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THE EFFECT OF HYDROPHOBIC–LIPOPHILIC INTERACTIONS ON CHEMICAL REACTIVITY. 11. THE DEPENDENCE OF THE WRAPPING-UP CAPABILITY OF SODIUM CARBOXYMETHYLAMYLOSE ON ITS DEGREE OF SUBSTITUTION

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ABSTRACT

The dependency of the wrapping-up capability of sodium carboxymethylamylose (NaCMA) on its degree of substitution (D.S. = 0·00, 0·12, 0·18, 0·24, 0·29, 0·35 and 0·41) have been studied, using two guest species, iodine and cetyl-trimethylammonium bromide (CTAB). The λ_{max} values of NaCMA-iodine helical inclusion complexes decrease with increasing D.S. values and the amounts of encapsulated iodine by NaCMA as measured by amperometric titrations also decrease with increasing D.S. values. With CTAB as the substrate, the largest number of binding sites, n, and the dissociation constants K_d have been determined by the method of surface tension versus the CTAB concentration plots. The results show that n decreases while K_d increases with increasing D.S. values. All these observations point to the fact that the wrapping-up capability of NaCMA decreases with increasing degrees of substitution. The results are discussed in terms of host–guest and host–solvent hydrophobic–lipophilic interactions as well as intramolecular hydrogen-bonding.

Although it has been known for many years that amylose or its derivative, e.g., sodium carboxymethylamylose (NaCMA), form helical inclusion complexes with various types of compounds,[1] it is only more recently that they have been used as flexible hosts to mimic the induced-fit and catalytic effects of enzymes.[2–4] The conformational, encapsulating and catalytic behaviors of amylose have been studied mainly in Me_2SO—H_2O mixtures with different volume fractions (ϕ) of the organic component,[4,5] whereas similar behaviors of NaCMA have been studied in aqueous solutions.[2] Hui, Gu and Jiang have established that NaCMA has a remarkable catalytic effect on the hydrolysis of two esters of *N*-alkyl-3-hydroxypyridinium iodides with long hydrocarbon chains. However, the NaCMA they used had only a fixed degree of substitution (D.S. = the number of carboxylate groups per glucose residue = 0·41) of the $-OH$ groups by the carboxylate groups, ($-OCH_2CO_2^-$, Na^+) which were believed to be the catalyzing groups, and it was not possible to speculate, let alone predict, whether an increase in D.S. would increase or decrease the catalytic efficiency of the corresponding NaCMA. There appeared to be two conflicting factors which might affect the catalytic efficiency: (1) if the carboxylate groups were truly the catalyzing groups, then an increased D.S. might bring about a higher catalytic efficiency. (2) An increased D.S. might

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lead to a decreased wrapping-up capability of the corresponding NaCMA and thus reduce its catalytic efficiency, since it had already been shown that helical inclusion complex formation was a prerequisite for catalysis.[2,4] Therefore, in order to address the complicated problem posed above, we have to answer the two interrelated questions independently. (1) Do the carboxylate groups actually catalyze the hydrolysis of esters? (2) How do the carboxylate groups affect the stability of the inclusion complexes? Certainly, the second question is of interest and importance in its own right, and the present work is an attempt to answer this question. A companion paper will try to tackle the first question.[6]

We have first prepared NaCMAs with graded degrees of substitution, i.e., D.S. = 0·00, 0·12, 0·18, 0·24, 0·29, 0·35, 0·41. Their encapsulating or wrapping-up capabilities have been evaluated by determining their abilities to form helical inclusive complexes with two guest species, viz., iodine and cetyl-trimethylammonium bromide (CTAB). The results are discussed in terms of host–guest and host–solvent hydrophobic–lipophilic interactions as well as intramolecular hydrogen-bonding.

EXPERIMENTAL AND RESULTS

Preparation of sodium carboxymethylamylose (NaCMA) with different degrees of substitution

NaCMA was prepared as reported.[2] The D.S. values, i.e. the number of carboxymethyl groups per glucose residue, was regulated by changing the relative amounts of reagents ($ClCH_2CO_2H$ and NaOH) and substrate (amylose). The results are summarized in Table 1.

