Calorimetric Investigation of Inclusion Complexes of Amylose with Long-Chain Aliphatic Compounds Containing Different Functional Groups

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ABSTRACT: The effects of different functional groups and chain lengths of straight-chain aliphatic compounds on their inclusion complexes with amylose have been investigated by differential scanning calorimetry. For a given functional group, increasing the number of carbons in the aliphatic chain increases the melting temperature T_m of its inclusion complex. For a given chain length, T_m is different for ionic and nonionic functional groups, but the specific nature of these groups has no significant effect. For nonionic ligands of a fixed chain length, two different complexes, whose T_m values differ by approximately 25 °C, may be formed. Either complex may be formed at the exclusion of the other by appropriately selecting the temperature of complex formation. For ionic ligands of a fixed chain length, only one complex can be obtained.

Introduction

It is well established that amylose, the linear component of starch, can form helical inclusion complexes with a variety of compounds in aqueous solution.[1] While the inclusion complex with iodine has been extensively studied and reviewed in the literature,[1-3] inclusion complexes with organic compounds such as fatty acids, alcohols, etc. have received less attention. These latter complexing ligands have been used primarily as a means of separating amylose from the noncomplexing branched component of starch, amylopectin.[4,5]

It was early recognized that steric factors related to the structure of the ligand are of critical importance in complex formation.[6] This was confirmed from X-ray diffraction studies, which revealed that the number of glucose residues per turn of the helix could vary with different ligands.[7-13] However, systematic investigations of how variations in the structure of the ligand affect complexation have not been reported.

In the present work, the complexation of amylose with straight-chain aliphatic compounds, containing between 4 and 18 carbons in the aliphatic chain, and different functional groups on the terminal carbon, have been compared by differential scanning calorimetry (DSC). Although DSC has been used extensively in investigations of other biological macromolecules,[14] its use for the elucidation of starch structure and complexes has only recently gained prominence, primarily due to the work of Donovan.[15]

Experimental Section

Calorimetric measurements were made on a Perkin-Elmer DSC-4 differential scanning calorimeter with Perkin-Elmer large-volume, high-pressure sample cells. The onset and maximum temperatures of the thermal transitions, as well as the heat flow ΔH associated with them, were determined with the standard software supplied by the instrument manufacturer.

All chemicals used as complexing ligands were purchased from Aldrich Chemical Co. and were of the highest purity available (generally 98% or better).

Complexation was studied with starch derived from corn, containing between 0% and 73% amylose, with the remainder being amylopectin. The amylose content of the starch samples was determined by iodometric titration. Since no complex formation was observed with pure amylopectin, and the temperatures of complex formation were independent of the amylose content of the starch, most experiments were carried out with corn starch of 55% amylose content, without isolating pure amylose from the naturally occurring amylose/amylopectin mixtures. Fatty acids naturally present in starch can also form inclusion complexes with amylose. Therefore, all starches were defatted with Me_2SO/methanol prior to use.

In its natural state starch occurs in a highly ordered, water-insoluble state, referred to as granular. When the granular starch is heated in the presence of sufficient water, the starch granule becomes disordered, allowing its constituent linear amylose and branched amylopectin to be dispersed in the water. This irreversible process, known as gelatinization, occurs over specific temperature ranges that are characteristic of the starch source (e.g., corn, potato, rice, etc.). Before any complexation of amylose can occur, the starch must first be gelatinized. Gelatinization was carried out directly in the DSC cells by heating mixtures of starch, water, and ligand (usually 12, 40, and 5 mg, respectively) to 150 °C and holding at that temperature for 10 min. The gelatinized samples were then cooled to 20 °C at the instrument's maximum cooling rate, to allow complex formation to occur, and rescanned from 20 to 150 °C at a rate of 15 °C/min, to note the thermal transitions for disordering of any complexes that may have formed. When the starch was gelatinized for less than 10 min at 150 °C, the gelatinization process was found to be incomplete. On subsequent heating–cooling cycles, which resulted in the disordering and re-forming of the complexes, the amount of complex that formed (as determined by the ΔH values of the transitions) increased on each subsequent cycle for several cycles, eventually reaching a maximum value, which remained constant with further heating–cooling cycles. With gelatinization times greater than 10 min at 150 °C, no increase in ΔH over that obtained in 10 min was observed. As further evidence for the completeness of the gelatinization process under the above conditions, it was found that the transition heat ΔH for disordering 1-decanol/amylose complexes increased linearly with the amylose content of the starch.

