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Thermochimica Acta 308 (1998) 123–130

thermochimica acta

Calorimetric investigations of the thermodynamic quantities of binding in bio-related molecular systems[1]

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Received 26 August 1996; received in revised form 22 April 1997; accepted 12 May 1997

Abstract

By using a flow microcalorimeter system, enthalpies ΔH^0 of binding or complex formation were determined in aqueous solutions for the following systems; self-association of α-chymotrypsin, bindings of 2-pyrrolidone and poly (*N*-vinyl-2-pyrrolidone) with hydrocarbon cosolutes, and complex formations in cyclodextrin-guest molecular systems. The other thermodynamic parameters, ΔG^0 and ΔS^0, were evaluated by the theoretical relations. A critical comparison of these parameters with the reported ones based on equilibrium methods were given. Significant dissimilarities were shown between two. It was assumed that these calorimetric determined parameters are much inclined to reflecting solution and binding states in aqueous solution. © 1998 Published by Elsevier Science B.V.

1. Introduction

It is a characteristic of calorimetry that generated heats in a calorimeter cell are proportional to the degree of physical and chemical changes irrespective of chemical species. This individuality has a point of advantage to determine accurate quantities brought by structural, binding's and reactional changes included medium effects in order to discuss their mechanisms, and studies by this means have been carried out often by many researchers so far.

In this paper, by using a highly sensitive and substantial mixing flow conduction microcalorimetry system [1,2], the heats of binding have been evaluated in several bio-related molecular systems as a function of the concentration $[X_0]$ of ligand or guest molecule [3,4]. Values of binding standard enthalpy changes ΔH^0 in each system were evaluated and then the other thermodynamic quantities (parameters) ΔG^0 and ΔS^0 were estimated according to some related equations [5,6]. The values ΔH of heat of binding were obtained after correcting of the heats ΔdH of dilution for each solution to be mixing.

A critical comparison of this calorimetric thermodynamic parameters with those based on equilibrium and van't Hoff methods is a problem, so that the two-state equilibrium data reported previously by many authors which consist of one equilibrium constant K were studied to be compared. The calorimetric investigations in this study are accounted for drawing an analogy of thermodynamic contributions between the binding or complexation in cyclodextrin (CD) -guest molecular system and the binding in biopolymer system, for the binding of 2-pyrrolidone (abbreviated to P) and poly (*N*-vinyl-2-pyrrolidone) (PVP) with hydrocarbon cosolutes in solution and for the com-

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[1] Presented at the 14th IUPAC Conference on Chemical Thermodynamics, held in Osaka, Japan, 15–30 August, 1996.

PII S0040-6031(97)00339-0

plexations or inclusions in some CD-guest molecular systems in aqueous media. Because of involving many force contributions to stabilize binding complexes, the parameters are complicated in content. Comparisons of those by equilibrium method with those by calorimetric one which are more faithful with binding processes may be useful for better understanding the mechanisms.

2. Experimental

α-Chymotrypsin, Miles Lot No. 16950, was used in this work after refining by dialyses. CD samples were recrystalized twice those of Wako Chemicals from water solutions. Water was distilled twice with potassium permanganate after ion-exchange treatment. Three kinds of PVP which are varied on average molecular weight M and the source were used. They were $\overline{M}$: 1.0×10^4 from Scientific Polymer Products. (PVP-1), $\overline{M}$: 1.0×10^4 from Aldrich Chemical. (PVP-2) and $\overline{M}$: 1.25×10^4 from Nakarai Chemical. (PVP-3), and dialytic refined in water. Just before preparation of solution, all the highest grade liquid reagents were refined by distillation under normal or reduced pressure. In the case of solid reagents, they were refined by recrystallization from each water solution and used.

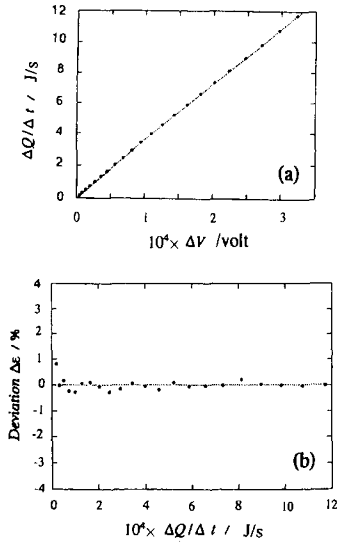

Fig. 1. An example of calibration constants ε.

