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ORIGINAL ARTICLE

Solubility enhancement of kinetin through host–guest interactions with cucurbiturils

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Abstract We explored the use of cucurbiturils to form inclusion complexes to overcome the solubility problems of kinetin, a plant cytokinin. Inclusion complexes between kinetin and Q[7], TMeQ[6] and HMeQ[6] in aqueous solution and in solid state were investigated by phase solubility studies, ^{1}H NMR and IR. The effects of pH and temperature on complex stability were also investigated. Phase solubility studies showed that kinetin solubility increased in a linear fashion as a function of Q[7] and TMeQ[6] concentrations. However, kinetin solubility increased first, then decreased as the HMeQ[6] concentration increased, and the maximum solubility of kinetin was achieved at 4.95 mM in HMeQ[6]. The solubility of kinetin as well as the stability constant of its complex with Q[7] were affected by the pH of the medium. The thermodynamic parameters of the complex formation were also determined, and it showed that the formation of the inclusion complexes between kinetin and Q[7] was enthalpy controlled, suggesting that hydrophobic and van der Waals interactions were the main driving forces. Moreover, we found that the size of the cavity of cucurbituril played an important role in the association process. The formation of inclusion complexes between Q[7], TMeQ[6] and HMeQ[6] with kinetin was confirmed by ^{1}H NMR, and IR spectroscopy showed the presence of inclusion complexes in solid state. Our results demonstrated that the complexation of kinetin with Q[n] could be used to improve the solubility of kinetin in aqueous solution.

Keywords Complexation · Kinetin · Phase solubility methods · Q[7] · TMeQ[6] · HMeQ[6]

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Introduction

Synthetic receptors, such as cyclodextrins (CDs) and calixarenes, have been extensively explored as drug carriers with the aim to enhance the solubility, stability, and bioavailability of drug molecules and to reduce their toxicity [1–3]. Cucurbituril [4] and its homologues [5–7], are a new family of synthetic receptors, which have been widely studied since the structure of cucurbituril (Q[6]) was determined and reported in 1981 [4]. The Q[n] family has common characteristic features, like a hydrophobic cavity and two open hydrophilic portals. It consists of different number of glycoluril units that are linked by pairs of methylene bridges. Cucurbit[7]uril(Q[7]) consists of seven glycoluril units. Tetramethyl cucurbituril (TMeQ[6]) is a modified Q[6] obtained by methyl substitution, partially methyl substituted cucurbit [6]uril (HMeQ[6]) has been prepared by using the monomethyl substituted glycoluril (Fig. 1). This chemical modification significantly increases the solubility of TMeQ[6] and HMeQ[6] over Q[6]. Different numbers of glycoluril units lead to different cavity sizes. The inner diameters of the hydrophobic cavity are approximately 7.2, 6.2 (long axes), and 5.9 Å for Q[7], TMeQ[6] and HMeQ[6], respectively. Thus, the varying

cavity and portal sizes enable the formation of inclusion or exclusion complexes with different organic or inorganic species through a combination of dipole-ion, hydrogen bonding and hydrophobic interactions. These studies in different periods of development of Q[n]s chemistry have been summarized in different reviews [8–10].

Cytokinins are important adenine derivatives that serve as hormones to control many processes in plants. They were discovered as factors that promote cell division in tobacco tissue cultures, and they also have been shown to regulate several other developmental events. Kinetin (Fig. 1) was isolated first in 1955 from autoclaved herring sperm DNA and has been treated as an artificial DNA rearrangement product [11, 12]. It can be easily cleaved off DNA at slightly acidic conditions, due to the acid lability of the glycosylic bond. Generally kinetin promotes growth, induces bud formation and bud development, controls apical dominance, and slows senescence. Although the effects of cytokinins in plants are well known, the mechanisms of their actions are still not well understood. Kinetin and other N6-substituted adenines show strong potential to be used in molecular medicine [13]. However, the solubility of kinetin is a problem. In cold water, ethanol, and methanol, the solubility of kinetin is very low. Even though its solubility increases as pH decreases below pK_1 (3.78) or increases above pK_2 (9.79), it is practically insoluble in distilled water [11, 12]. The low aqueous solubility of kinetin hinders its biological applications. Some cucurbit[n]urils have been used as complexing agents to increase the aqueous solubility and stability of poorly water-soluble drugs [14]**.** In the present report, we described the preparation and characterization of kinetin inclusion complexes formed with Q[7] and modified cucurbiturils (TMeQ[6] and HMeQ[6]) and their aqueous solubility. Both ^{1}H-NMR and IR spectroscopy demonstrated the formation of inclusion complexes.

