fixed at 624 μM (1.5%). All of the experimental solutions were kept in well stoppered, Corning-glass bottles; each of the bottles and solutions was thoroughly bubbled with nitrogen gas. After equilibration, the solutions were removed from each container and very quickly transferred to wide test-tubes kept immersed in the thermostatic bath, and having an arrangement for continuous passage of nitrogen gas. Resistances of the solutions were measured under the conditions described.

Equilibrium-dialysis experiments. — In equilibrium-dialysis experiments, a fixed amount (8 mL) of 3.8% polymer solution was placed inside the dialysis bag, and the alkali solutions were varied outside, maintaining the same range of concentration and the same total volume (total, inside and outside the dialysis bag) as used in the conductometric experiments. Controls contained the same volume of pure water, instead of polymer solution, inside the dialysis bags. After equilibration (4–6 h), the concentrations of the outer solutions of the controls and the samples were determined by measuring the conductance. A loss in conductance of the sample solutions relative to the controls was observed. Because the solutions of the bases were dilute, a linear, conductance–concentration relationship was assumed, and the concentrations of free base were evaluated from a least-squares, calibration curve constructed from the controls. The results of the equilibrium-dialysis experiments agreed fairly well with the results of the direct-conductance method. Both methods were adopted for study of NaOH binding. For KOH and $Ca(OH)_2$ interactions, only the direct-conductance method was used.

Viscosity experiments. — The viscosity experiments were performed at 30°. Here, the polysaccharide concentration was varied between 3.8 and 23.0 mg/mL, *i.e.*, above and below the concentrations used for the conductometric experiments. The media used were 5mM and 0.05M aqueous NaOH and NH_4OH solutions.

RESULTS

Conductance and viscosity of amylose–strong base mixtures. — Like simple carbohydrate–salt and carbohydrate–base systems[11–13], the conductance of NaOH, KOH, and $Ca(OH)_2$ decreased in the presence of amylose (see Fig. 1). After equilibrium was attained, the conductance remained unchanged for a long period of time. Titration of the bases with standard oxalic acid solution did not show any consumption of them by the amylose, indicating weak, physical (adsorption type) binding of them with the amylose chain. It is known[19] that the conductance of an electrolyte may be lessened in a solution containing macromolecules and suspensoids, due to higher resistance to ion migration on account of the increased viscosity of the medium. This must be taken into account prior to quantitizing the loss of ions through com-

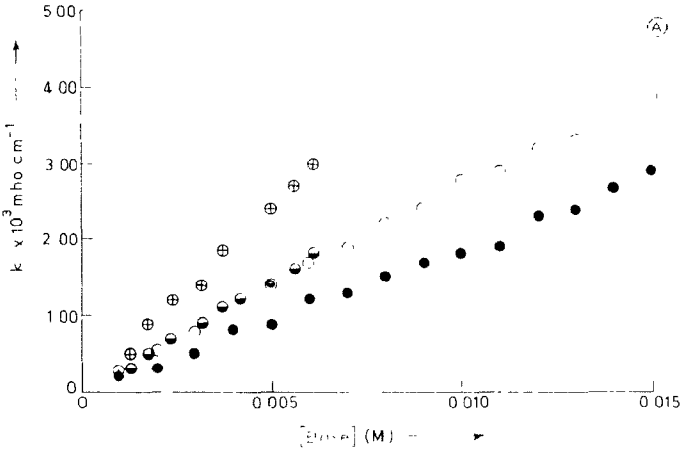

Fig. 1. Variation of specific conductance of NaOH and $Ca(OH)_2$ at different concentrations in the absence, and presence, of amylose at 303 K; concentration of amylose kept fixed at 1.5%. [Key: open circle, NaOH in absence of amylose; closed circle, NaOH in presence of amylose; circle with cross, $Ca(OH)_2$ in absence of amylose; half-filled circle, $Ca(OH)_2$ in presence of amylose.]