Experiments to determine the effects of complex formation temperature T_f on the melting temperatures of the resulting complexes were carried out by reheating the samples described above (still in the sealed DSC sample cells) to 150 °C, to disorder any complexes present, then quickly cooling to the desired T_f (e.g., 100, 80, 60 °C, etc.), and maintaining at that temperature for 15 min. This was followed by quickly cooling to 20 °C and then rescanning at a rate of 15 °C/min to 150 °C to determine the melting temperatures and transition heats of the formed complexes.

Typical thermograms obtained in the disordering of 1-decanol/amylose complexes (formed at different temperatures) are shown in Figure 1, where heat flow is plotted as a function of temperature and endotherms appear as positive peaks. The transition enthalpies ΔH were calculated from the areas under the endotherms in units of cal/g of amylose. The reported melting temperatures T_m of the complexes are the endotherm onset temperatures.

Results and Discussion

DSC has been utilized to investigate the complexation of amylose with a variety of straight-chain aliphatic compounds containing between 4 and 18 carbons, with different functional groups on the terminal carbon. Probably

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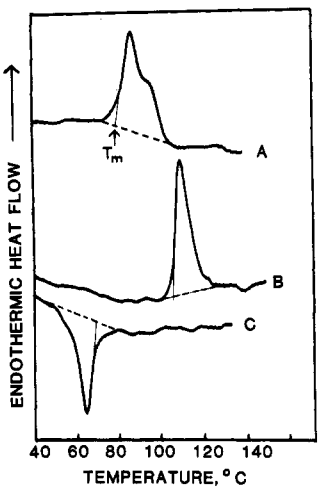

Figure 1. Typical thermograms obtained in disordering 1-decanol/amylose complexes formed by quickly cooling from 150 to 20°C (A) and formed at 80 °C (B). Thermogram C shows the formation of the low-temperature complex at a cooling rate of 15 °C/min.

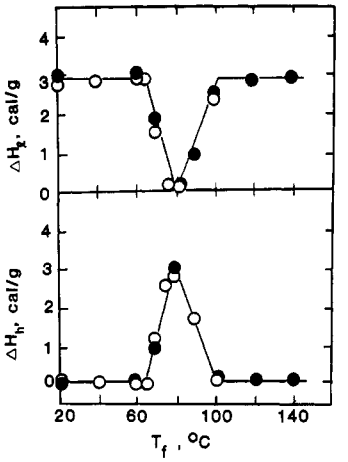

Figure 2. Transition heats for disordering of high-temperature (ΔH_h) and low-temperature (ΔH_l) 1-decanol/amylose complexes as a function of the formation temperature T_f, for decanol/amylose weight ratios C_w of 0.6 (○) and 1.3 (●).

the most noteworthy finding from this investigation is that the same complexing ligand can form two different complexes with amylose, depending on the conditions at which complexation is allowed to occur. This is illustrated in Figure 1, which shows thermograms obtained in the disordering of 1-decanol/amylose complexes formed at different temperatures. When formed by quickly cooling from 150 to 20 °C, a complex with a melting temperature of 82 °C is obtained (thermogram A). When the complex is formed at 80 °C, by quickly cooling from 150 to 80 °C and holding at that temperature for 15 min, a complex with a melting temperature of 106 °C is observed (thermogram B).

