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Thermodynamics of molecular recognition of nitrogen heterocycles. Part 1. Interaction of imidazole and imidazolium cation with α-cyclodextrin and β-cyclodextrin

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Abstract

The microcalorimetric technique has been used to study the interactions of imidazole and imidazolium cation with α-cyclodextrin and β-cyclodextrin. Binding constants of imidazole to α-cyclodextrin were found to be approximately 6 times greater than those for β-cyclodextrin. The binding constants increased on addition of sodium acetate and sulfate to buffer solutions and decreased strongly in the presence of tetrabutylammonium chloride. There is no reliable evidence for complexation of the imidazolium cation with α-cyclodextrin and β-cyclodextrin.

The standard enthalpy for the complexation of imidazole with α-cyclodextrin has been estimated as $\Delta_r H^{\ominus}_{aq} = -22.4 \pm 0.5$ kJ mol^{-1}.

INTRODUCTION

Chemical simulation of biospecific interactions is of much current interest, both in connection with the synthesis of artificial receptors and enzymes (see, for instance, refs 1–3) and from the standpoint of thermodynamics [4–6]. In particular, molecular recognition of nitrogen heterocycles, which form part of various biological molecules such as nicotinamide coenzymes, nucleotides, amino acids and proteins, attracts considerable attention (see, for instance, refs 7–10). Here we present the first paper in the projected series "Thermodynamics of molecular recognition of nitrogen heterocycles", devoted to the determination of thermodynamic parameters of the interaction of imidazole and imidazolium cation with α- and β-cyclodextrins. Cyclodextrins have been chosen as host molecules since their structure and solution behavior are well known [11,12], and the importance

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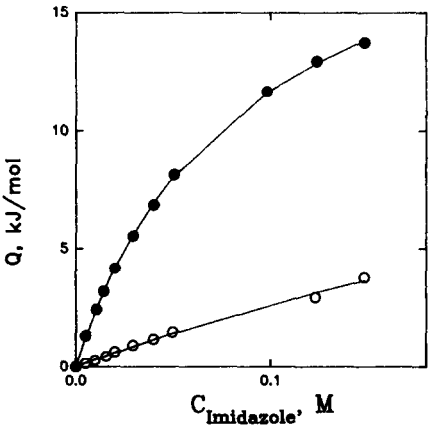

Fig. 1. Heat effects for the interactions of imidazole (0.01 M ammonia buffer solution, pH 9.5) with α-cyclodextrin (●) and β-cyclodextrin (○). The heat effects (Q) are calculated per 1 mole of cyclodextrin.

of thermodynamic parameters for understanding the nature of intermolecular interactions responsible for the host–guest complexation with cyclodextrins has been recognized [13–17].

EXPERIMENTAL

The interaction of imidazole (Sigma; 99%, used without further purification) with α-cyclodextrin and β-cyclodextrin (Aldrich; water content 0.9 and 13%, respectively, used without further purification) was studied in 0.01 M ammonium buffer (pH 9.5), and that of imidazolium cation in 0.5 M acetate buffer (pH 4.0). Experimental microcalorimetric detection was carried out in the flow–mix cell of a Bioactivity Monitor (LKB-2277).

Figure 1 presents the heat effects of the interactions of imidazole with α-cyclodextrin (4.883 mM) and β-cyclodextrin (4.341 mM). Evidently imidazole binds to α-cyclodextrin much more tightly than to β-cyclodextrin. Detailed results of the microcalorimetric experiments are given in Tables 1 and 2, respectively. These data were used for the least-squares estimations of the respective enthalpies and binding constants assuming complexation in the ratio 1 : 1. Calculations were performed with an IBM AT computer and the SigmaPlot program according to the equation

$$W = (W_{max} K'^{c} c_{Im})/(1 + K'^{c} c_{Im}) \quad (1)$$

where W is the heat production (μW) of imidazole interaction with a cyclodextrin, W_{max} is its limit at high imidazole concentration, K'^{c} is the concentration binding constant at a definite composition of buffer solution and c_{Im} is the imidazole concentration.

