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Thermodynamics of Cycloamylose–Substrate Complexation

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The thermodynamics of cycloamylose–substrate binding is analyzed in terms of the Nemethy–Scheraga hydrophobic bond picture, and the analysis points to a substantial contribution from van der Waals–London dispersion forces interactions. In addition, the origins of the rather "unusual" entropy term associated with cycloamylose–substrate binding is assigned to rotational restrictions imposed on the cycloamylose cavity on substrate complexation

INTRODUCTION

The cycloamyloses, a group of cyclic oligosaccharides containing from six to eight α-1,4-linked glucose units, offer an excellent opportunity to study some of the forces responsible for enzyme-substrate-like binding (*1*). They have been shown to form a variety of complexes in aqueous solution in which guest molecules are contained in the cycloamylose's hydrophobic cavity (*2*). The stability of the complex has been shown to be dependent on both the size and charge (*3*) of the guest molecule and on the size of the oligosaccharide's cavity. Although these oligosaccharides have been extensively and successfully developed as active site models (*1*), they have been almost completely neglected as systems for studying the hydrophobic bond.

As a hydrophobic bond model, the cycloamyloses offer a number of important advantages the dissociation constants for a large number of cycloamylose–substrate complexes can be accurately measured with a number of different techniques (*4*), the host–guest relationship can be defined in the solid phase with X-rays (*5*) and in the aqueous phase with ^{1}H and ^{13}C nmr (*2, 4*), conformational changes in the cycloamyloses on complexation can be measured with nmr; and the cavity can be synthetically modified with some facility (*6*). In this paper we apply the Nemethy–Scheraga (*7*) hydrophobic bond theory to thermodynamic data obtained for a variety of cycloamylose–sodium *p*-nitrophenolate complexes to see if the thermodynamic parameters observed for cycloamylose–substrate complexation are in agreement with what the Nemethy–Scheraga theory predicts.

EXPERIMENTAL

Materials. The cycloamyloses and *p*-nitrophenol were obtained from Aldrich Chemical Co. The *p*-nitrophenol was crystallized from chloroform.

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Sample preparation. Stock solutions of the cycloamyloses were made up in sodium phosphate buffer at pH 11.0 ± 0.02, $I = 0.5$. The sodium *p*-nitrophenolate concentration was held at a constant 5.0×10^{-5} *M* and the cyclohexaamylose, dodecakis-2,6-*O*-methylcyclohexaamylose, cycloheptaamylose, and tetradecakis-2,6-*O*-methylcycloheptaamylose concentrations were varied between 0.20 and 10.0 m*M*.

Determination of cycloamylose–substrate binding constants by visible spectroscopy. The change in absorbance of sodium *p*-nitrophenolate was measured as a function of cycloamylose concentration over a wavelength range of 5100–3400 Å using a Cary Model 14 recording spectrophotometer with a thermostated cell compartment. Absorbance values for every 10 Å were recorded on punch cards via a Cary interphase connected to an IBM keypunch.

The data were treated according to a least-squares curve fit for determination of K_{diss}.

Computer analysis of absorbance data. A Fortran program was written to make best possible use of the massive absorbance data available. Rather than single out data from one wavelength for calculation of K_{diss}, the computer made it possible to use data from a whole range of wavelengths (within which the absorbance differences were acceptably large) and to obtain a series of dissociation constants for each run. The advantage in this was twofold: First, it provided a broad sampling of results such that any unwanted wavelength dependence or large fluctuation in results could be detected and dealt with; second, it provided a more meaningful final result, namely, the mean of all acceptable dissociation constants obtained.

The program used entails roughly three sections (this program is available on request):

(i) The data and necessary parameters, i.e., the number of solutions, the cycloamylose–substrate concentrations in each solution, the starting wavelength, the number of cards for each absorbance spectrum (ca. 200 Å per card), and the temperature, are read in. Then the absorbance differences between each solution and the blank are calculated at each wavelength and corrected for baseline differences between the two. Absorbance differences which are too small are not used in subsequent calculations.