Experimental

Materials

Kinetin was obtained from Sigma and used without further purification. Cucurbit[n]urils (Q[7], TMeQ[6] and HMeQ[6]) were prepared and purified according to the published methods [5, 15, 16]. All other reagents were of analytical grade and were used as received.

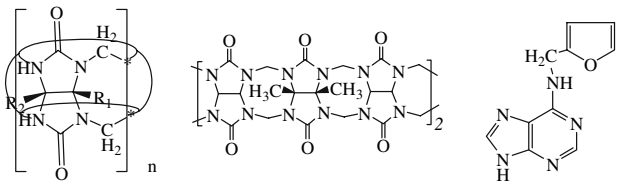

Fig. 1 Structures of cucurbit[n]urils and kinetin

pH solubility profiles

The same amounts of kinetin, which were in large excess of the amount to reach saturation, were added to 50 mL of water. The pH was adjusted to various values at 20 °C with HCl and NaOH. The solutions were thermostatically shaken, allowed to settle, and filtered. The absorbance of the clear solution was then measured at 267 nm. The concentration of each solution was determined using appropriate calibration curves.

Phase solubility studies

Phase solubility was measured according to the method of Higuchi and Connors [17]. Briefly, excess amounts of kinetin were added to aqueous solutions containing various concentrations of Q[7], TMeQ[6] and HMeQ[6] (0–16.0 mM). Samples were maintained at 20 °C, vibrated for 24 h, and allowed to stay for 7 days until equilibrium was reached. Afterwards, samples were filtered (0.45 μm) and appropriately diluted with H_2O. The absorbances at 267nm were measured on an Agilent 8453 spectrophotometer. The kinetin calibration curve was generated at pH 9.40. Apparent 1:1 stability constants (K) were determined from the initial part of the straight-line of the phase solubility diagrams:

$$K = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where (S_0) is the free kinetin aqueous solubility. The molar ratios of the complexes were determined by UV absorbance at 267 nm. Each experiment was conducted in triplicate.

Gibbs and Van't Hoff equations were used to estimate the thermodynamic parameters ΔH^o, ΔS^o and ΔG^o according to:

$$\Delta G^o = -RT\ln K \quad (2)$$

$$\ln K = \Delta S^O/R - \Delta H^O/RT \quad (3)$$

A plot of ln (K) versus 1/*T* produces: Slope $= -\Delta H^o/R$ and intercept $= \Delta S^o/R$.

^{1}H NMR measurements

To study the host–guest complexation of Q[n] and kinetin, 2.0–2.5 $\times$ 10^{-3} mmol samples of Q[7] in 0.5–0.7 mL D_2O with increasing concentrations of kinetin were prepared.

The corresponding ^{1}H NMR spectra were recorded at 20 °C on a VARIAN INOVA 400 spectrometer.

Preparation of the inclusion complexes and physical mixtures

The kinetin inclusion complexes were prepared at a 1:1 molar ratio. The solution was stirred at 20 °C for 24 h and then filtered, and the filtrate was freeze-dryed to obtain the inclusion complexes. The physical mixtures were prepared by simply blending Q[7] and kinetin with 1:1 molar ratio uniformly in a mortar.

Infrared spectroscopy(IR) measurement

IR spectra were recorded on an IR Presting-21 (SHIMADZU, Japan) spectrophotometer. Samples in KBr disks were prepared with a hydrostatic press at a force of 5T/cm^2 for 3 min. The scanning range was 4000–400 cm^{-1} and the resolution was 4 cm^{-1}.

Results and discussion

Profiles of solubility versus pHs

Figure 2 shows the pH-solubility profiles of kinetin at 20 °C. The reported pKa values of kinetin are 3.78 and 9.79. Solubility was much higher when the pH decreased below pK_1 (3.78) or increases above pK_2 (9.79). Thus, in this study, we investigated the interactions between Q[n] and kinetin in the pH range of 3–11 with the phase solubility method.