TABLE 1

Heat effects for the interactions of buffered solutions of imidazole and α-cyclodextrin [a]

Dilution [b]			Mixing [c]	Interaction [d]	
Initial concentration (mmol kg^{-1})	Final concentration (mmol kg^{-1})	Heat production (μW)		Heat production (μW)	Heat effect (kJ^{-1} mol^{-1})
10.6	5.2	0.0	18.4	18.4	1.32
21.5	10.6	0.4	34.1	33.7	2.41
29.2	14.4	0.4	44.7	44.3	3.17
40.5	20.0	0.4	58.2	57.8	4.14
59.8	29.5	1.9	79.3	77.4	5.54
80.8	39.9	0.5	96.1	95.6	6.85
102.2	50.5	−0.4	113.6	114.0	8.16
198.6	98.1	13.4	176.7	163.3	11.69
251.0	123.9	13.1	194.2	181.1	12.97
300.9	148.6	17.5	209.5	192.0	13.75

[a] 0.01 M ammonia, pH 9.5; the heat of mixing of two identical buffer solutions and that of mixing buffer with 4.883 mmol kg^{-1} α-cyclodextrin solution at flow rate 10.30 g h^{-1} was 2.9 μW.

[b] Heat effects of dilution of imidazole solutions.

[c] Heat effects of mixing of imidazole and α-cyclodextrin solutions, corrected for the heat of mixing of two identical buffer solutions (μW).

[d] Heat effects of interaction of imidazole with α-cyclodextrin.

TABLE 2

Heat effects for the interactions of buffered solutions of imidazole and β-cyclodextrin [a]

Dilution [b]			Mixing [c]	Interaction [d]	
Initial concentration (mmol kg^{-1})	Final concentration (mmol kg^{-1})	Heat production (μW)		Heat production (μW)	Heat effect (kJ mol^{-1})
10.3	5.1	0	1.8	1.8	0.14
20.1	9.9	0.4	3.9	3.5	0.28
31.7	15.7	0.4	5.9	5.5	0.44
40.7	20.1	0.4	8.4	8.0	0.64
59.8	29.5	0	11.6	11.6	0.93
81.1	40.0	0	14.6	14.6	1.18
100.5	49.6	−0.3	17.9	18.2	1.47
249.1	123.0	12.7	48.9	36.2	2.92
300.6	148.4	13.7	60.3	46.6	3.75

[a] 0.01 M ammonia, pH 9.5; the heat of mixing of two identical buffer solutions and that of mixing the buffer with 4.341 mmol kg^{-1} β-cyclodextrin solution at a flow rate of 10.30 g h^{-1} was 2.9 μW.

[b] Heat effects of dilution of imidazole solutions.

[c] Heat effects of mixing of imidazole and α-cyclodextrin solutions, corrected for the heat of mixing of two identical buffer solutions (μW).

[d] Heat effects of interaction of imidazole with α-cyclodextrin.

The following reaction enthalpies and binding constants for the complexation of imidazole with α-cyclodextrin and β-cyclodextrin respectively were found: $\Delta_r H^{pH\,9.5}$ (ammonia buffer, 0.01M) = -21.8 ± 0.2 kJ mol^{-1}; $K'^{c} = 11.7 \pm 0.2$ M^{-1}, and $\Delta_r H^{pH\,9.5}$ (ammonia buffer 0.01M) = -16 ± 4 kJ mol^{-1}; $K'^{c} = 1.9 \pm 0.6$ M^{-1}. Theoretical curves calculated with these parameters (solid lines in Fig. 1) show good agreement with the experimental data.

Thus, the weaker binding of imidazole to β-cyclodextrin is completely explicable by the less negative reaction enthalpy. This may be the result of a better fitting of the imidazole molecule into the smaller cavity of α-cyclodextrin.

To obtain more precise values of the reaction enthalpy and the binding constant with α-cyclodextrin as the host, we determined the complexation heats of imidazole with α-cyclodextrin in four independent experimental series.

The parameters calculated from the first series are given above. Calculations from the other three series gave the following results: $\Delta_r H^{pH\,9.5}$ (ammonia buffer, 0.01 M) = -21.3 ± 0.2; -20.6 ± 0.2; -22.9 ± 0.2 (kJ mol^{-1}) and $K'^{c} = 12.4 \pm 0.2$; 12.3 ± 0.2; 11.8 ± 0.2 (M^{-1}). Experimental conditions were as in the first series. The enthalpy value averaged from the four series is equal to -21.7 ± 0.5 kJ mol^{-1} ($K'^{c} = 12.1 \pm 0.2$ M^{-1}), where the error is the mean standard deviation. It should be pointed out that the meaning of errors in the SigmaPlot calculations is not quite clear. Comparison of errors found in the averaging procedure with those from SigmaPlot calculations shows the latter to be 2.5 times smaller than the former for enthalpies and to be the same for binding constants.

In subsequent measurements only one experimental series was used for determination of the thermodynamic parameters at the given conditions, and the respective corrections were made for the calculated errors of enthalpy values. In view of this correction, the enthalpy for the case of β-cyclodextrin may be given without an indication of the precision.