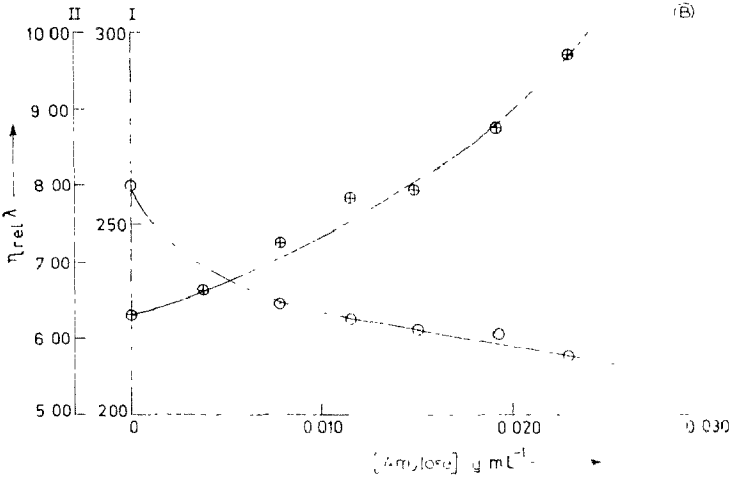

Fig. 2. Variation of Walden product with amylose concentration, for amylose–NaOH and amylose–NH_4OH systems at 303 K. Concentration of NaOH kept fixed at 5mM, and concentration of NH_4OH kept fixed at 0.05M. [Key: open circle, amylose–NaOH system (Scale I); circle with cross, amylose–NH_4OH system (Scale II).]

plexation with the macromolecule (*viz.*, the amylose), and may be tested by calculating the Walden product (the product of the equivalent conductance and the solution viscosity $\lambda\eta$). The implication is that a decrease in conductance is compensated for by an increase in viscosity, yielding $\lambda\eta \approx$ constant. The Walden product for the present amylose–strong base systems did not show any constancy; at a constant concentration of base, it decreased significantly with increasing concentration of

amylose. This decrease was sharp in the beginning (see Fig. 2). The non-constancy of $\lambda\eta$, and its onward decrease meant more diminution in the conductance (through loss of ions) than that demanded by the viscosity increase. Amylose thus binds a strong base, forming a complex.

Quantitative evaluation of the binding process. — The binding of the bases with amylose may be quantitatively estimated by measuring the conductance of the mixture at equilibrium. To minimize the effect of viscosity, a low concentration of amylose was used: the total diminution in conductance was thus taken to be entirely due to the binding of the base with the polymer. An independent evaluation of the binding was also made by adopting the equilibrium-dialysis method. The data were analyzed in the light of Langmuir's binding isotherm. At a constant temperature, assuming n equivalent, non-interacting sites on the amylose chain for the base to bind to, this isotherm, at low coverage, at equilibrium, is represented by Eq. *1*.

$$\frac{\left(\frac{C_b}{C_p}\right)}{\left(\frac{C_b}{C_p}\right)_{max}} = \frac{K_A C_f}{1 + K_A C_f} \tag{1}$$

Now, since

$$\left(\frac{C_b}{C_p}\right)_{max} = n$$

(the maximum number of ligands capable of binding with the polymer), Eq. *1* becomes

$$\left(\frac{C_p}{C_b}\right) = \frac{1}{K_A n\, C_f} + \frac{1}{n}, \tag{2}$$

where C_b is the number of moles of the base bound to C_p moles of amylose, C_f is the moles of free base per liter at equilibrium, and K_A is the intrinsic binding-constant per site. For dilute solutions of a strong base (fully dissociated), the specific conductance k is linearly proportional to concentration; thus,

$$C_f = mk_p, \tag{3}$$

and

$$C_b = m(k_o - k_p), \tag{4}$$

where k_o and k_p are the specific conductance of the base in the presence and absence of amylose, and C_b is the concentration of the base bound to amylose. In terms of Eqs. *3* and *4*,

$$C_f = \frac{k_b}{m}, \text{ and } C_b = \left(\frac{k_o - k_p}{m}\right).$$

Eq. *2* then becomes

$$\frac{m\,C_p}{(k_o - k_p)} = \frac{1}{K_A\,n}\left(\frac{m}{k_p}\right) + \frac{1}{n} \quad (5)$$

Consequently, a plot of

$$\frac{m\,C_p}{(k_o - k_p)} \textit{ versus } \frac{m}{k_p}$$

should yield the values of K_A and n from the slope and the intercept. Representative, least-squares plots are shown in Figs. 3–5. This procedure is equivalent to the double-reciprocal plotting of Klotz *et al.*[26].