Calorimetry system in this study is the same conduction flow type one which was reported previously [1,2] and eighteen Melcor thermomodules were connected in series in order to obtain a high sensitivity. In this experiment,

$$\Delta Q/\Delta t = \varepsilon \cdot \Delta V \tag{1}$$

the deviation $\Delta\varepsilon$ of calibration constant ε at 298.15 K was within ±0.22% in water system (Fig. 1). Here, ΔV denotes the electric potential from base line in volt and $\Delta Q/\Delta t$ is heating power which is standard electric heat to be supplied per unit time in J/s. Actual flow rates of two solutions to be mixed were both constant, about the same rate 6 ml/h in this experiment. Generated heat, 10^{-4}–10^{-7} J/s, was calculated from directly proportional relation and determined by using ε value in each solution system.

3. Results and discussion

Calorimetrically determined thermodynamic parameters of bindings or complexations are expressed generally by the following equations for brevity. In these equations, it is beyond that a close relation between concentration and activity is disregarded owing to simplifying and dilute solution systems. A single equilibrium constant K is supposed, and still more, it was applied to the equations on the assumption that solution circumstances or pH and the others are almost not influenced with the progress of bindings or complexations.

$$\Delta dH(\infty) = -\Delta H^0 + \left(\frac{-\Delta H^0}{K}\right)^{1/2}\left(\frac{\Delta dH(\infty)}{m_i}\right)^{1/2} \tag{2}$$

$$Q = \frac{[X]}{1+K[X]}\Delta H^0 K \tag{3}$$

$$[X] = [X_0] - \frac{Q[M_0]}{\Delta H^0} \tag{4}$$

$$\frac{1}{Q} = \frac{1}{\Delta H^0} + \frac{1}{\Delta H^0 K}\cdot\frac{1}{[X_0]-[MX]} \tag{5}$$

$$\Delta G^0 = -RT\ln K = \Delta H^0 - T\Delta S^0$$

Eq. (2) is shown for self-associated system [5] and Eqs. (3)–(5) are shown for binding system [6]. $\Delta dH(\infty)$ is the value at infinite dilution from initial concentration m_i. Here, $Q(=-\Delta H)$ is measured generate heat, K equilibrium constant of the binding system, $[M_0]$ initial concentration of subjected binding molecules, and, $[X_0]$ and $[X]$ initial and final concentrations of ligand molecules, respectively. For Eq. (5) in this study, the middle concentration $[X_0]_{av}$ between initial and final concentrations to be diluted or binded was used in our results. The Eq. (5) is shown by Eq. (6) as an approximation and confirms well to our following calorimetric results within

$$\frac{1}{Q}=\frac{1}{\Delta H^0}+\frac{1}{\Delta H^0 K}\cdot\frac{1}{[X_0]_{av}} \qquad (6)$$

the limits of this experimental concentration.

3.1. A comparison of the temperature dependence of ΔH^0 and ΔS^0 between α-chymotrypsin self-association and α-cyclodextrin-p-aminobenzoic acid inclusion systems.

A provisional study on the conceptual model of protein association was carried out on the thermodynamics of the association processes and on the signs and magnitudes of the thermodynamic parameters in terms of molecular forces and of small molecule interactions [7]. They have expressed that specifically and most importantly the sources of the negative values observed for ΔH^0 and ΔS^0 require delimination. Many contributions to the parameters are existent to be analyzed. In this field, the following empirical key relations have been made at 298 K, neglecting the changes in electrostatic charges, hydrogen bonding and temperature dependent equilibria [8,9]. Unitary changes of ΔC_p^0 of heat capacity and ΔS^0 of entropy are expressed as the sum of hydrophobic, vibrational and conformational contributions [8].

$$\Delta C_p = \Delta C_p(\text{hydro}) + \Delta C_p(\text{vib}) \qquad (7)$$

$$\Delta S_u = \Delta S_u(\text{hydro}) + \Delta S_u(\text{vib}) + \Delta S_u(\text{conform}) \qquad (8)$$

$$\Delta S_u(\text{vib}) = 1.05\Delta C_p(\text{vib})(273\text{–}373\,\text{K}) \qquad (9)$$

$$\Delta C_p(\text{hydro}) = \frac{1.05\Delta C_p - [\Delta S_u - \Delta S_u(\text{conform})]}{1.31} \qquad (10)$$