(ii) A least-squares curve fit is used to calculate the best possible dissociation constant from the available data. Only the data at wavelengths with four or more acceptable absorbance differences are used in these calculations. Each K_{diss}, once calculated, is written along with the wavelength and number of points used.

(iii) The mean and standard deviations over the set of all dissociation constants are calculated; then, for subsequent use in a van't Hoff plot, both $1/T$ and $\ln(K_{diss})$ are calculated.

Synthesis of tetradecakis-2,6-O-dimethylcycloheptaamylose and dodecakis-2,6-O-dimethylcyclohexaamylose. These compounds were synthesized as described in our earlier work (*8*).

Determination of thermodynamic parameters. The enthalpies and entropies of association were determined using van't Hoff plots. The $\ln \bar{K}_{diss}$ values as measured at 5, 15, 20, 25, 35, and 45 °C were plotted against $1/T$, and a least-squares fit was applied. The slope of this line is $-\Delta H/R$, and the intercept is $\Delta S/R$. The corresponding ΔG^0 values were taken from the best-fitting line for each van't Hoff plot ($\Delta G = -RT\ln K_{eq}$).

RESULTS AND DISCUSSION

Cycloamylose–Substrate Binding Energy Components

There have been several suggestions as to the origins of the cycloamylose–substrate binding energy: van der Waals–London dispersion forces (*1*), release of cycloamylose strain energy (*9*), and release of enthalpy-rich cavity water (*1*).

The importance of the van der Waals–London dispersion forces component in the overall cycloamylose–substrate binding energy is based on a linear relationship between the polarizability of a group of structurally similar substrates and the stability of the complexes which they form in aqueous solution. The strain energy theory, however, is based entirely on observations made in the solid phase; namely, one of the glucose rings has been shown to be orthogonal to the others, generating strain in the cyclic oligosaccharide. This strain, which would be released upon substrate complexation, would provide a driving force for the process.

The high-energy water concept, like the van der Waals–London dispersion forces theory, is based on solution studies. Bender observed that the cycloamylose–substrate complexation was an unusual hydrophobic interaction in that it was associated with a favorable ΔH and an unfavorable negative ΔS term. The unusual ΔH component was assigned to the release of "enthapy-rich" cavity water on cycloamylose–substrate complexation.

Although it seemed likely to us that all of these forces were contributing to the binding energy, in a recent study we were unable to demonstrate (*10*) that either the release of high-energy cavity water or cycloamylose strain energy contribute significantly to the binding of *p*-nitrophenol and sodium *p*-nitrophenolate-like compounds. Although these findings may be representative of only polar substrate–cycloamylose complexes, studies of the sodium *p*-nitrophenolate adducts have a major advantage in that, unlike other cycloamylose complexes, the structure of the sodium *p*-nitrophenolate cycloamylose complexes in aqueous solution has been established using both ^{13}C and ^{1}H nmr. This was accomplished by observing changes in the chemical shift of both the host and guest molecules in the complex relative to the unbound components and by measuring intermolecular nuclear Overhauser effects (*2*) in the complexes. Together the data provided a measure of both the direction and extent to which the substrate penetrates the cavity, thus allowing one to calculate the number of water molecules displaced from the cavity and from the substrate surface on complexation. Without such information, meaningful interpretation of thermodynamic binding data is somewhat tenuous. It is the purpose of this paper to analyze the thermodynamics of cycloamylose–substrate binding in terms of "hydrophobic bond" theory in light of these structural studies.

Entropy of Association

The entropies of association for the sodium *p*-nitrophenolate cyclohexaamylose, dodecakis-2,6-*O*-methylcyclohexaamylose, cycloheptaamylose, and tetradecakis-2,6-*O*-methylcycloheptaamylose complexes are listed in Table 1. The negative values for the cyclohexaamyloses suggest that the entropy of formation is largely associated with a loss in the cycloamylose's mobility and not with the normal increase in solvent entropy associated with hydrophobic bond formation. However, the positive entropies of formation for the cycloheptaamylose complexes suggest there is very little loss in the oligosaccharide mobility on complexation.