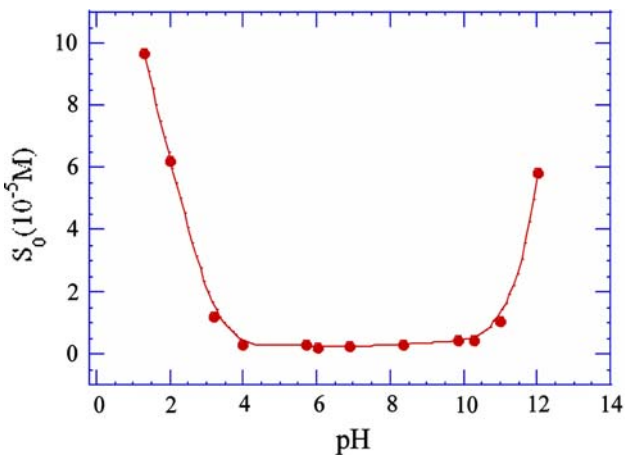

Fig. 2 Solubility profiles of kinetin under different pH at 20 °C

Effects of Q[n] on the solubility of kinetin

The effects of cucurbituril on the aqueous solubility of kinetin were evaluated using the phase solubility method. Figure 3 shows the phase diagrams of kinetin with three different types of Q[n] in aqueous solutions at 20 °C. The solubility of kinetin increased linearly as a function of Q[7] and TMeQ[6] concentrations. These phase solubility diagrams (PSDs) are classified as type A_L by Higuchi [17], which denotes a linear increase in solubility. In contrast, HMeQ[6] showed a type B_S solubility curve, which denotes an initial increase in solubility and a later decrease in solubility, because of the limited solubility of the complexes.

Based on the phase solubility diagrams, the association constants (K_{11}) for the different inclusion complexes were determined using Eq. (1), assuming a 1:1 stoichiometry (see Table 1). The stability of the kinetin inclusion complexes is in the order of HMeQ[6]>TMeQ[6]>Q[7], demonstrating the importance of the size fit between the host and the guest in the complexation process.

The highest association constant was observed for HMeQ[6], indicating that kinetin interacts strongly with HMeQ[6] due to the suitable cavity size (diameter 5.9 Å)

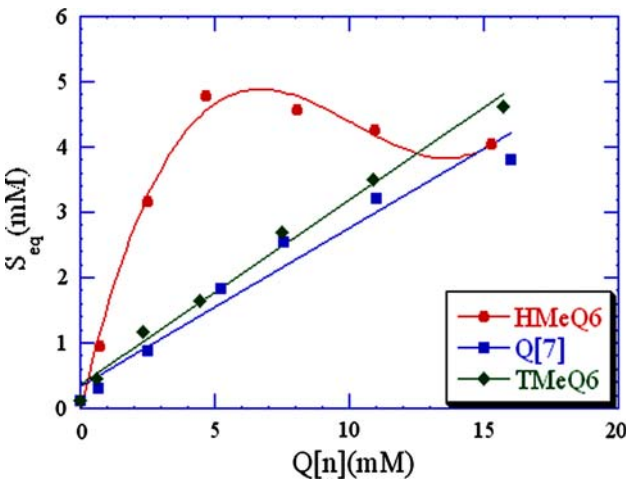

Fig. 3 Phase solubility diagrams of kinetin versus concentrations of Q[7], TMeQ[6] and HMeQ[6], obtained in water at 20 °C

Table 1 Complex formation constants of kinetin with Q[7], TMeQ[6] and HMeQ[6] calculated from PSDs (Fig. 3)

	Equation	R	K_{11} (M^{-1})	PSD type
Q[7]	Y = 0.239x + 0.339	0.9776	2.86×10^3	A_L
TMeQ[6]	Y = 0.260x + 0.351	0.9944	3.19×10^3	A_L
HMeQ[6]	Y = 0.953x + 0.256	0.9933	1.84×10^5	B_S

*A_L stands for a linear PSD, B_S stands for PSDs with a descending portion according to Higuch and Connors [17]

and the higher solubility. Q[7] had the lowest affinity with kinetin, presumably because kinetin formed loose complexes with the hydrophobic cavity of Q[7] (diameter 7.3 Å). On the other hand, although the smaller cavity size of TMeQ[6] allows suitable room for encapsulation, but because of lower solubility, its affinity with kinetin was less than that of HMeQ[6]. Therefore, HMeQ[6] appear to be the beset host molecule for kinetin encapsulation in our study.

In the aqueous solutions of Q[n]–kinetin complexes, the free kinetin molecules are in equilibrium with the kinetin molecules entrapped within the cavity. Thus, with the increase of the concentration of Q[n], more kinetin molecules will be captured from the aqueous solution into the hydrophobic cavities of the Q[n]. Therefore, more kinetins are being dissolved in water and the solubility of kinetin increases with the increased concentration of Q[n]. The concentration of kinetin in HMeQ[6] solution reached 4.95 mM, a 43-fold increase over that in water. A 35-fold increase in solubility was achieved when 16 mM Q[7] solution was used. HMeQ[6] is the most effective candidate to solubilize kinetin (Tables 2, 3, 4).