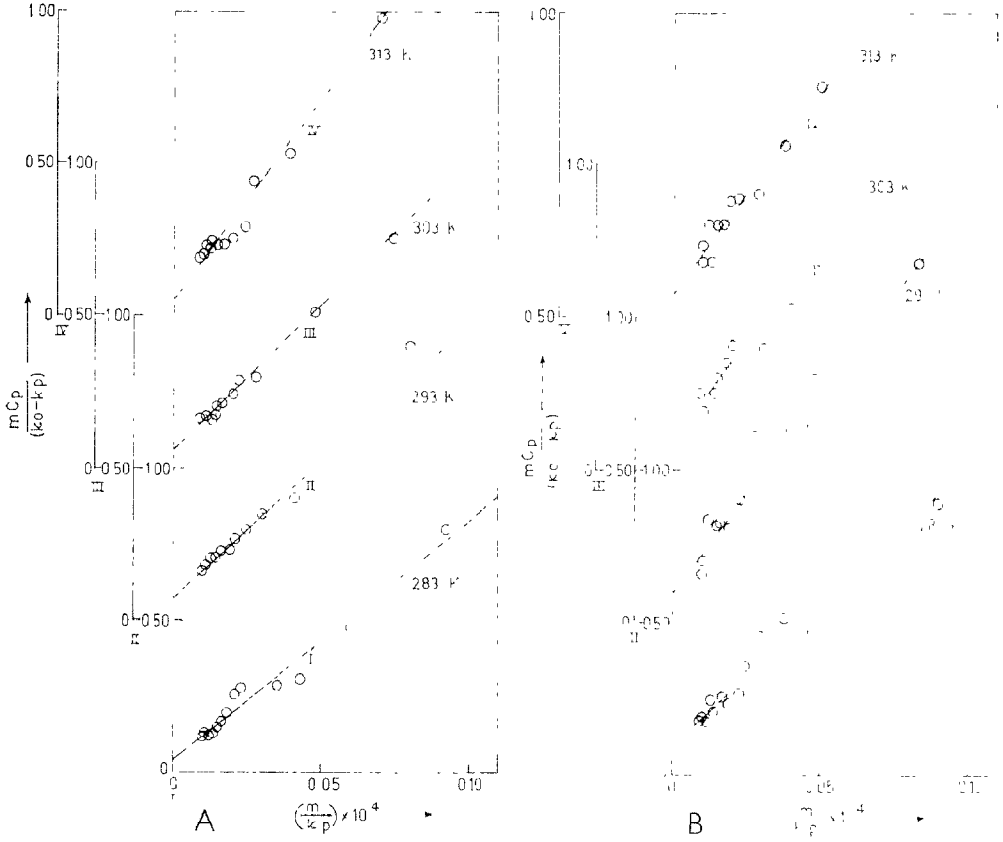

Fig. 3. A. Conductometrically evaluated, rearranged, Langmuirian isotherm plot for amylose–NaOH system at fixed amylose concentration of 1.5%. Time of equilibrium: at 283 K, 4 h; 293 K, 3 h; 303 K, 2 h; and 313 K, 2 h. B. Equilibrium-dialytically evaluated, rearranged, Langmuirian isotherm plot for amylose–NaOH at constant amylose concentration of 1.5%. Time of equilibrium: at 283 K, 6 h; 293 K, 6 h; 303 K, 4 h; and 313 K, 4 h.