In this paper, a comparison from above standpoint between α-CD host-guest inclusion system as a biorelated molecule and α-chymotrypsin (α-CT) self-association as a biopolymer material or enzyme systems was carried out if it has no exception to the application. Direct calorimetric determinations of thermodynamic parameters were performed on both the systems in α-CD-p-aminobenzoic acid (p-ABA) inclusion and α-CT dissociation [10]. The results are shown in Figs. 2 and 3. Further, the parameters are shown in Table 1 and the analyzed results from the

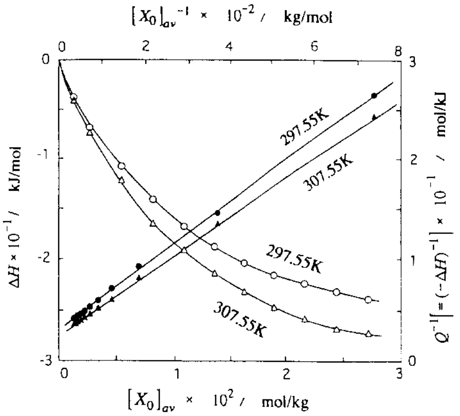

Fig. 2. Molar enthalpies (○,△) of the interaction of α-CD with p-aminobenzoic acid in aq. solution. Values are corrected for enthalpies of dilution and the reciprocal plots (●,▲) according to Eq. (6).

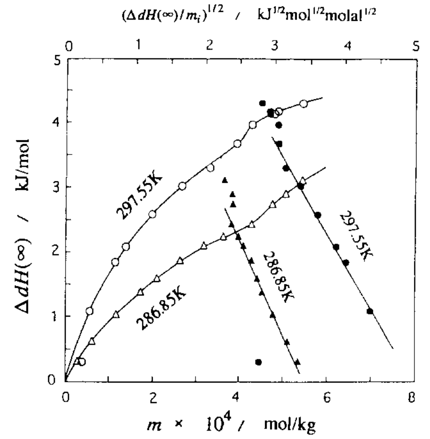

Fig. 3. Molar heats of $\Delta dH(\infty)$ of dilution (○,△) at infinite dilution of α-chymotripsin in 0.05 M phosphate buffer and 0.2 M KCl aq. solution at pH 7.80 as a function of protein concentration, and the plots (●,▲) according to Eq. (2).

Table 1
Thermodynamic parameters for binding or association

System	Temp./K	ΔG^0/(kJ/mol)	ΔH^0/(kJ/mol)	ΔS^0/(kJ/K mol)	ΔC_p^0/(J/K mol)
α-CD-p-ABA	297.55	−10.9	−32.5	−73	−670
	307.55	−11.0	−39.2	−92	
α-CT association	286.85	−16.1	−63.5	−165	−916
	297.55	−19.0	−73.3	−183	

Table 2
Analysis of the data in Table 1 in terms of hydrophobic and vibrational contributions

System	Temp./K	ΔC_p^0 (hydro)/(J/K mol)	ΔC_p^0 (vib)/(J/K mol)	ΔS^0 (hydro)/(J/K mol)	ΔS^0 (vib)/(J/K mol)
α-CD-p-ABA	297.55	−481	−189	+125	−198
	307.55	−467	−203	+121	−213
α-CT association	286.85	−608	−308	+158	−323
	297.55	−594	−322	+155	−338

parameters are listed in Table 2. As shown in the latter Table, both large negative values of ΔC_p^0 and ΔS^0 in both systems are compared in terms of hydrophobic and vibrational contributions, respectively. They are disclosing as follows; the values of ΔC_p^0(hydro), ΔS^0(hydro) and ΔS^0(vibr) in the ΔS^0 value of CD-*p*-ABA system are shown as the comparatively much larger content than those of α-CT association system. On the other hand, it is shown that CT association system has the larger content for ΔC_p^0(vibr) than that for the system.