Table. 1 The dependence of the D.S. of NaCMA on the amounts of $ClCH_2CO_2H$ and NaOH used

amylose (g)	NaOH (g)	$ClCH_2CO_2H$ (g)	D.S.	NaCMA yield (g)	$(M)_n \times 10^{-4}$
100	23	27	0·12	60	5·1
100	28	34	0·18	70	5·1
50	17	10	0·24	40	5·15
100	40	50	0·29	80	5·15
50	25	25	0·35	40	5·2
50	30	30	0·41	45	5·2

The molecular weight ($(M)_n$), D.S. and straight-chain purity of the amyloses used

$(M)_n$: A previously described viscosity method was used to determine the molecular weight.[7] The results are: amylose, $(M)_n = 5{\cdot}0 \times 10^4$, degree of polymerization, D.P. = 309. the molecular weights of NaCMA are given in Table 1.

The D.S. values of NaCMA was determined by conductance measurements.[2]

Determination of straight-chain purity of the amylose used. Pure straight-chain amylose should absorb 20% (weight) of iodine.[2] By the procedure of Larson,[8] it was found that the amylose absorbed 18·4%, thus the straight-chain purity of the amylose used was 92%.

Measurement of the λ_{max} and absorbance (A) of NaCMA-iodine complex

Four milligrams of NaCMA with different D.S.'s was dissolved in 100 ml redistilled water; 15 ml of this solution was introduced to a 25 ml-volumetric flask, then 5 ml of 0·2 N HCl, 2 ml of 0·01 N I_2-KI solution were added to the same flask; finally, the total volume of the aqueous solution was made up to 25 ml. The λ_{max} and absorbance A were measured on a Perkin–Elmer 559 spectrophotometer. The results are shown in Table 2 and Figure 1.

Table 2. The λ_{max} and A values of the NaCMA-iodine complexes for NaCMA with Different D.S. values, 35 °C

D.S.	0·00	0·12	0·18	0·24	0·29	0·35	0·41
λ_{max}	630	613	606	598	592	584	/
A		0·815	0·780	0·735	0·650	0·418	/

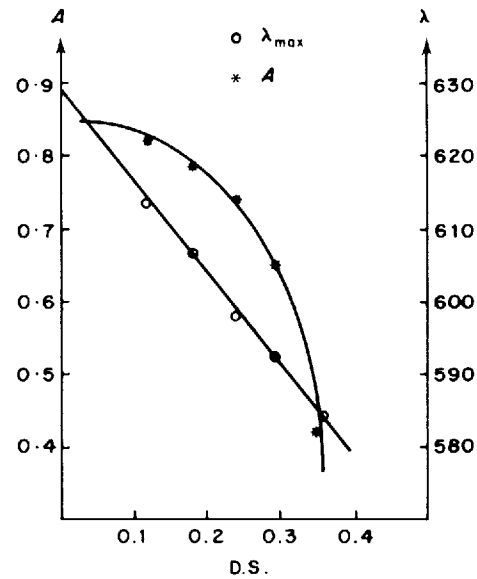

Figure 1. The dependence of λ_{max} and A values on the D.S. of NaCMA

Amperometric determination of the amount of iodine encapsulated by NaCMA with different D.S. values

The amount of iodine complexed with NaCMA was measured by the previously reported method.[2,8] About 30 mg of NaCMA with different D.S.'s was dissolved in 25 ml of 1 N NaOH, then 10 ml of 0·5 N KI was introduced. The solution was diluted to 100 ml with redistilled water and transferred to a 250 ml beaker equipped with a magnetic stirrer. The solution was titrated with 0·005 N KIO_3 at room temperature and the titration curves are shown in Figure 2.

In Figure 2; the current in milliamperes (mA) was plotted against the volume of the 0·005 N aqueous KIO_3 used in the titration. The dashed line represents the titration in the absence of

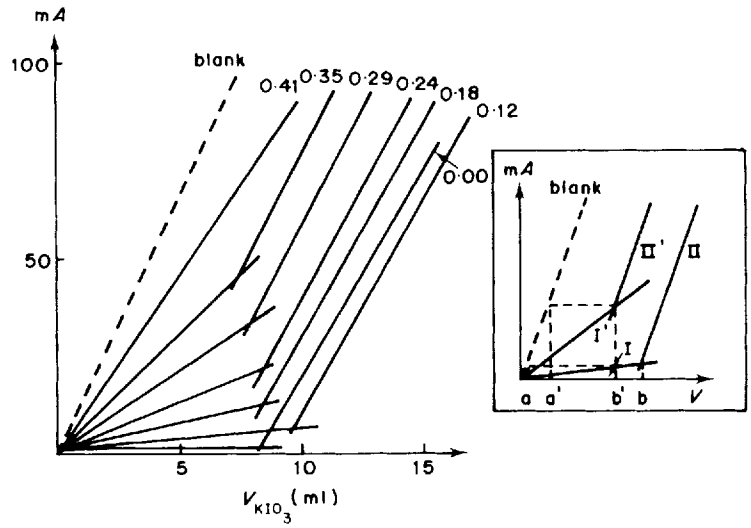

Figure 2. The current (mA) vs. volume (V_{KIO_3}) titration curves for iodine in the presence of NaCMA with different D.S. values