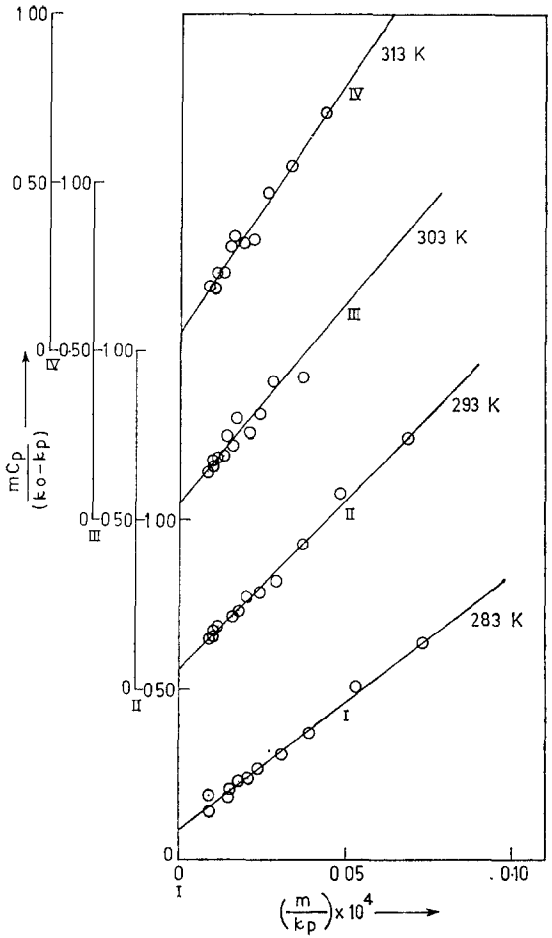

Fig. 4. Conductometrically evaluated, rearranged, Langmuirian isotherm plot for amylose–KOH system at constant amylose concentration of 1.5%. Time of equilibrium: at 283 K, 8 h; 293 K, 6 h; 303 K, 5 h; and 313 K, 3 h.

The values of K_A and n obtained from direct measurements of the conductance of the base in the absence and presence of amylose, as well as those derived from the results of equilibrium-dialysis experiments, are given in Table I. Both sets of data agree well for the NaOH–amylose system, corroborating that the assumptions made were valid. For the other strong bases, KOH and $Ca(OH)_2$, evaluation of K_A and n was made from direct-conductance measurements, the tedious, equilibrium-dialysis method not being used. In Table I, the number, n_o, of D-glucosyl residues required to bind one molecule of the base is also listed. The value of n_o was obtained by dividing n by the total number of D-glucosyl residues constituting the amylose chain.

Conductance and viscosity of amylose–weak base. — It was observed that, unlike the strong-base system, the specific conductance of NH_4OH increased in the

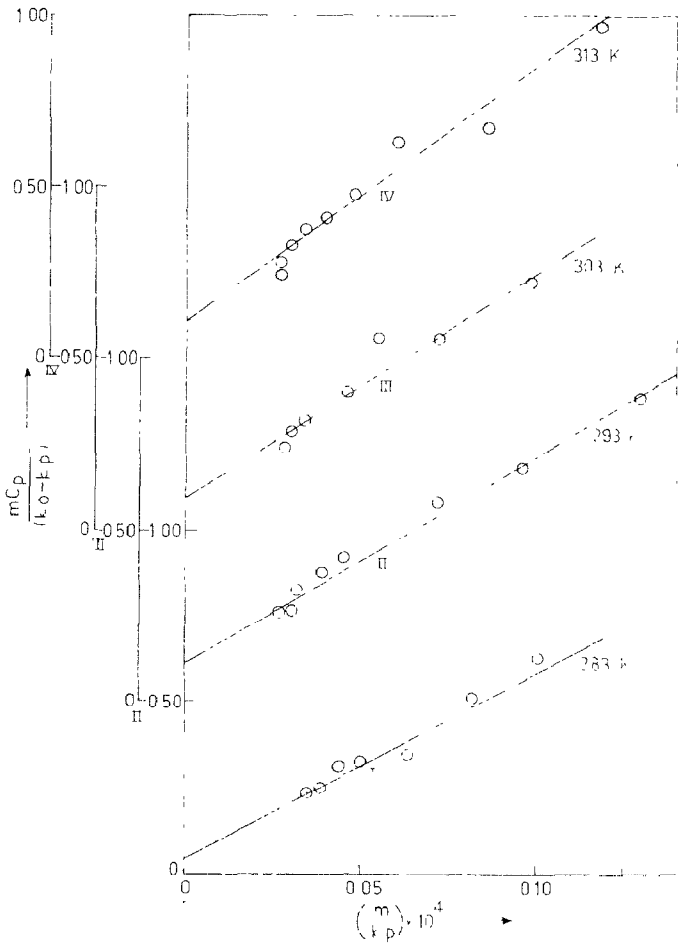

Fig. 5. Conductometrically evaluated, rearranged, Langmuirian isotherm plot for amylose–$Ca(OH)_2$ system. Time of equilibrium: at 283 K, 6 h; 293 K, 6 h; 303 K, 4 h; and 313 K, 3 h.