3.2. Binding interactions of 2-pyrrolidone and poly(N-vinyl-2-pyrrolidone) with hydrocarbon cosolutes in solution

The interactions of PVP or P with hydrocarbon molecules in aqueous media have been of great interest in biochemical studies, because PVP regards as one of the model protein materials [11]. It is known that PVP in aqueous solution displays a strong binding affinity toward dissolved various cosoluted aromatic compounds. Such cosolute bindings by water soluble vinylpolymers display analogous effects. This interactions between both hydrocarbon segments of the polymers and the cosolutes have been explained by the concept of hydrophobic bond. In this field, the change of standard thermodynamic quantities of bindings had been measured by means of binding equilibria, dialysis and isothermal distillation, and their temperature dependences [12]. A critical comparison of these calorimetric quantities and those based on equilibrium and van't Hoff methods are partly referred. The quantities for the systems, P- and PVP- resorcinol (Res) in water and P-2-naphthol (Naph) in methanol were evaluated calorimetrically, at 298.15 K.

Fig. 4 shows calorimetric measured values of P- and PVP- Res aqueous solution systems as a function of $[\mathrm{Res}]_{\mathrm{av}}$ values. In the figure, parentheses show the values to be corrected on the heats ΔdH of dilution for

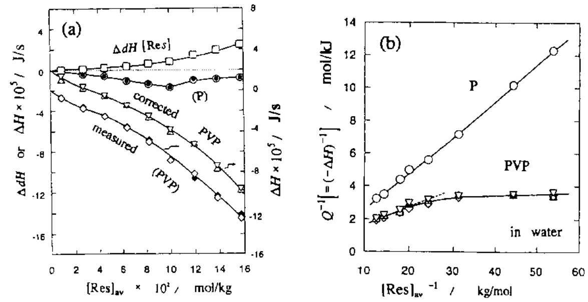

Fig. 4. (a) Calorimetric values of ΔH as a function of molar concentration for P- and PVP-Res systems in water, at 298.15 K. (b) Double reciprocal plots in P- and PVP-Res systems. △: PVP-1, ▽: PVP-2, ◇: PVP-3.

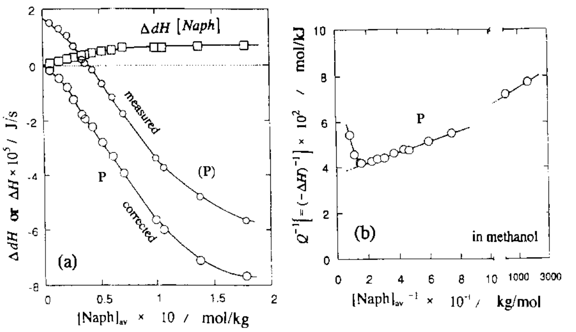

Fig. 5. (a) Calorimetric values of ΔH as a function of molar concentration for P-Naph system in methanol, at 298.15 K. (b) Double reciprocal plots in P-Naph system.

P and PVP aqueous solutions. Endothermic values of ΔdH, for Res aqueous solution are shown as a curve in Fig. 4(a). In the cases of P and PVP solutions, the fixed concentration solutions were diluted and interacted in mixing tubes, respectively. The average values of ΔdH were as follows; P:-7.22×10^{-6}, PVP:-5.42×10^{-6} J/s in this mixing tube. On the bases of these corrected values, double reciprocal plots according to Eq. (6) were shown in Fig. 4(b). Following the same procedure, no parentheses P is shown for the corrected value and the double reciprocal relation from the values on Fig. 5(a) were obtained and shown in Fig. 5(b) for P-Naph methanol solution system. The average value of ΔdH for P in methanol solution was shown as $+1.63\times10^{-5}$ J/s.

In Fig. 4(a and b) the values of ΔH for three kinds of PVP polymers were shown as almost the same and even the double reciprocal plots in Fig. 4(b). It was difficult to make exact distinctions among those three polymers. Because the polymers may cause some segmental cohesions with the progress of cosolute bindings, the double reciprocal plots are not conformable to Eq. (6) as shown in Fig. 4(b). In the case of P-Naph methanol solution system, the double reciprocal plots in Fig. 5(b) swerved from Eq. (7) at more than about 0.1 mol/kg Naph concentration. This phenomenon is considered to be concerned with phase separation or clathrate formation. All evaluated calorimetric thermodynamic quantities in this study are shown in Table 3 and the reported values, even though measured temperature is slightly different, by equilibrium methods are shown graphically and written numerically in Fig. 6 [12].