Effect of pH on the complex formation constant (K_{11})

The influence of pH on the apparent association constant of the Q[7]–kinetin inclusion complex, and on the efficacy of the solubility enhancing effect was studied. The PSDs obtained in water at different pHs are shown in Fig. 4. The K_{11} constants at pH 5.72, pH 6.89 and pH 8.32 were 2.61×10^3, 1.72×10^3 and $1.44 \times 10^3 M^{-1}$, respectively, where kinetin was predominantly neutral and/or anionic. The association constant of ionized kinetin at pHs 3.21 was lower (6.40×10^2 M^{-1}), where it existed as positive ion species. Generally, both the hydrophobic cavity interaction and the portal ion–dipole interaction of the host Q[7] and the ionized kinetin led to a complex formation, the competition between the bulk protonated water with the carbonyl of the portals of the host could weaken the interaction of the Q[7] and kinetin.

Thermodynamics

In principle, evaluation of K over a significant temperature range can be used to calculate the enthalpy (ΔH^o) and entropy (ΔS^o) of association. These parameters can provide insight into the driving forces responsible for the binding interaction. Therefore, we measured the K values from the solubility measurements at 295, 299, 303, 310 and 315K.The PSDs of kinetin obtained at different temperatures are shown in Fig. 5. The corresponding complex formation constants (K_{11}), the solubilities of kinetin in the absence of Q[n] (So) and the calculated thermodynamic parameters (ΔH^o, ΔS^o and ΔG^o) are listed in Table 5.

Figure 5 shows that the solubility of kinetin increased linearly with the concentration of Q[7] at different temperatures, indicating an A_L type of PSDs according to Higuchi [17]. Moreover, K values decreased with the increase of temperature, suggesting that the complex was less stable with the increase of temperature. The changes of Gibbs free energy, enthalpy and entropy are all negative. These data suggested that complex formation ($\Delta G = -18.80 \sim -19.48$ kJ/mol) was largely driven by enthalpy ($\Delta H = -34.61$ kJ/mol) in the presence of an unfavourable entropy ($\Delta S = -29.90$ J/mol K), which were attributed to the van der Waals and hydrophobic interactions.

Table 2 Increase of kinetin solubility by complexing with Q[7]

Q[7] (mM)	0	0.66	2.49	5.22	7.58	10.91	16.25
S_{eq} (mM)	0.11	0.30	0.89	1.83	2.55	3.22	3.81
S/S_0	1.00	2.73	8.09	16.64	23.18	29.27	34.63

Table 3 Increase of kinetin solubility by complexing with TMeQ[6]

TMeQ[6] (mM)	0	0.66	2.53	4.86	8.15	11.87	17.15
S_{eq} (mM)	0.11	0.46	1.17	1.65	2.70	3.51	4.63
S/S_0	1.00	4.18	10.64	15.00	24.54	31.91	42.09

Table 4 Increase of kinetin solubility by complexing with HMeQ[6]

HMeQ[6] (mM)	0	0.75	2.68	4.95	8.57	11.63	16.29
S_{eq} (mM)	0.11	0.96	3.16	4.79	4.58	4.27	4.04
S/S_0	1.00	8.73	28.73	43.54	41.64	38.81	36.73

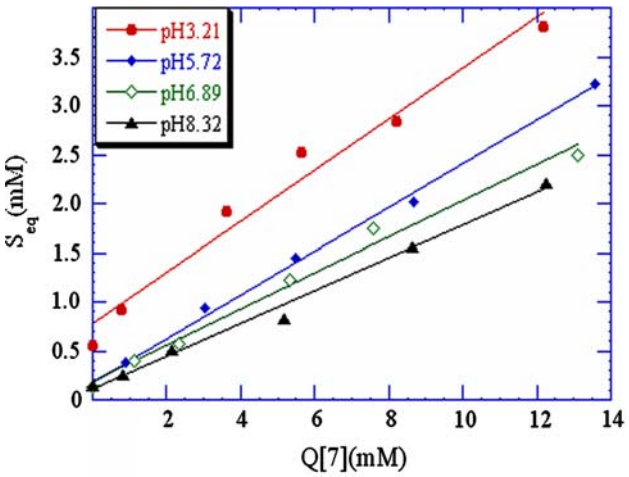

Fig. 4 Phase solubility diagrams of the kinetin/Q[7] system at pH 3.21, 5.67,6.86 and 8.32 at 20 °C