NaCMA. Almost all of the curves consist of two intersecting straight lines, I and II, or I′ and II′ (see inset). The II, II′, ... lines roughly parallel the dashed line for uncomplexed ('free') iodine. The inset also shows how the values of b, b′, ... and a, a′, ... are defined, thus b, b′, ... represent the total amounts of iodine, a, a′ ... represent the amounts of free iodine, and b–a, b′–a′, represent the amounts of encapsulated iodine. If Y% = weight percent of complexed iodine, N = normality of the KIO_3 solution, and W = weight (g) of dried NaCMA, then the percentage of iodine encapsulated by NaCMA with a particular D.S. is equal to N × (b–a) × 127 × 100%/W. The results are listed in Table 3.

Table 3. Dependence of the percentage of complexed iodine (Y%)[a] on the D.S. of NaCMA.

D.S.	0·00	0·12	0·18	0·24	0·29	0·35	0·41
Y%	18·2	16·5	14·7	12·2	10·2	8·0	/

[a]uncertainty: ± 2%.

Determination of the largest number of binding sites, n, and the intrinsic dissociation constants, K_d*

The largest number of binding sites, n, and the intrinsic dissociation constants, K_d*, were obtained from surface tension (σ) versus surfactant concentration curves according to the method developed by Hui and Gu.[9] The surfactant was cetyl-trimethylammonium bromide (CTAB). For NaCMA with different D.S.'s, the solvent was 2% aqueous NaCl; for amylose, which is not soluble in water, the solvent was 0·5 Naq. NaOH. The σ vs. [CTAB] for NaCMA with D.S. = 0·12, 0·18, 0·24, 0·29, 0·35 and 0·41 are plotted in Figure 3.; similar plots for amylose and NaCMA with D.S. = 0·12 in 0·5 N aqueous NaOH are shown in Figure 4.

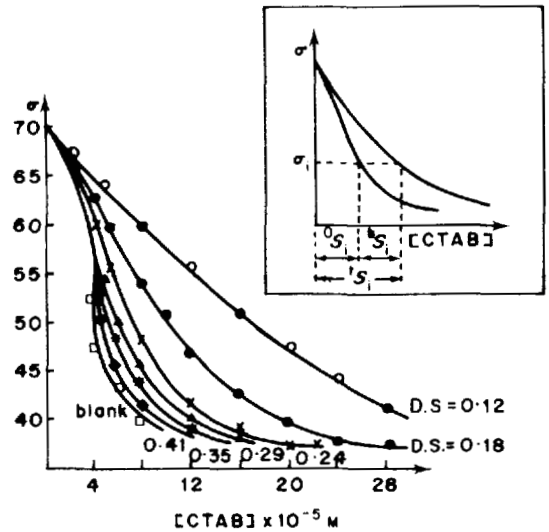

Figure 3. Surface tension vs. [sufactant] plots for NaCMA with different D.S. values. [NaCMA] = $2{\cdot}1 \times 10^{-5}$M

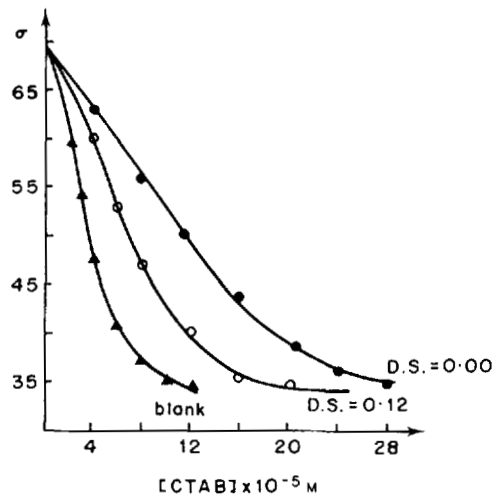

Figure 4. Surface tension vs. [surfactant] plots for amylose, in 0·5 N aq. NaOH. [amylose] = $1{\cdot}3 \times 10^{-5}$M