Table 3
Evaluated thermodynamic quantities of binding, at 298.15 K

System	ΔG^0/ (kJ/mol)	ΔH^0/ (kJ/mol)	$T\Delta S^0$/ (kJ/mol)
P – Res in water	−1.32	−2.66	+1.34
PVP – Res in water	−6.34	−1.34	+4.99
P – Naph in methanol	−12.4	−26.4	−14.0

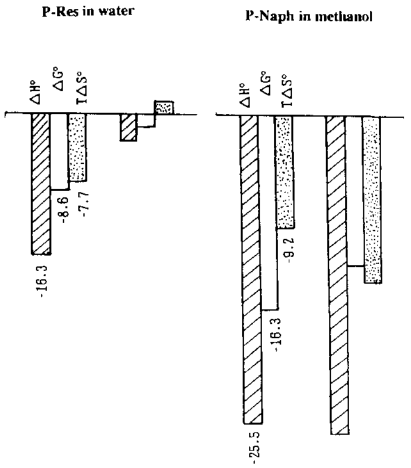

Fig. 6. Thermodynamic parameters by calorimetry (right) as compared with those by equilibrium method (left).

Differences of the evaluated thermodynamic values between calorimetry and equilibrium method are pointed out as a concluding remark. The values are almost the same in methanol solvent system in this study. However, they are different significantly in the case of water solvent system. It is a matter of course that almost parts of this difference are due to the results of hydrophobic bond formation, $\Delta H^0>0$, $\Delta S^0>0$. Calorimetric thermodynamic parameters reflect well stabilizing processes and they are contributing to our thermodynamic description of binding stability phenomenon.

3.3. Complex formations in cyclodextrin-guest molecular systems

Thermodynamic parameters of the guest molecular inclusion or complexation by CD have been measured by various methods: circular dichroism, X-ray crystallography, NMR, spectrophotometry and so on.

Because the mechanisms are complicated, many studies on the elucidation and analysis have been carried on. It seems that there are essential features which are uncertain and the details of inclusion or complexation are still at an experimental stage with the analyses. For the β-CD adduct system, it has been reported previously that a calorimetric study is indicated either no inclusion or complexation takes place or a negligible temperature change in calorimeter cell occurs during the complexation [13]. On the other side, a study by using induced circular dichroism has been reported that the sign and intensity of CD are quite sensitive to the orientation of the guest chromophor in β-CD cavity but the less intensity [14]. A study by spectrophotometry has been also reported for the β-CD adduct systems [15].

In the following calorimetric studies, almost the values of interaction heat were possible to obtain without corrected heats of dilution on guest molecular solutions, $\ll 10^{-7}$ J/s, except 1-propanol aqueous solutions. For that reason, the values do not draw intentionally and only the net values of interaction are shown below. In this study, thermodynamic parameters in several CD complex formation systems were determined from Eq. (7) on the basis of heats of interaction and listed in Table 4. They are shown with the parameters in parentheses which obtained by the other methods. The reported values of parameter except calorimetric method were appreciably different from our results.

Fig. 7 shows the complexation heats of α-CD with 1-pentanol in aqueous medium as a function of the concentration $[X_0]_{av}$ of guest molecular solution. The heats of dilution to be corrected are shown as comparatively large so that the net heats of complex formation were obtained making up an account. Both heat of dilution and complexation are evident from the figure to be discontinuity in the vicinity of 4×10^{-4} mol/kg concentration. Judging from this behavior of heats of dilution, it is regarded to be the change of 1-pentanol aqueous solutions structures. Evaluated ΔH^0 value, about −2 kJ/mol, is not so good agreement with the other reported calorimetric value, −14 kJ/mol [16,17]. The values of $T\Delta S^0$ was evaluated as +18 kJ/mol. The origin of discrepancy in the ΔH^0 value might be attributed to the differences of measured concentration, of activity, difficulty of the correction by the heat of dilution and so on.