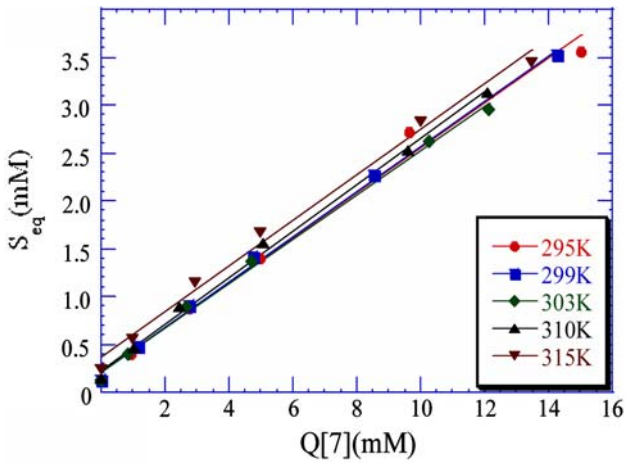

Fig. 5 Phase solubility diagrams of the kinetin/Q[7] system at different temperatures (295 K, 299 K, 303 K, 310 K and 315 K)

Table 5 Complex formation constants (K_{11}) and the thermodynamic parameters of the Q[7]/kinetin system at pH 5.67

T (K)	S_0 (mM)	K_{11} (M^{-1})	ΔG^o (kJ/mol)	ΔH^o (kJ/mol)	ΔS^o (J/mol K)
295	0.11	2.79×10^3	−19.48	−34.61	−29.90
299	0.12	2.57×10^3	−19.54		
303	0.12	2.52×10^3	−19.75		
310	0.14	2.28×10^3	−19.95		
315	0.24	1.30×10^3	−18.80		

^{1}H NMR study of the inclusion complexes

^{1}H NMR spectroscopy is a powerful tool to study the structures of inclusion complexes. It is well known that insertion of a guest molecule into the hydrophobic cavity of cucurbituril can affect the chemical shifts of the guest protons. Figure 6 shows the ^{1}H NMR spectra of kinetin in the absence (a) and in the presence of 1 equivalent (b), 2 equivalents (c), and 5 equivalents (d) of Q[7]. The undeuterated protons H_a–H_f of kinetin were detected.

The protons H_b, H_c and H_d on the furfural ring moiety and the methylene protons (H_a) of kinetin underwent a gradually upfield shift with increasing equivalent of Q[7] (from bottom to top), suggesting that Q[7] encapsulated the furan ring and the methylene moiety into its cavity with a fast ingress and egress exchange ratio. Moreover, the protons H_e and H_f on the adenine ring showed a downfield shift. Chemical shift changes of certain proton resonances of the guest or host with increasing or decreasing equivalents of the guest or host have been used to study host–guest interaction. However, it was difficult to determine the accurate chemical shift and integration of the guest hydrogen for the Q[7]–kinetin interaction system due to the broad proton resonances of the guest.

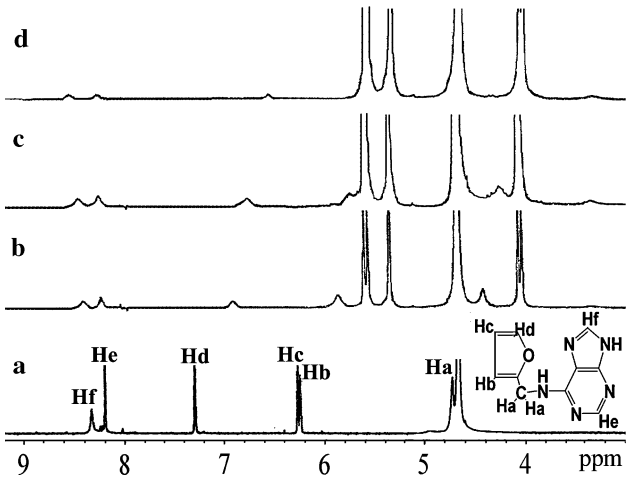

Fig. 6 ^{1}HNMR spectra (400 MHz, D_2O) of kinetin in the absence (a) and in the presence of 1 equivalent (b), 2 equivalents (c), and 5 equivalents (d) of Q[7]

When complexed with host TMeQ[6] and HMeQ[6], the furfural ring and methylene moiety of guest kinetin were also entrapped in the cavity of the host. ^{1}HNMR spectra of kinetin in the absence and in the presence of TMeQ[6] and HMeQ[6] are shown in Figs. 7 and 8, respectively. The proton H_d on the furan ring moiety showed an upfield shift by ~0.8ppm with increasing equivalents of cucurbiturls (from bottom to top). Moreover, the protons H_b and H_c on the furfural ring showed an upfield shift and merged in the 5–6 ppm region. The methylene proton (H_a) was also upfielded shift by ~0.2 ppm. These data suggested that the furan ring and the methylene group were also encapsulated into the cavity of the cucurbiturils.