Due to the reversibility of the complexation process, repeated heating and cooling cycles allow for the disordering and quantitative re-forming of either of these complexes, or a mixture of both, depending on the formation temperature T_f. When T_f was varied in 10 °C increments between 20 and 120 °C and held at each T_f temperature for 15 min, no complexes other than the ones melting at 82 or 106 °C could be observed. These will respectively be referred to as the low- and high-temperature complexes. That the observed thermal transitions are indeed due to the disordering of inclusion complexes with amylose was verified by performing the same experiments with 1-decanol and the noncomplexing component of starch, amylopectin, for which no thermal transitions were observed.

The enthalpy change ΔH associated with disordering a given complex is a measure of the amount of complex present. Figure 2 shows the variation in ΔH for the high (ΔH_h) and low (ΔH_l) temperature 1-decanol/amylose complexes as a function of formation temperature. Between 20 and 60 °C, only the low-temperature complex is formed. As T_f is increased from 60 to 80 °C, the amount of low-temperature complex formed decreases, disappearing at 80 °C, and the amount of high-temperature complex formed increases, reaching a maximum at 80 °C. With further increases in T_f the trends are reversed; i.e., ΔH_l increases and ΔH_h decreases. When T_f is above 100 °C, again primarily the low-temperature complex is obtained. This behavior is independent of the decanol concentration, with identical trends being observed for decanol to amylose weight ratios, C_w, of 0.6 and 1.3. The identical maximum ΔH values of 2.9 cal/g for both samples indicate that in both cases the alcohol concentration is in excess of the stoichiometric amount required for complete complexation of the amylose. In the presence of excess ligand the reaggregation of amylose chains, referred to as retrogradation, appears to be absent, even after several months. However, with less than stoichiometric quantities of the ligand, as when $C_w = 0.15$ and the maximum value of ΔH for disordering the complex is only 1.6 cal/g, after only a few days of storage at room temperature thermal transitions due to disordering of retrograded starch, in addition to those due to melting of the complexes, are observed in the thermograms.

As is illustrated in Figure 2, the maximum ΔH value for disordering either form of the complex (in the presence of excess 1-decanol) is 2.9 cal/g when only one form of the complex is present. Under conditions where a mixture of both complexes is obtained, the sum $\Delta H_h + \Delta H_l$ also equals 2.9 cal/g. This constancy of ΔH suggests that both complexes involve the same stabilizing forces and that the difference between them is entropic. This further leads to the speculation that the high- and low-temperature complexes may both be helical inclusion complexes that differ in the number of glucose units per turn of the helix.

Although primarily structures with six residues per helical turn have been reported for amylose in the presence of a variety of complexing ligands, experimental evidence also supports the existence of an amylose helix with larger dimensions. From X-ray diffraction measurements it has been shown that while the sixfold helix is obtained with linear alcohols, seven- and eightfold helices form to accommodate bulkier ligands, such as branched alcohols[8,9] or α-napthol.[10] Only in a few instances have different helical forms been observed with the same ligand. For Me_2SO[11] and propionic acid[12] either six- or sevenfold helices could be obtained, depending on the amount of water present. Perhaps the high- and low-temperature decanol/amylose complexes also result from a different distribution of water within the helical cavity. Preliminary experiments in our laboratory suggest that this may be the case. The relative proportions of the high- and low-temperature complexes were found to be different from those in Figure 2 when the amount of water was varied, although the melting temperatures and total ΔH of the two complexes were not affected. This possibility is being investigated further.