Table 4
Evaluated calorimetric thermodynamic parameters of the complexations in cyclodextrin-guest molecular systems, at 298.15 K. The values in parentheses show the non-calorimetric reported results

System	ΔG^0/ (kJ/mol)	ΔH^0/ (kJ/mol)	$T\Delta S^0$/ (kJ/mol)
α-CD-1-Pentanol	−20	−2	+18
	−24	−14	+10 [a]
	(−14)	(−16)	(−5) [b]
α-CD-Benzoic acid	−10	−42	−32
	(−14)	(−42)	(−28) [c]
β-CD-Benzoic acid	−11	−18	+7
	(−14)	(−22)	(−8) [d]
α-CD-p-Aminobenzoic acid	−22	−57	+36
	(−16)	(−44)	(−28) [e]
β-CD-p-Aminobenzoic acid	−23	−18	+5
	(−15)	(−23)	(−8) [f]
α-CD-Benzaldehyde	−16	−313	−297 [g]
	+19	+24	+5 [h]
α-CD-Methylorange	−17	−66	−49
	(−27)	(−26)	(+1) [i]
β-CD-Methylorange	−17	−58	−41
γ-CD-Methylorange	−21	−41	−2

[a] Ref. [16]
[b] Ref. [15]
[c] Ref. [20]
[d] Ref. [20]
[e] Ref. [14]
[f] Ref. [14]
[g] This calorimetric result in conc. range
[h] This calorimetric result in dilute range
[i] Ref. [21]

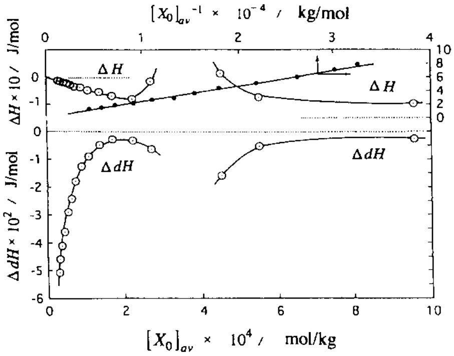

Fig. 7. Values of the heats ΔdH of dilution to be corrected, net complexation enthalpies ΔH and the reciprocal plots in α-CD-1-Pentanol system, at 298.15 K.

However, the tendency of positive ΔS^0 values in α-CD-n-alkanol system was approved. For the binding phenomena, noncalorimetric methods ought to give

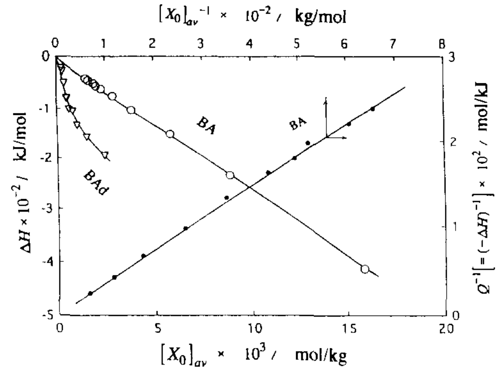

Fig. 8. Calorimetric values of ΔH in α-CD-BA (○) and -BAd (▽), and the double reciprocal plots in BA (●) system at 298.15 K. On the BAd system, see in Fig. 9

the more proper association constant than calorimetric one but the other many contributions include in calorimetric data. Fig. 8 is shown for the α-CD-benzoic acid (BA) system. The data of α-CD-benzaldehyde (BAd) system are plotted in the same figure for ready comparison with the former inclusion system, and the double reciprocal plots are illustrated in the next figure, Fig. 9, because of their concentration range. In Fig. 9, data of the systems: α-CD-p-ABA, -BAd, β-CD-BA and -p-ABA are shown in the same figure. Evaluated thermodynamic parameters are all listed together in Table 4. Recognizable behaviors and the consequent parameters are found in the figure and the table.

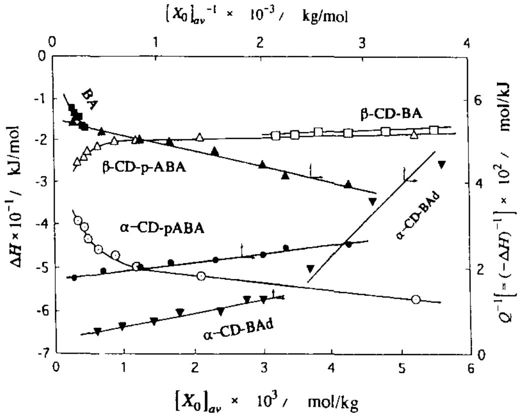

Fig. 9. Calorimetric values of ΔH in α-CD-pABA (○) and BAd (▽), and in β-CD-pABA (△) and -BA (□) systems at 298.15 K. The black-finished symbols are shown on the double reciprocal plots, respectively.