As shown in Figure 3, the amount of total surfactant (CTAB) in the system is ${}^{t}s_i$, in the absence of added amylose or NaCMA the amount of CTAB in the solution which has a surface tension of say, σ_i, is ${}^{0}s_i$, thus the amount of CTAB which is bound by amylose or NaCMA is ${}^{b}s_i = {}^{t}s_i - {}^{0}s_i$. If Γ_i, is defined as ${}^{b}s_i/c$, where c = molar concentration of amylose or NaCMA, then n and K_d^* can be obtained by application of the Langmuir isotherm: $1/\Gamma_i = 1/n + K_d^*/n \times {}^{0}s_i$, i.e. they can be determined by $1/\Gamma_i$ vs. $1/{}^{0}s_i$ plots, as shown in Figures 5 and 6. The values of n, K_d^* and K_d (K_d^*/n) thus obtained are summarized in Tables 4 and 5.

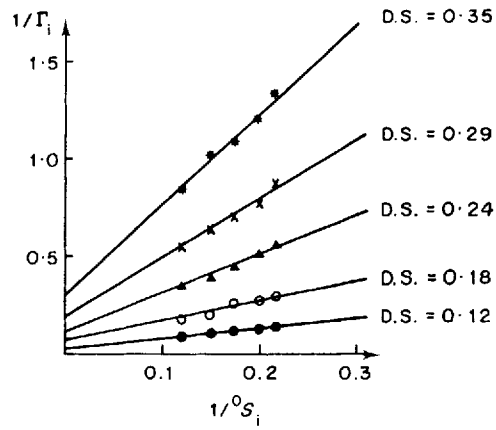

Figure 5. The correlation of $1/\Gamma_i$ with $1/^0s_i$

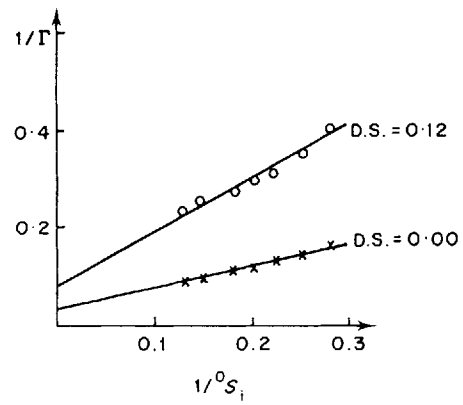

Figure 6. The correlation of $1/\Gamma_i$ with $1/^0s_i$

Table 4. The values of n, K_d*, K_d, for NaCMA with different D.S.'s in 2% NaCl aqueous solution[a]

D.S.	0·12	0·18	0·24	0·29	0·35
n	33·3	15·9	9·1	5·9	3·9
K_d* (10^{-4}M)	1·67	1·75	1·85	1·92	2·00
K_d (10^{-4}M)	0·05	0·11	0·21	0·32	0·50

[a]The uncertainty is 10% for D.S. = 0·35 and within 7% for others.

DISCUSSION

The fact that amylose or NaCMA form blue-to-purple helical inclusion complexes with iodine has been established and studied for years.[10–14] At a fixed concentration of the host species, the amount of iodine bound inside the helical cavities of amylose or NaCMA is directly proportional to the absorbance (A) at the λ_{max} of the inclusion complex, thus the absorbance A is a useful indicator of the relative amounts of encapsulated iodine.[9,12,15] On the other

Table 5. The values of n, K_d^*, and K_d for amylose in 0·5 N NaOH aqueous solution[a]

D.S.	0·00	0·12
n	27·8	11·4
K_d^* (10^{-4}M)	1·19	1·25
K_d (10^{-4}M)	0·04	0·11

[a]the uncertainty is ± 5%.

hand, Bailey and Whelan[16] have established that λ_{max} values increase with the average chain length (degree of polymerization, D.P.) of the host molecules, whereas, Rao and Foster have observed a blue shift (from 600 to 550 nm) for the λ_{max} if amylose is substituted with carboxymethyl groups. The only NaCMA they used had a fixed D.S. of 0·80, and they believed that the blue shift was caused by the shortening of the average length of the helical segments.[17–19]