The salt effects on thermodynamic parameters of imidazole complexation with α- and β-cyclodextrins were studied with sodium acetate, sodium sulfate and tetrabutylammonium chloride as the added electrolytes.

The results of microcalorimetric experiments performed in 0.01 M ammonia buffer solution (pH 9.5) in the presence of 0.5 M sodium acetate for α- and β-cyclodextrins are given in Tables 3 and 4, respectively, and in Fig. 2. Calculations made as described above (solid curve in Fig. 2) lead to the following values: $\Delta_r H^{pH\,9.5}$ (ammonia buffer 0.01 M + 0.5 M CH_3COONa) = -22.6 ± 0.5 kJ mol^{-1} ($K'^{c} = 14.0 \pm 0.6$ M^{-1}) and $\Delta_r H^{pH\,9.5}$ (ammonia buffer 0.01 M + 0.5 M CH_3COONa) = -15 kJ mol^{-1} ($K'^{c} = 2.6 \pm 0.3$ M^{-1}). Apparently, the binding constants for both α-cyclodextrin and β-cyclodextrin increase on addition of 0.5 M sodium acetate to the buffer solution, whereas the reaction enthalpies for α-cyclo-

TABLE 3

Heat effects for the interactions of buffered solutions of imidazole and α-cyclodextrin [a]

Dilution [b]			Mixing [c]	Interaction [d]	
Initial concentration (mmol kg^{-1})	Final concentration (mmol kg^{-1})	Heat production (μW)		Heat production (μW)	Heat effect (kJ mol^{-1})
11.8	5.8	0	20.3	20.3	1.46
23.0	11.4	0.7	44.0	43.3	3.11
34.0	16.8	1.2	61.6	60.4	4.34
46.1	22.8	1.0	79.2	78.2	5.62
68.9	34.0	5.5	107.0	101.5	7.29
93.0	45.9	8.0	131.4	123.4	8.87
115.0	56.8	8.7	147.1	138.4	9.94
179.5	88.7	12.0	182.7	170.7	12.27
223.4	110.4	11.2	208.8	197.6	14.20
298.3	147.3	24.2	233.4	209.2	15.03
347.7	171.7	18.0	240.5	222.5	16.00

[a] 0.01 M ammonia + 0.5 M CH_3COONa, pH 9.5; the heat of mixing of two identical buffer solutions and that of mixing of buffer with 4.866 mmol kg^{-1} cyclodextrin solution at a flow rate of 10.30 g h^{-1} was 2.9 μW.

[b] Heat effects of dilution of imidazole solutions.

[c] Heat effects of mixing of imidazole and α-cyclodextrin solutions, corrected for the heat of mixing of two identical buffer solutions (μW).

[d] Heat effects of interaction of imidazole with α-cyclodextrin.

dextrin are equal within experimental error both in the presence and in the absence of the electrolyte.

Likewise, the complexation of imidazole with α-cyclodextrin was studied in the presence of 0.5 M sodium sulfate (an electrolyte possessing stronger salting-out effect than sodium acetate [18]); see Fig. 2. The reaction enthalpy was estimated as $\Delta_r H^{pH\,9.5}$ (ammonia buffer 0.01 M + 0.5 M Na_2SO_4) = -22.9 ± 0.5 kJ mol^{-1} ($K'^{c} = 17.1 \pm 0.2$ M^{-1}).

It is noticeable that the binding constants increase on passing from 0.01 M ammonia buffer solution to the same buffer containing 0.5 M sodium acetate or sulfate. Taking into account that the error in the enthalpy is the mean standard deviation and, therefore, the 95% confidence limits are at least twice as high, the complexation enthalpies appear to be in good agreement in all media employed. (Similar effects were described previously for the inclusion of *p*-nitrophenol into α-cyclodextrin in the presence of inorganic salts [19]).

Thus, the binding enthalpy remains invariant within the experimental errors in the presence of 0.01–0.5 M levels of various electrolytes. It seems reasonable to expect the same value of the enthalpy in pure aqueous solution also. It should be emphasized that at pH 9.5 both imidazole and α-cyclodextrin exist in solution as neutral monomeric molecules. Furthermore, we took account of the heat effects of reactant dilution in our

TABLE 4

Heat effects for the interactions of buffered solutions of imidazole and β-cyclodextrin [a]