On binding forces of the complex formation in CD-guest molecular systems, many origins which contain the strain energy [18] and the high energy water [19] and so on have been proposed and it seems there are many points which are uncertain up to the present. In general, however, the inclusion processes are affected by hydrophobic and van der Waals interactions for apolar systems as primarily important binding forces [15]. In this calorimetric study, several features of thermodynamic parameters were compared with those by based on equilibrium methods. They are as in the followings. Significant dissimilarities were shown between above two methods, because the parameters contain some electrostatic interactions.

In the case of α-CD-BA system, the parameters determined by calorimetry almost agreed with those by equilibrium methods. On the contraly, α-CD-*p*-ABA, β-CD-BA and -*p*-ABA systems showed obviously as the positive entropy changes. In the case of interacting complex formations, signs of the parameters may change generally as follows, respectively, according to their process of complexation; partial immobilization: $\Delta G>0$, $\Delta S<0$, $\Delta C_p<0$, hydrophobic: $\Delta G<0$, $\Delta \mathrm{H}>0$, $\Delta S>0$, $\Delta C_p<0$, electrostatic: $\Delta G<0$, $\Delta H{\sim}0$, $\Delta S>0$, van der Waals and hydrogen bond: $\Delta G<0$, $\Delta H<0$, $\Delta S<0$, $\Delta C_p<0$ [7]. In the cases of hydrophobic and electrostatic interactions individually, the sign is positive and due to govern by positive entropy. On the case of above system α-CD-BA, which has all negative parameters, the values almost agree with those of equilibrium methods so that it can be considered that there is little effect of electrostatic interaction. It may be explained that above system is complexed largely by van der Waals interaction. On the latter systems, the absolute values of exothermic ΔH^0 decreased and the positive ΔS^0 values were recognized markedly. These phenomena are understanding that hydrophobic interactions play a primary role for the complexations. The systems might be reflected in good fitting conditions to the cavity size and electrostatic field, respectively. All parameters are also obtained for the systems α-, β- and γ-CD-methyl-orange systems (Fig. 10) and they may have a possibility to be complexed the van der Waals force with electrostatic interactions because parameter in these systems differ largely from those in the reported

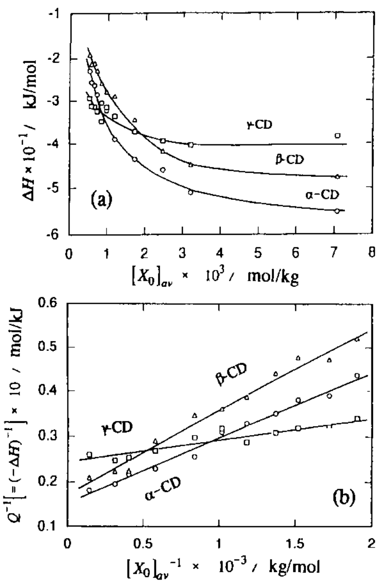

Fig. 10. (a) Calorimetric values of ΔH in α-, β- and γ-CD-methylorange systems at 298.15 K. (b) Double reciprocal plots in the same systems.

values. In the α-CD-BAd system, both ΔH_0 and ΔS^0 values were extremely large negative as the reactive interactions based on active aldehyde group are supposed. On the ability of cyclodextrin to bind several anions have been reported [13]. Preceding these investigations, the values of ΔC_p^0 for the association of α-chymotrypsin and the complex formation of α-cyclodextrin with *p*-aminobenzoic acid were determined to be −916 and −670 J/K mol respectively, and compared to some extent. It was recognized that only the value of ΔC_p^0 (vibr) for the α-chymotripsin system was more contributed than the α-cycrodextrin system, and the values of ΔC_p^0 (hydro), ΔS^0(hydro) and ΔS^0 (vibr) in the α-cyclodextrin system were shown to be more contributed relatively than those in former system. For cycrodextrin complex formation, the importance of hydrophobic binding to the thermodynamic parameters may be comprehended in particular.

From the experimental facts described above, especially in Table 4, we may find the difference between equilibrium and calorimetry methods. The former method is substantially different from the latter one in respect to the direct and accurate binding quantity shown by the equilibrium constant *K*, while the latter calorimetric method is more sensitive and more faithful to the physical and chemical changes than the former one in all over the binding reaction. This reason must bring about the difference of this binding thermodynamic parameters.

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