presence of amylose, and this result was confirmed by repeated, careful experiments. This phenomenon seems to be a general property of polyoxygen-centered non-electrolytes, because both D-glucose and poly(ethylene glycol) increase the conductance of ammonia solution. Such results are exemplified in Fig. 6. As expected, the Walden product ($\lambda\eta$) for the NH_4OH–amylose system showed a reversed trend; it sharply increased, instead of decreasing (see Fig. 2). Such an increase was expected, due to the increased dissociation of NH_4OH by amylose, D-glucose, and poly(ethylene glycol). When compared on the basis of equivalent D-glucosyl residues, the effect of D-glucose was observed to be greater than that of amylose. Quantitative treatment of the conductance data is difficult at this stage. An indirect treatment of simultaneous binding of NH_4OH with amylose and its polymer-affected, increased dissociation-constant has been attempted in the next section.

TABLE I

THERMODYNAMIC PARAMETERS OF AMYLOSE–STRONG BASE SYSTEMS

	Amylose–NaOH								*Amylose–KOH*				*Amylose–$Ca(OH)_2$*			
	Conductometry				*Dialysis*				*Conductometry*				*Conductometry*			
Temp. (K)	283	293	303	313	283	293	303	313	283	293	303	313	283	293	303	313
n	23	14	17	20	10	9	10	12	20	8	10	9	12	16	20	22
n_0	6	11	9	7	16	16	14	12	7	18	15	16	13	9	8	7
K_A	55.2	79.4	62.3	38.4	116.3	81.0	75.3	63.6	93.0	201.9	151.9	148.3	113.2	60.5	43.6	30.6
$-\Delta G°$ (kJ.mol^{-1})	9.5	10.7	10.5	9.6	11.5	10.8	11.0	10.9	10.7	13.0	12.7	13.1	11.2	10.1	9.6	9.0
$-\Delta H°$ (kJ.mol^{-1})	23.0	23.0	23.0	23.0	14.2	14.2	14.2	14.2	16.6	16.6	16.6	16.6	25.9	25.9	25.9	25.9
$-\Delta S°$ (J.mol^{-1}.deg^{-1})	47.7	42.0	41.3	43.0	10.4	11.6	10.6	10.5	20.8	12.3	12.9	11.3	51.9	54.1	53.8	54.2

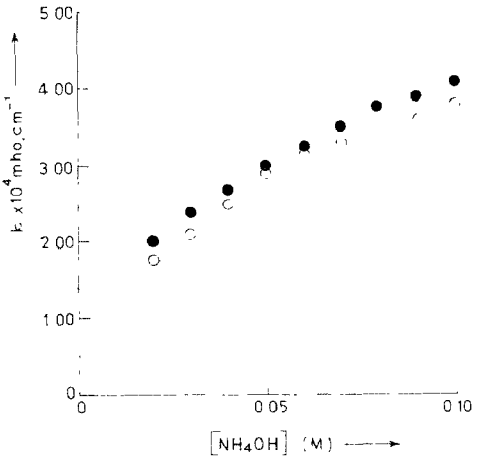

Fig. 6. Concentration-dependent variation of specific conductance of NH_4OH in the presence, and absence, of amylose at 303 K. Concentration of amylose kept constant at 1.5%. [Key: open circle, NH_4OH in the absence of amylose; closed circle, NH_4OH in the presence of amylose.]