Formation of the high-temperature complex is a much slower process than that of the low-temperature complex, requiring a minimum of 15 min at the appropriate temperature. Stoichiometric formation of the low-temperature

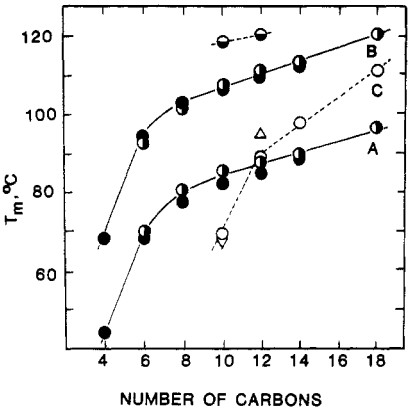

Figure 3. Melting temperatures T_m of amylose inclusion complexes formed with aliphatic compounds of different chain lengths containing OH (●), COOH (◑), COONa (○), OSO_3Na (△), or SO_3Na (▽) groups on the terminal carbon and diols (⊖) with the hydroxyl groups on the first and terminal carbons.

complex, however, occurs instantaneously upon cooling to the appropriate temperature. With cooling rates of 50, 20, 15, 10, or 5 °C/min, where complex formation may be monitored by observing the appearance of an exotherm (curve C, Figure 1), only a single transition with an onset temperature of 67 °C and a ΔH value of −2.9 cal/g is observed. Upon reheating, a single endotherm with an onset temperature of 82 °C is observed. It is interesting to note that the formation temperatures of both complexes are lower than their corresponding melting temperatures, with the difference between T_m and T_f being 24 °C for the high-temperature complex and 15 °C for the low-temperature complex. Similar thermal hysteresis has been observed for sodium palmitate/amylose complexes,[16] investigated by DSC and optical rotation.

That the apparent formation of two different complexes with decanol is not an experimental artifact that can only occur in the closed DSC cell was verified by larger scale preparations of the two complexes. DSC analysis of the resulting products confirmed that the high- and low-temperature complexes could be isolated and that they were stable at ambient conditions for a period of at least several months.

Other straight-chain aliphatic alcohols investigated, containing between 4 and 14 carbons, display the same behavior as decanol; i.e., for each chain length, two different inclusion complexes, with melting temperatures differing by approximately 25 °C, are observed. The melting temperatures of the complexes vary with the aliphatic chain length, as shown in Figure 3. For 1-butanol, complexes with melting temperatures of 42 and 68 °C are obtained. With increasing chain length the melting temperatures of both the high- and low-temperature complexes increase. When there are between four and eight carbons the increase in T_m is sharp, becoming much more gradual with further increases in chain length. For alcohols containing more than 14 carbons, no complex formation is observed. However, this is not surprising since the solubility of alcohols in water decreases with increasing chain length and a prerequisite for the formation of amylose inclusion complexes is at least some solubility of the complexing agent in the aqueous solvent. In each case, low-temperature complex formation was fast, while high-temperature complex formation required 15 min. The optimum temperature for formation of the high-temperature complex for each alcohol was similar to the melting temperature of the low-temperature complex; e.g., with butanol, solely the high-temperature complex was obtained when T_f was 40 °C.

With each of the diols investigated, 1,10-decanediol and 1,12-dodecanediol, only a single complex, which forms quickly on cooling, can be obtained. As shown in Figure 3, the melting temperatures of these complexes are significantly higher than those of either the low- or high-temperature complexes of the corresponding monofunctional alcohols. It is particularly interesting that ΔH for disordering the 1,10-decanediol complex is 5.8 cal/g, which is exactly twice the 2.9 cal/g value observed for the complex with 1-decanol. These data seem to indicate that the hydroxyl group plays an important role in stabilizing the helix.

The effects of different functional groups on complex formation have also been investigated. Carboxylic acid derivatives exhibit the same behavior as the alcohols. Two complexes, whose melting temperatures are nearly identical with those of the corresponding alcohols, are observed for each acid, as shown in Figure 3 (curves A and B). However, for the fully neutralized sodium salts, the melting temperatures of their amylose complexes are quite different from those of their acids. Only a single complex can be formed for each salt, with a melting temperature significantly different from either the high- or low-temperature complex of its nonionic analogue. With other ionic groups on the aliphatic chain, i.e., with the sodium sulfates or sulfonates, the T_m values are identical with those of the carboxylic salts of the same chain length (curve C, Figure 3). (For the eight-carbon derivatives, no complex could be observed with either the sodium carboxylate or sulfonate). In view of the differences in the properties of ionic polymers and their nonionic analogues, the observed differences in the melting temperatures of the ionic and nonionic amylose complexes are not surprising. When formed with an anionic complexing group, the helical inclusion complex can probably be viewed as a pseudopolyelectrolyte. Electrostatic effects are then dominant in dictating the behavior of the complex, with the specific nature of the ionic group being much less significant.