The above ^{1}H NMR spectra showed that the furfural ring protons of kinetin exhibited a higher upfield shift when

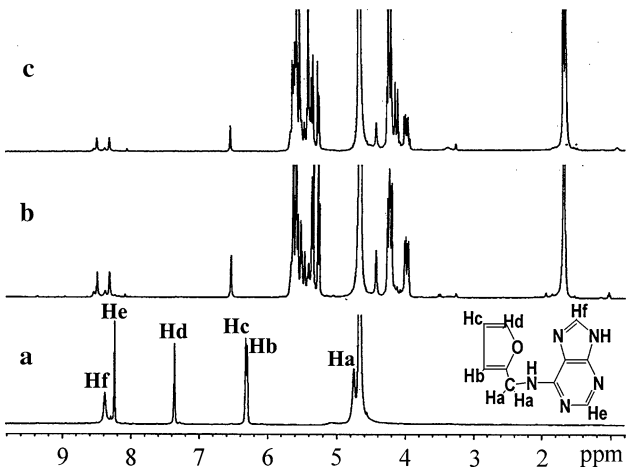

Fig. 7 ^{1}H NMR spectra (400 MHz, D_2O) of kinetin in the absence (a) and in the presence of 1.0 equivalent (b), and 3.0 equivalents (c) of TMeQ[6]

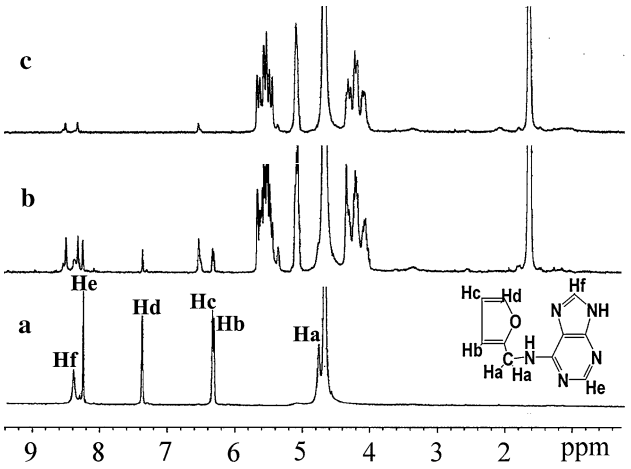

Fig. 8 ^{1}H NMR spectra (400 MHz, D_2O) of kinetin in the absence (a) and in the presence of 1.0 equivalent (b), 4.0 equivalents (c) of HMeQ[6]

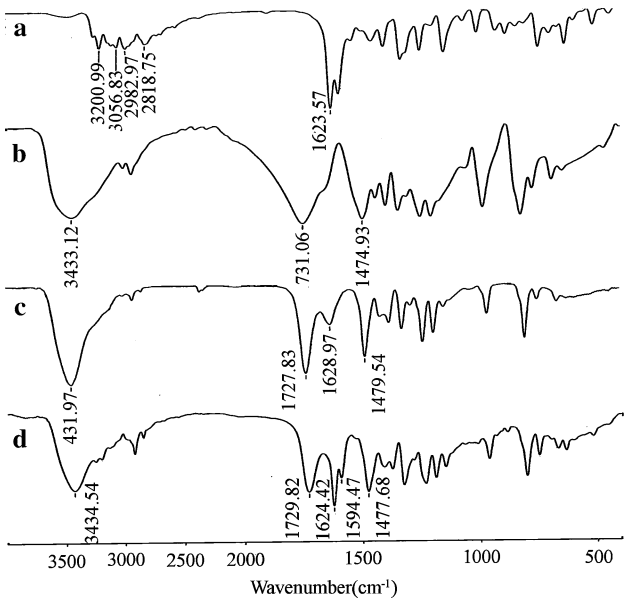

Fig. 9 FT-IR spectra of kinetin (a), Q[7] (b), inclusion complex (c), and physical mixture of Q[7] and kinetin (1:1) (d)

complexed with TMeQ[6] and HMeQ[6] (~0.82 ppm) than when complexed with Q[7] (~0.73 ppm), indicating that the complexation with the flexible TMeQ[6] and HMeQ[6] were stronger than with the symmetric and rigid Q[7]. Adenine protons (H_d and H_e) of kinetin exhibited weak downfield shifts, suggesting that the adenine ring was likely located at the outside cucurbituril cavity.