DISCUSSION

Parallel to the binding of salts and bases with polyhydroxy compounds[11–13], amylose also binds bases under normal conditions. The binding is moderate, and the order of the equilibrium constant is in the range of 40–110. The number of moles of D-glucosyl residues necessary to associate with 1 mole of base falls in the range of ~10–20. The binding sites are, therefore, scattered, and are expected to be non-cooperative. Very poor fitting of the experimental data with Hill's equation also supported this. It is known from independent, equilibrium and kinetics studies that native amylose exists as a random-coil chain that is transformed into helices on complexation with various ligands, for example, iodine[20,21] and alcohols[22]. The chain is somewhat shortened by this process, and the viscosity of the solution is lessened; some nonaqueous solvents, on the other hand, increase the viscosity of an amylose solution by their presence[21–23]. Bases also increase the viscosity[7–10], and the random-coil chain thus becomes elongated, probably through segmental repulsion that is a consequence of scattered charges on the polymeric chain by way of association of the base with the polymer. With reference to our previous observations, the effective charge of the base-complexed amylose chain is negative. In 0.5M KOH, the intrinsic viscosity $[\eta]$ increases by 11%. This may be the percent increase of the overall radius of gyration of the biopolymer (the observed increase[21] was 8%). The thermodynamics of alkali binding also suggest less rigidity. A small, negative entropy of complexation in the range of 10–50 kJ.mol^{-1}.deg^{-1} has been obtained. The temperature-dependence of K_A yielded an enthalpy in the range of −12 to −25 kJ.mol^{-1} for this binding process. The binding of base is, thus, partly entropy-controlled. Binding of iodine to amylose[21] is associated with a negative enthalpy (~70 kJ.mol^{-1})

and a negative entropy-change ($\sim$165 J.mol^{-1}.deg^{-1}). The process thus favors an ordered situation; *i.e.*, a coil to helix transition associated with structured water around the helix. The comparatively much smaller negative entropy in the present case favors randomness for the complexed-amylose coil, and less structured-water around the chain. Greater solvent-affinity of the base-bound amylose indicates this, as reflected in the increased intrinsic viscosity of the polymer in an alkaline medium. The iodine complex is much less hydrophilic, and is apt to organize water structure around it[24], resulting in a significant, negative entropy of complexation.

The interaction of a weak base with amylose is peculiar, with regard to the increased specific conductance of NH_4OH in the presence of the polymer. The presence of hydroxyl groups (broadly speaking, oxygen centers) is the cause of such conductance enhancement, because both D-glucose and poly(ethylene glycol) also helped to increase the conductance of an aqueous NH_4OH solution, but how this

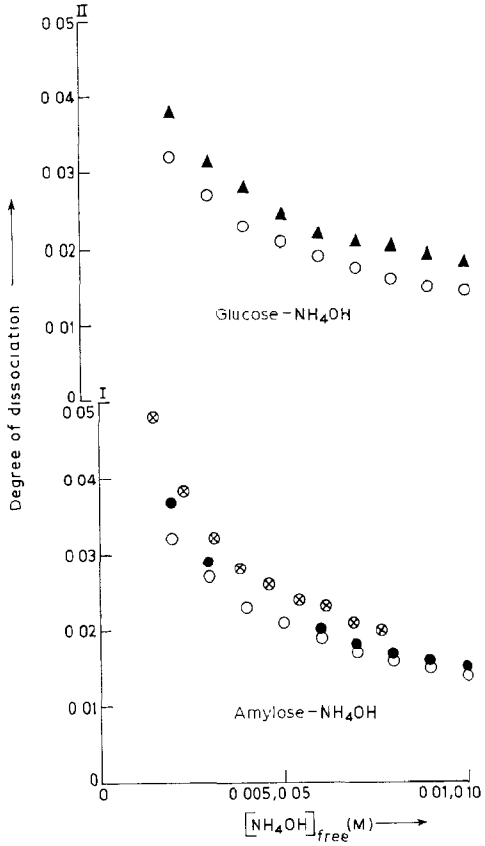

Fig. 7. Concentration-dependent variation of the degree of dissociation of NH_4OH in the presence, and absence, of carbohydrates (1.5% of amylose; 1.6% of D-glucose) at 313 K. [Key: open circle, α in absence of amylose and glucose; closed circle, α in presence of amylose, when no binding was considered; circle with cross, α' in presence of amylose when binding was considered; closed triangle, α in presence of D-glucose, when no binding was considered.]

happens is not yet clearly understood. Several mechanisms appear possible. One model could be the binding of part of the base to amylose and enhancement of the dissociation of the rest, thus imparting increased conductance through more ion-production. The influence of a non-electrolyte on the dissociation of weak acid is documented in the literature. Urea has been observed to decrease the pK_A of acetic acid[25]; urea is a very weak base, and it affects the dissociation of a weak acid. The present system is a corollary to this: the hydroxyl groups of carbohydrates are weakly acidic, and so they may affect the dissociation of a weak base.