Dilution [b]			Mixing [c]	Interaction [d]	
Initial concentration (mmol kg^{-1})	Final concentration (mmol kg^{-1})	Heat production (μW)		Heat production (μW)	Heat effect (kJ mol^{-1})
11.8	5.8	0	2.3	2.3	0.19
23.0	11.4	0.7	6.2	5.5	0.45
34.0	16.8	1.2	9.5	8.3	0.68
46.1	22.8	1.0	11.9	10.9	0.90
68.9	34.0	5.5	20.2	14.7	1.21
93.0	45.9	8.0	28.9	20.9	1.72
115.0	56.8	8.7	32.8	24.1	1.99
179.5	88.7	12.0	48.2	36.2	2.98
223.4	110.4	11.2	52.2	41.0	3.38
298.3	147.3	24.2	75.1	50.9	4.19
347.7	171.7	18.0	77.2	59.2	4.88

[a] 0.01 M ammonia + 0.5 M CH_3COONa, pH 9.5; the heat of mixing of two identical buffer solutions and that of mixing of buffer with 4.245 mmol kg^{-1} cyclodextrin solution at a flow rate of 10.30 g h^{-1} was 2.9 μW.

[b] Heat effects of dilution of imidazole solutions.

[c] Heat effects of mixing of imidazole and α-cyclodextrin solutions, corrected for the heat of mixing of two identical buffer solutions (μW).

[d] Heat effects of interaction of imidazole with α-cyclodextrin.

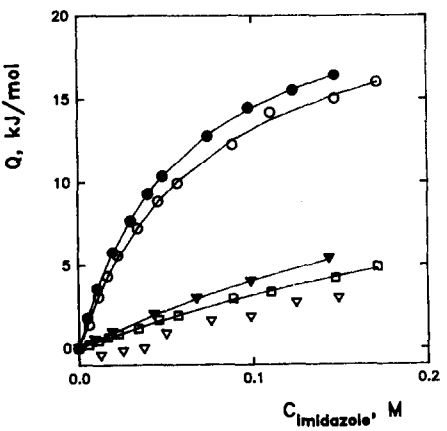

Fig. 2. Heat effects (Q, kJ mol^{-1}) for the interactions of imidazole with α-cyclodextrin (●, ○, ▽, ▼) and β-cyclodextrin (□) in various buffer solutions. ○, □: 0.01 M ammonia buffer solution (pH 9.5) containing 0.5 M sodium acetate; ●: 0.01 M ammonia buffer solution (pH 9.5) containing 0.5 M sodium sulfate; ▼, ▽: 0.01 ammonia buffer solution (pH 9.5) containing 0.1 M and 0.5 M tetrabutylammonium chloride, respectively. The heat effects (Q) are calculated per 1 mole of cyclodextrin.

calculations. All the arguments given above allow us to conclude that the standard enthalpy of the complexation of imidazole with α-cyclodextrin is $\Delta_r H^{\ominus}_{aq} = -22.4 \pm 0.5$ kJ mol^{-1}.

Addition of 0.5 M and even 0.1 M tetrabutylammonium chloride leads to a strong decrease in the imidazole binding to α-cyclodextrin, Fig. 2. Such a reversed effect of this salt may be a result of both its salting-in nature [18] and the competition of the hydrophobic cation and imidazole for the cyclodextrin cavity. The marked effect observed in our case is hardly explicable by simple electrolyte influence. Probably there is competition between butyl chains and imidazole molecules for the binding to α-cyclodextrin. This explanation is to some extent supported by Barone's experiments [14], which showed fairly good binding of n-butanol to α-cyclodextrin ($K'^{c} \approx 100$ M^{-1}).

The complexation of imidazolium cation with α-cyclodextrin was studied in 0.01 M and 0.5 M acetate buffer solutions (pH 4.0). The former buffer was rejected because the heat effects of imidazolium acetate dilution in this medium exceeded 1000–1500 μW, hampering measurement of the effects of heat of complexation owing to high background (for comparison, the heat effects of imidazole dilution (Tables 1–4) did not exceed 20 μW). The heat effects of reactant dilution in 0.5 M acetate buffer were much lower, about 300–350 μW.