Most of the water in the cycloamylose cavity as well as some of the water surrounding the sodium *p*-nitrophenolate guest molecule is stripped away on complex formation. Based on calculations of the number of water molecules contained in the cycloamylose cavities when in aqueous solution (*11*) and on the direction and depth of sodium *p*-nitrophenolate penetration as determined using nmr (*2*, *4*), one can estimate the number of water molecules released to the bulk solvent on complexation (Table 2). With the Nemethy–Scheraga expression, $\Delta S^0_w/\Delta Y^s = 0.67$ eu, it is possible to approximate the entropy changes, ΔS^0_w, associated with the displacement of a particular number of ΔY^s water molecules from between two hydrophobes when the hydrophobes come together (Table 2). It is clear from the fact that these entropies are positive that the ΔS associated with the interaction of the molecules themselves is even more negative than the experimentally determined numbers indicate, which is probably due largely to a loss of rotational freedom in the cycloamylose molecules.

TABLE 1

THERMODYNAMIC PARAMETERS FOR SODIUM *p*-NITROPHENOLATE-CYCLOAMYLOSE COMPLEXES[a]

Cycloamylose	K_{diss} at 25°C (M)	ΔG^0 (kcal/mol)	ΔH (kcal/mol)	ΔS (eu)
Cyclohexaamylose	5.0×10^{-4}	-4.47 ± 0.03	-9.06 ± 0.06	-15.7 ± 0.2
Dodecakis-2,6-*O*-dimethylcyclohexaamylose	1.27×10^{-4}	-5.05 ± 0.04	-10.06 ± 0.08	-16.8 ± 0.3
Cycloheptaamylose	1.59 ± 10^{-3}	-3.82 ± 0.03	-3.79 ± 0.06	$+0.1 \pm 0.2$
Tetradecakis-2,6-*O*-dimethylcycloheptamylose	1.24×10^{-3}	-3.98 ± 0.04	-3.30 ± 0.08	$+2.3 \pm 0.3$

[a] Values are given for 25°C and represent averages for three sets of runs.

Models show that rotation about the glycosidic linkage of cyclohexaamylose is restricted when a large guest molecule such as sodium *p*-nitrophenolate "fills" the cavity and could thus explain the negative entropy of cycloamylose–substrate association. The entropy of complexation would be expected to be less negative for the cycloheptaamylose- than for the cyclohexaamylose–sodium *p*-nitrophenolate complexes because of the larger diameter of the cycloheptaamylose cavity. The rotation about the glycosidic linkages is less restricted by sodium *p*-nitrophenolate penetration of the cycloheptaamylose cavity than of the cyclohexaamylose cavity and would result in a smaller negative entropy term contribution to the overall entropy of complexation. Furthermore, since the substrate penetrates the cycloheptaamylose cavity further than it does the cyclohexaamylose cavity, releasing a larger number of water molecules to the bulk solvent, the penetration will be associated with a larger positive entropy component.

To further verify that these ideas about the differences in the relative contribution of the entropy term to the overall free energy of complexation for the cycloheptaamylose and cyclohexaamylose complexes were correct, we examined the entropy of complexation for the sodium *p*-nitrophenolate, 2,6-*O*-permethylated cycloamylose analogs. The magnitude and direction of the effect of cycloamylose methylation on the entropy of substrate complexation was in keeping with the above entropy arguments for each of the cycloamyloses.