It is interesting that the shape of the T_m vs. carbon number curve is qualitatively the same for the ionic and nonionic complexes, showing the initially sharp increase in T_m followed by a much smaller rate of increase with increasing carbon number. However, the sharpest change in slope appears between 10 and 12 carbons for the salts, and between 6 and 8 carbon atoms for the nonionic alcohols and acids. When curves C and B in Figure 3 are compared, it is apparent that the ionic complexes melt at consistently lower temperatures than their high-temperature nonionic analogues. With increasing chain length, these differences decrease, i.e., for the 10-carbon derivatives the difference is 40 °C, for the 14-carbon derivatives the difference is 20 °C, and for the 18-carbon derivatives the difference is 10 °C. The effect of the ionic group seems to become less significant with increasing chain length, and curves B and C approach each other as the number of carbons increases. For any given chain length the melting temperature of a complex formed with the salt corresponds to one expected for an alcohol or unneutralized acid of shorter chain length. These trends suggest that perhaps the presence of an ionic group results in a shorter portion of the aliphatic chain participating in the amylose inclusion complex than in the nonionic analogue. An investigation of the stoichiometry of complexation with ionic and nonionic materials should provide insight about the validity of this suggestion.

The effect of partial neutralization of the different carboxylic acids is shown in Figure 4, where the melting temperatures of complexes formed with acids containing

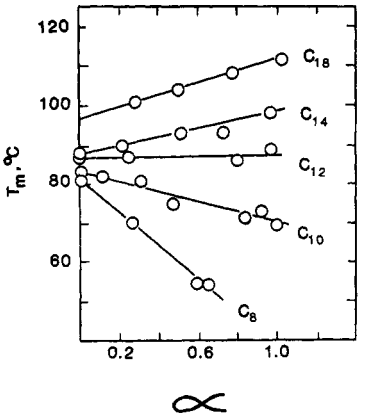

Figure 4. Effect of fractional neutralization α of carboxylic acids of different chain lengths on the melting temperatures T_m of their amylose complexes.

between 8 and 18 carbons in the aliphatic chain have been plotted as a function of their degree of neutralization α. Both high- and low-temperature complexes are observed when α is less than 0.3, and only a single complex is observed for $\alpha > 0.3$. However, melting temperatures for only the low-temperature complexes are plotted in Figure 4. For acids containing more than 12 carbons in the aliphatic chain, T_m increases linearly with α, for 12 carbons, T_m is independent of α, and for less than 10 carbons, T_m decreases with α. although at the present time it is difficult to postulate a reason for these trends, it is certain that they are due to the changing ionic character of the complexing agent with changing α and not due to differences in the helical structure of amylose as a result of the changing hydrogen ion concentration at the different degrees of neutralization. This was concluded by observing the complexation of amylose with nonionic 1-decanol in solutions buffered at pH 4, 7, and 10. At each pH, both high- and low-temperature complexes were observed, with melting temperatures identical with those reported in Figure 3 for unbuffered aqueous solutions of 1-decanol.

Summary

From DSC experiments it has been shown that the melting temperatures of amylose complexes formed with straight-chain aliphatic compounds vary with the number of carbons in the aliphatic chain. Although large differences between the melting temperatures of ionic and nonionic ligands of a given chain length are observed, the specific nature of the ionic or nonionic group has no significant effect. The ability of a given complexing liquid to form more than one form of the complex with amylose has been demonstrated. Perhaps it is this ability to form different complexes at different temperatures that has sometimes resulted in conflicting conclusions in the literature regarding the structures of some amylose complexes.

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