The heat effects of mixing of imidazolium acetate buffer solutions (0.5 M, pH 4.0) with the 0.5 M acetate buffer solution and with the latter containing α- or β-cyclodextrin are presented in Figs. 3 and 4, respectively. The dilution heat effect is seen to be practically uninfluenced by the

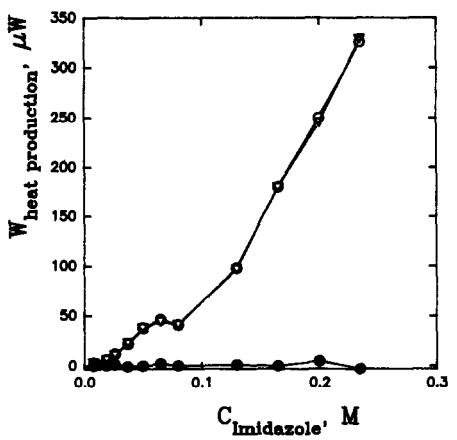

Fig. 3. Heat production (W) on (a) mixing of 0.5 M acetate buffer solution (pH 4) with similar solutions containing increasing concentrations of imidazolium acetate (▽) (W_1); (b) mixing of 0.005 M α-cyclodextrin in 0.5 M acetate buffer solution (pH 4) with similar solutions containing increasing concentrations of imidazolium acetate (○) (W_2); (c) interaction of α-cyclodextrin with imidazolium acetate (●) ($W_3 = W_2 - W_1$).

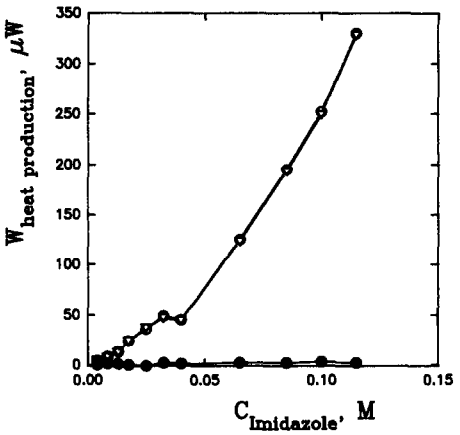

Fig. 4. Heat production (W) on (a) mixing of 0.5 M acetate buffer solution (pH 4) with similar solutions containing increasing concentrations of imidazolium acetate (▽) (W_1); (b) mixing of 0.005 M β-cyclodextrin in 0.5 M acetate buffer solution (pH 4) with similar solutions containing increasing concentrations of imidazolium acetate (○) (W_2); (c) interaction of β-cyclodextrin with imidazolium acetate (●) ($W_3 = W_2 - W_1$).

presence of the cyclodextrins. This may be caused either by the lack of binding or by the binding heat effects being close to zero.

To determine the role of the counter ion in the interaction of imidazolium cation with cyclodextrins, we used imidazolium perchlorate instead of the acetate, because perchlorate anion has been shown to form complexes with cyclodextrins [13]. Indeed, imidazolium perchlorate interacts with α- and β-cyclodextrins with a non-zero heat effect (Fig. 5). Similar

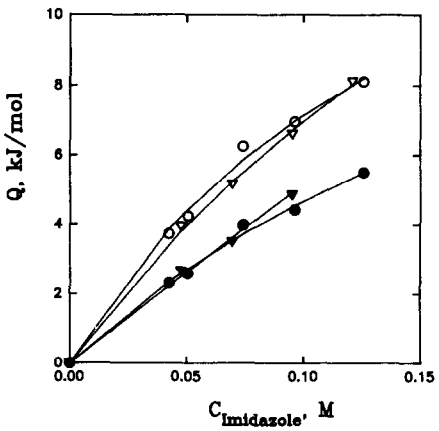

Fig. 5. Heat effects (Q, kJ mol^{-1}) for the interactions imidazolium perchlorate (●, ○) and sodium perchlorate (▼, ▽) (0.5 M acetate buffer solution, pH 4.0) with α-cyclodextrin (○, ▽) and β-cyclodextrin (●, ▼). The heat effects (Q) are calculated per 1 mole of cyclodextrin.

effects were obtained in the case of sodium perchlorate, however (Fig. 5). Hence these effects may be attributed to the binding of perchlorate anion to cyclodextrins, and this approach also did not prove the complexation of imidazolium cation by cyclodextrins.

CONCLUSION

The microcalorimetric study has shown the complexation enthalpy of imidazole with α-cyclodextrin to be $\Delta_r H^{\ominus}_{aq} = -22.4 \pm 0.5$ kJ mol^{-1}. The binding constants of imidazole to α- and β-cyclodextrins have been found to increase on passing from the ammonia buffer solution (pH 9.5) to a similar solution containing 0.5 M CH_3COONa and then to that containing 0.5 M Na_2SO_4. The K'^{c} values for the complexation of imidazole with α-cyclodextrin are 12.1 ± 0.2 M^{-1}, 14.0 ± 0.6 M^{-1} and 17.1 ± 0.2 M^{-1} in the respective media. There is no reliable evidence for the complexation of imidazolium cation by α-cyclodextrin or β-cyclodextrin.

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