TABLE 2

NEMETHY–SCHERAGA PARAMETERS FOR SODIUM *p*-NITROPHENOLATE–CYCLOAMYLOSE COMPLEXES[a]

Cycloamylose	ΔH_w^{0b}	ΔS_w^{0c}	ΔY^s	ΔF_w^{0b}	$(\frac{1}{2})E_{RW}\Delta Y^{sb}$	$Z_R E_R{}^b$	$\Sigma\Delta F_{rot}$	$\Delta F_{HO}^{0\ b}$	$\Delta F_{obs}{}^b$
Cyclohexaamylose	3.36	14.1	21	−0.966	2.4	−5.25	ΔF_{rot}^2	> -3.75	-4.45 ± 0.6
Dodecakis-2,6-*O*-dimethylcyclohexaamylose	4.32	18.09	27	−1.242	2.7	−6.75	ΔF_{rot}^1	> -5.29	-5.12 ± 0.2
Cycloheptaamylose	6.56	27.4	41	−1.87	6.56	−10.25	ΔF_{rot}^4	> -5.56	-3.84 ± 0.11
Tetradecakis-2,6-*O*-dimethylcycloheptamylose	8.32	34.84	52	−2.392	8.32	−13.0	ΔF_{rot}^3	> -7.07	-3.97 ± 0.11

[a] $\Delta F_{H\emptyset}^0 = \Delta F_w^0 - (\frac{1}{2})E_{RW}\Delta Y^s + Z_R E_R + \Sigma\Delta F_{rot}$.

[b] Values are given in kilocalories per mole at 25°C.

[c] Values are given in entropy units at 25°C.

The difference in the entropy of complexation between the cyclohexaamylose–sodium *p*-nitrophenolate adduct and the dodecakis-2,6-*O*-methylcyclohexaamylose–sodium *p*-nitrophenolate adduct was nearly within experimental error. In the dodecakis-2,6-*O*-methylcyclohexaamylose complexation of sodium *p*-nitrophenolate, the cyclohexaamylose-2-*O*-methyl groups can make two different and opposing contributions to the entropy of formation. The stripping away of solvent molecules from between the 2-*O*-methyls and the substrate will result in a positive entropy contribution, while a negative contribution will arise from rotational restrictions placed on the methyl groups by the substrate. Both of these effects are likely to be small and cancel one another to some extent.

Any rotation of the 2-*O*-methyl groups toward the center of the cyclohexaamylose cavity would be hindered by the presence of the sodium *p*-nitrophenolate guest, resulting in a greater negative entropy of association. However, the movement of the methyl groups is already quite restricted by the neighboring hydroxyls. Consequently, any restrictions placed on the cavity methyls by the substrate are likely to be small. Furthermore, simple calculations show that only an additional five water molecules would be released to the bulk solvent from the methylated cycloamylose relative to the unmodified cyclodextrin.

The positive difference in the entropy of complexation between the cycloheptaamylose and the tetradecakis 2,6-*O*-methylcycloheptaamylose–sodium *p*-nitrophenolate complexes, although small, suggests the importance of the release of cycloamylose cavity water to the bulk solvent.

Because of the increased diameter of the cycloheptaamylose cavity and therefore the increased distance between sodium *p*-nitrophenolate and the 2-*O*-methyl groups, there is no rotational restriction placed on the methyls at all by the guest molecules. However, the stripping of the water molecules from between the methyls and guest molecules, about 11 water moelcules, should result in a positive entropy contribution. Using the previous expression, $\Delta S^0_w/\Delta Y^s = 0.67$ eu, this would mean approximately an additional 7.4 eu. The observed difference, although smaller than this, is in the correct direction, and of reasonable magnitude (Table 1). Finally, regarding any loss of freedom in the substrate, its likely that translational losses are about the same in both the cycloheptaamylose and cyclohexaamylose complexes. Although rotational losses should be greater for the cyclohexaamylose complexes because the cavity is smaller and the substrate fits more tightly, dynamic coupling experiments have shown that aromatic rings rotate freely in the cavity (*11*).

Enthalpy of Association

Water structure plays an integral role in the overall ΔG of formation of the "hydrophobic bond," contributing to both the favorable ΔS of association as well as to the ΔH component. Because of the dependence of this water structure on temperature, the ΔH of hydrophobic bond formation is also temperature dependent, with the result that van't Hoff plots for such associations should be curved and not linear. This dependence of ΔH on temperature for hydrophobic associations has been shown both theoretically and experimentally (*7*). However, it is clear from Fig. 1 that the van't Hoff plots for the various cycloamylose–sodium *p*-nitrophenolate complexes are not curved,

suggesting that the driving forces for cycloamylose–substrate complexations are not simple entropy-controlled hydrophobic bond forces.