Our results, using NaCMA with seven D.S. values, are summarized in Table 2 and Figure 1. They clearly show that both λ_{max} and A values decrease with increasing D.S. In fact, the λ_{max} is linearly related to D.S. (λ_{max} = 629·45 – 130·28 D.S., r = 0·9996). Therefore, we can safely conclude that at fixed concentrations of host guest species, both the amount of encapsulated iodine and the average length of the helical segments decrease with the increasing number of carboxylate groups (D.S.), An interesting observation can be made if we compare our data with those of Bailey's.[16] This is done in Table 6, e.g., at D.S. = 0·00, our λ_{max} is 630 nm, this λ_{max} corresponds with that of Bailey's amylose with a D.P. of 315; at D.S. = 0·18, the λ_{max} value (606 nm) corresponds to that of Bailey's amylose with D.P. of 90. In other words, viewed in the perspective of the wrapping-up capabilities of the host molecules, the net effect of increasing the number of carboxylate groups (D.S.) appears to be equivalent to cutting up the host molecules into shorter pieces, even though the acual average chain length of our NaCMAs changed only slightly.

A second methodology for the quantitative evaluation of the amount of encapsulated iodine is the amperometric titration of an aqueous solution of NaCMA and KI with aqueous KIO_3 (cf. experimental). The results from this approach, as shown in Table 3, are in full agreement with the above-mentioned spectral data, i.e., the amount of bound iodine decreases with increasing D.S.

Two other notions of great importance to our study are, the largest number of binding sites, n and the dissociation costant, K_d^* or K_d ($K_d = K_d^*/n$). The values of n and K_d^* can be obtained from σ vs [surfactant] curves followed by the Langmuir-isotherm treatment. (cf. experimental). Our results are summarized in Tables 4 and 5. They show that n decreases while K_d increases with increasing D.S. Evidently, the wrapping-up capability of NaCMA, whether expressed in terms of the number of effective binding sites (helical segments) or the stability of the inclusion complexes, is definitely impaired by the presence of the hydrophilic carboxylate groups.

In aqueous solutions, dissolved NaCMA molecules probably form random coils in which ordered helical segments are connected by disordered segments.[1a,20] If the degree of polymerization of the NaCMA macromolecule is large enough, then at a fixed D.S. value, the number and average length of the helical segments will depend on the pH and ionic strength of the medium.[17,20] Although previous workers used and studied NaCMA with only a single D.S. value (Rao and Foster, 0·8; Brant and Min, 0·55; Hui and Gu, 0·41),[2,17,19] they had

already proposed that subsitution by carboxylate groups would shorten the helical segments. While the aforesaid proposition might appear to be an acceptable rationalization for all our experimental findings, it would be an incomplete and too simplistic rationalisation. Other factors should also be taken into consideration. For instance, the 2- and 3-positions of glucose, particularly the 2-positions, are known to be the main sites of substitution,[7] and it has also been pointed out that in the helical conformations of amylose, intramolecular hydrogen bonding occurs between the 2-position of one glucose residue and the 3-position of a neighboring glucose unit.[7] Thus substitution by the carboxylate groups would reduce the number of these intramolecular hydrogen bonds and consequently impair the capability of amylose to form helices. Furthermore, the steric effects of the bulkier carboxymethyl groups might also somewhat disfavor the formation of helical conformations.[19] However, we believe the crucial factors or aspects of our problem are the hydrophobic and lipophilic interactions, and there are at least three types of interactions which are of importance, namely, (1) host–solvent, (2) guest–solvent, and (3) host–guest interactions. Using only one substrate (CTAB) in aqueous solution, the guest–solvent interactions can be omitted in our discussion. The host–solvent hydrophobic interactions will certainly be reduced by substitution because the anionic carboxylate groups are more hydrophilic than the un-ionized (and intramolecularly H-bonded) hydroxyl groups.[4] The reduced hydrophobic interactions coupled with the reduced number of intramolecular H-bonding sites (*vide supra*) will certainly reduce both the number and the average length of the helical segments of NaCMA, and therefore, the larger the D.S. value, the greater the corresponding reductions. On the other hand, lipophilic interactions between host and guest molecules also contribute to host–guest interactions.[15] These attractive interactions will also be reduced by the negatively charged carboxylate groups, thus larger D.S. values also disfavor the formation of helical inclusion complexes. In short, all our experimental observations, i.e., increased degree of substitution will decrease the λ_{max} of the iodine-complex, decrease the amount of encapsulated iodine, decrease the largest number of binding sites (n) and decrease the stability of the helical inclusion complex of CTAB (increase the K_d values), can all be understood in terms of hydrophobic–lipophilic interactions and intramolecular H-bonding, and perhaps, also of steric effects of the larger carboxylate groups. In a companion paper,[6] we will show that an increase in D.S. will also impair the catalyzing ability of the NaCMA in the hydrolysis of long-chain esters.

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