FT-IR studies of the inclusion complexes

Pseudorotaxane, polyrotaxane and host–guest inclusion complexes based on cucurbit[n]urils have been extensively characterized with ^{1}H NMR, UV–vis, fluorescence, and mass spectroscopy. However IR is rarely used to characterize those compounds [18]. In this study, FT-IR was used to characterize the structures of the free guest kinetin, hosts Q[7], TMeQ[6] and HMeQ[6], the inclusion complexes of host–kinetin, and the physical mixtures of the hosts and kinetin. The obtained IR data supported the formation of the inclusion complexes of Q[7]–kinetin, TMeQ[6]–kinetin and HMeQ[6]–kinetin (referring to Figs. 9–11).

The IR spectrum of kinetin showed the presence of a peak at 1623 cm^{-1} (C–C, furan ring). The IR spectra of Q[7] showed prominent absorption bands at 3433 cm^{-1} (N–H) and 1731 cm^{-1} (C=O). In the IR spectrum of Q[7]–kinetin inclusion complex, the stretching vibration peak of kinetin at 1623 cm^{-1} was shifted to 1628 cm^{-1}, indicating interaction between kinetin and Q[7]. However, in the physical mixture Q[7] and kinetin, the bonds appeared at 1624 cm^{-1}, a slight shift compared with the kinetin, suggesting there is no obvious interaction between kinetin with Q[7] (Fig. 9a–d). In the TMeQ[6]–kinetin and HMeQ[6]–kinetin system, the peak of furan ring at 1623 cm^{-1} were

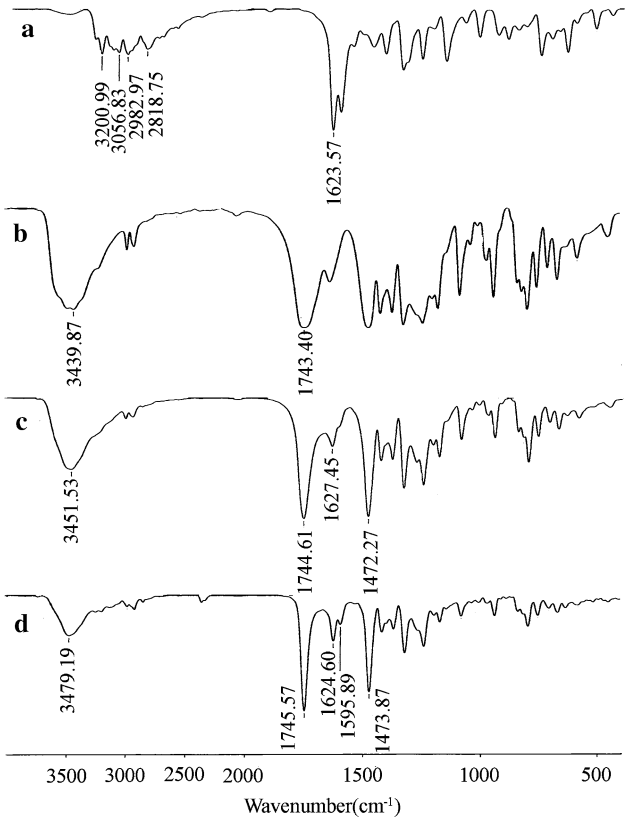

Fig. 10 FT-IR spectra of kinetin (a); TMeQ[6] (b), inclusion complex (c), and physical mixture of TMeQ[6] and kinetin (1:1) (d)

shifted to 1627 cm^{-1} and 1625 cm^{-1} respectively, suggesting the inclusion complexes were formed in solid state (Figs. 10a–d and 11a–d).

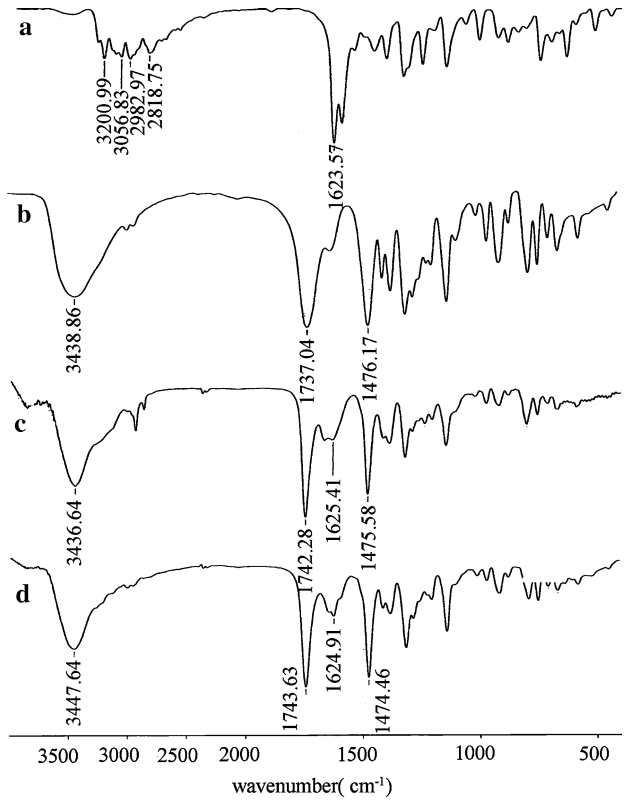

Fig. 11 FT-IR spectra of kinetin (a); HMeQ[6] (b); inclusion complex (c); and physical mixture of HMeQ[6] and kinetin (1:1) (d)