In their work with urea, Bull *et al.*[25] calculated the values of the degree of dissociation of acetic acid from the conductance ratios (ratio of the equivalent conductance of a finite concentration of acetic acid to the equivalent conductance of it at infinite dilution, λ/λ_0). We calculated the degree of dissociation (α) of NH_4OH at different concentrations in the absence, and presence, of amylose, by assuming that the base does not bind with the polymer, and that only the dissociation equilibrium is affected by it. In Fig. 7, these values are compared with the apparent degree of dissociation of NH_4OH. Next, an attempt was made to treat the data by considering that amylose simultaneously binds ammonia and affects its dissociation constant. An attempt to evaluate the binding of the base by the equilibrium-dialysis method proved fruitless. Estimation of the loss of NH_4OH (due to binding with the polymer) by titrating with a standard acid was irreproducible, as were measurements of the pH. To process the data, we therefore assumed that the extent of binding of NH_4OH with amylose parallels that of the strong bases, which have almost identical binding-patterns (see Table I). Thus, the fraction of NH_4OH bound to amylose was calculated from the relation between the free and total base observed for NaOH.

$$C_{free} = 0.768\ C_{total} \qquad (6)$$

TABLE II

DEGREES OF DISSOCIATION OF NH_4OH IN THE ABSENCE, AND PRESENCE, OF CARBOHYDRATES AT 303 K WHEN THE CARBOHYDRATES DO NOT BIND NH_4OH

$[NH_4OH]_{total}$ (M)	α_{NH_4OH}	α_{NH_4OH} *in amylose*	α_{NH_4OH} *in* D-*glucose*
0.02	0.032	0.037	0.038
0.03	0.027	0.029	0.031
0.04	0.023	0.033	0.028
0.05	0.021	0.027	0.025
0.06	0.019	0.020	0.022
0.07	0.017	0.018	0.021
0.08	0.016	0.017	0.020
0.09	0.015	0.016	0.019
0.10	0.014	0.015	0.018

TABLE III

DEGREE OF DISSOCIATION OF NH_4OH IN THE PRESENCE OF THE CARBOHYDRATES AT 303 K WHEN THE CARBOHYDRATES BIND NH_4OH

α'_{NH_4OH} *in amylose*	$[NH_4OH]_{free}$ (M)	$[NH_4OH]_{total}$ (M)	$[NH_4OH]_{free}$ (M)	α'_{NH_4OH} *in* D-*glucose*
0.048	0.015	0.02	0.006	0.158
0.038	0.023	0.03	0.010	0.124
0.032	0.037	0.04	0.014	0.096
0.028	0.038	0.05	0.018	0.077
0.026	0.046	0.06	0.026	0.059
0.024	0.054	0.07	0.029	0.057
0.023	0.061	0.08	0.035	0.053
0.021	0.069	0.09	0.041	0.047
0.020	0.077	0.10	0.048	0.042

A knowledge of the concentration of the free base then helped in evaluating the true, equivalent conductance of NH_4OH by the relation,

$$\lambda_{free} = \frac{1000 \times \text{sp. conductance}}{C_{free}} \qquad (7)$$

The ratio of λ_{free} to λ_o then yielded another set of degrees of dissociation, α'. These α' values are also presented in Fig. 7. As expected, $\alpha' > \alpha$ at all concentrations of NH_4OH: these α' values may not actually be true, but they represent a quantitative approach as to the influence of amylose on the dissociation equilibrium of NH_4OH. These calculated values of degree of dissociation are recorded in Tables II and III. In this connection, the α' values of NH_4OH in a D-glucose environment were calculated on the basis of a 1:1 complex between the base and the carbohydrate, and assuming the binding constant to be 30 (the average of the binding constants of D-glucose with strong bases[13]). All of these values (also recorded in Tables II and III) are significantly greater than those obtained in an amylose environment. Stronger interaction of the free reducing sugar (D-glucose) units than of their condensed form (as in amylose) is envisaged.

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