The enthalpy contribution from the stripping away of the water molecules between the cycloamylose and substrate molecules on complexation can be approximated from the relationship (*9*) $\Delta H^0_w/\Delta Y^s = 0.16$ kcal, where ΔY^s has the same meaning as previously described (Table 2). The positive sign of the calculated ΔH^0_w clearly indicates that the experimental ΔH of association between the two molecules themselves would be even more negative than determined, by the amount $-|\Delta H^0_w|$. If the water molecules in the cycloamylose cavity were very "enthalpy-rich," a negative ΔH of association would

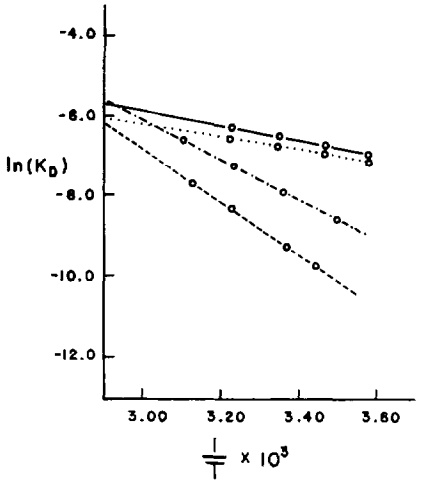

FIG. 1. Van't Hoff plots for sodium *p*-nitrophenolate complexes: —·—·—, cyclohexaamylose; – – –, dodecakis-2,6-*O*-methylcyclohexaamylose; ———, cycloheptaamylose; · · · · ·, tetradecakis-2,6-*O*-methylcycloheptaamylose.

be consistent with the expulsion of this cavity water upon substrate complexation. There are, however, a large number of cycloamylose–substrate binding constants inconsistent with this idea. For example, although sodium isobutyrate, sodium cyclohexanecarboxylate, and sodium *p*-nitrophenolate all have substantially different geometries, they would all displace about the same amount of water from the cyclohexaamylose cavity and thus should have similar binding constants, but they do not. The binding constants are $2.2 \pm 0.3 \times 10^{-1}$, $1.9 \pm 0.3 \times 10^{-2}$, and $5.0 \pm 0.8 \times 10^{-4}$ *M*, respectively (*1*). It is, of course, true that sodium isobutyrate takes up less space than the other substrates, but the remaining spaces are too small for water molecules to occupy.

If, however, London dispersion forces contribute to the binding of the substrate, the negative enthalpy of association ΔH_a, the difference in the ΔH_a values between the cycloheptaamylose and cyclohexaamylose complexes, and the effect of cycloamylose methylation on the ΔH_a values are understandable. Because of the dependence of these forces on the distance between the two interacting species (r^{-6}), any substrate which fits into and binds to cyclohexaamylose is likely to bind more weakly in the cycloheptaamylose cavity owing to greater diameter of the heptamer's cavity and the greater distance between the host and guest molecules. Methylation then of the cyclohexaamylose should result in a more negative ΔH of complexation, while methylation of the cycloheptaamylose should have little effect on the ΔH of complexation (Table 1).

Hydrophobic Bond Analysis

According to the Nemethy–Scheraga picture (*9*), the free energy of formation of the hydrophobic bond $\Delta F^0_{H\emptyset}$ can be separated into two components, ΔF^0_w and ΔF^0_H, a contribution from the change in water structure and a contribution from a change in the state of the hydrophobes; i.e., $\Delta F^0_{H\emptyset} = \Delta F^0_w + \Delta F^0_H$. The ΔF^0_w term can be estimated from the approximation $\Delta F^0_w/\Delta Y^s = -0.046$ kcal/mol, while the ΔF^0_H is broken up into three components: (1/2) $E_{RW}\Delta Y^s$, the energy loss of the water–solute interactions; $Z_R E_R$, the hydrocarbon interaction energy; and $\Sigma\Delta F_{rot}$. The most troublesome aspect of such an approximation is choosing the correct values for the above-defined terms. The ΔY^s and, therefore, the Z_R components ($Z_R = (1/2)\Delta Y^s$) can be easily estimated from our earlier nmr studies (*3*) and our calculations on the number of water molecules contained in the cycloamylose cavities (*2*, *8*) (Table 2).

Sodium *p*-nitrophenolate penetrates the cyclohexaamylose cavity from the wide 2,3-hydroxyl side, nitro-end first, to the extent that the meta protons of the substrate do not enter the cavity beyond a point where they are in contact or nearly in contact with the cyclohexaamylose C-3 hydrogens. However, the same substrate penetrates the cycloheptaamylose cavity completely. This means that approximately 6 and 11 molecules of water are displaced from the respective cavities. Using literature values for the number of water molecules surrounding the benzyl group (*7*), the number of water molecules stripped from the phenolate surface was calculated by assuming that its insertion is approximated by inserting the benzyl group completely into the cycloheptaamylose cavity or partially into the cyclohexaamylose cavity. The results of the calculations are indicated in Table 2.

Although it is impossible to accurately quantitate the ΔF_{rot} component, certain qualitative observations can be made. Nemethy and Scheraga have pointed out that the ΔF_{rot} is controlled by the ΔS_{rot} and that the positive ΔH_{rot} term is very small. Based on our early arguments regarding the ΔS_{rot} losses for the various cycloamylose–substrate complexes, the ΔF_{rot} order for the sodium *p*-nitrophenolate complexes should be dodecakis-2,6-*O*-methylcyclohexaamylose (ΔF^1_{rot}) $\geq$ cyclohexaamylose (ΔF^2_{rot}) > tetradecakis-2,6-*O*-methylcycloheptaamylose (ΔF^3_{rot}) > cycloheptaamylose (ΔF^4_{rot}). The data in Table 2 then establishes that the stability of the cycloamylose–sodium *p*-nitrophenolate complexes should be in the order tetradecakis 2,6-*O*-methylcycloheptaamylose > cycloheptaamylose > cyclohexaamylose $\geq$ dodecakis-2,6-*O*-methylcyclohexaamylose. However, the observed stability of these complexes is quite different from the predicted stability.

Although the ΔY^s and therefore the Z_R terms are certainly accurate, the $\Delta F^0_w/\Delta Y^s = -0.046$ ratio may be somewhat small. This ratio should be the same or very similar for each of the cycloamylose systems considered. However, the E_R terms are likely to differ substantially from one cycloamylose complex to the next. The E_R term, or the energy of interaction between the hydrophobes, is a van der Waals–London dispersion forces interaction and can be described by a Lennard–Jones 6–12 potential function, $U(r) = \varepsilon_0[2(ro/r)^6 - (ro/r)^{12}]$, showing a strong inverse dependence on internuclear distances (*7*). With the cycloheptaamylose system, 7.0 Å in diameter, the distance between the cavity wall and the substrate is greater than in the cyclohexaamylose system, 4.5 Å in diameter; consequently the E_R for cycloheptaamylose should be much smaller than the

E_R for cycloheptaamylose, and this would explain the observed order for the cycloamylose–substrate dissociation constants.

This analysis is consistent with our earlier nmr and binding constant studies of a number of cycloamylose–substrate complexes (*10*).

CONCLUSION

The observed thermodynamic binding parameters show that cycloamylose–substrate binding energy is not associated with a normal hydrophobic interaction i.e., an entropy-controlled phenomenon, but rather an enthalpy-controlled equilibrium. Furthermore, a qualitative analysis of the binding in terms of the Nemethy–Scheraga hydrophobic bond picture suggests that van der Waals–London dispersion forces are largely responsible for the differences in binding energy between the cyclohexa- and cycloheptaamylose complexes. Because of the structural information now accessible through nmr regarding the cycloamylose–substrate complexes in solution, these systems represent an excellent opportunity to examine some of the current theories relating to the "hydrophobic bond" in general.

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