Conclusion

We investigated inclusion complex formation between kinetin and Q[7], TMeQ[6] and HMeQ[6] in aqueous solution and in solid state by phase solubility studies, ^{1}H NMR and IR. The effects of pH and temperature on complex stability were also explored. Phase solubility studies showed that kinetin solubility increased in a linear fashion as a function of Q[7] and TMeQ[6] concentrations. The kinetin solubility in HMeQ[6] first increase, then decreased, and the maximum solubility was achieved at 4.95 mM with HMeQ[6]. The solubility of kinetin as well as the association constant of its complex with Q[7] are found to be affected by the pH of the medium. It was also found that the interaction between kinetin and the cucurbituril weakened as the temperature increased. Moreover, the formation of the inclusion complexes was found to be enthalpy controlled, suggesting that hydrophobic and van der Waals interactions were the main driving forces. ^{1}H-NMR study confirmed the formation of the kinetin inclusion complexes with Q[7], TMeQ[6], and HMeQ[6]. IR spectroscopy showed the presence of inclusion complexes in solid state. Our results demonstrated that the complexation of kinetin with Q[n] could be used to improve the solubility of kinetin in aqueous solution.

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References

1. Uekama, K., Hirayama, F., Irie, T.: Cyclodextrin drug carrier systems. Chem. Rev. **98**, 2045–2076 (1998).
2. Rauterkus, M.J., Krebs, B.: Pentanuclear platinum(II) macrocycles with nucleobases. Angew. Chem. Int. Ed. **43**, 1300–1303 (2004).
3. Liu, Y., Tao Y.-Q., Cao Y.-Y., Liang J., Gu J.-Y., Shi X.-F.: Study on supramolecular chemistry of calixarene (IV): liquid membrane transport and molecular recognition to ATP by p-tert-butylcalix[4]arene. Acta Chim. Sinica. **60**, 1111–1115 (2002).
4. Freeman, W.A., Mock, W.L.: Cucurbituril. J. Am. Chem. Soc. **103**, 7367–7368 (1981).
5. Kim, J., Jung I.-S., Kim S.-Y., Lee E., Kang J.-K., Sakamoto S., Yamaguchi K., Kim K.: New cucurbituril homologues: syntheses, isolation, characterization, and X-ray crystal structures of cucurbit[n]uril (n = 5, 7, and 8). J. Am. Chem. Soc. **122**, 540–541 (2000).
6. Day, A.I., Arnold, A.P., Blanch, R.J., Snushall, B.: Controlling factors in the synthesis of cucurbituril and its homologues. J. Org. Chem. **66**, 8094–8100 (2001).
7. Lee, J.W., Samal, S., Selvapalam, N., Kim, H.-J., Kim, K.: Cucurbituril homologues and derivatives: new opportunities in supramolecular chemistry. Acc. Chem. Res. **36**, 621–631 (2003).
8. Lagona, J., Mukhopadhyay, P., Chakrabarti, S., Isaacs, L.: The cucurbit[n]uril family. Angew. Chem. Int. Ed. **44**, 4844–4870 (2005).
9. Kim, K., Selvapalam, N., Oh, D.H.: Cucurbiturils-a new family of host molecules. J. Incl. Phenom. Macrocycl. Chem. **50**, 31–36 (2004).
10. Kim, K., Selvapalam, N., Ko, Y.H., Park, K.M., Kim, D., Kim, J.: Functionalized cucurbiturils and their applications. Chem. Soc. Rev. **36**, 267–279 (2007).
11. Miller, C.O., Skoog, F., Von Saltza, M.H., Strong, F.M.: Kinetin, a cell division factor from deoxyribonucleic acid. J. Am. Chem. Soc. **77**, 1392–1393 (1955).
12. Miller, C.O., Skoog, F., Okumura, F.S., Von Saltza, M.H., Strong, F.M.: Isolation, structure and synthesis of kinetin, a substrate promoting cell division. J. Am. Chem. Soc. **78**, 1375–1380 (1956).
13. Jan, B., Frank, Brian, M., Clark F.C.: Kinetin—a multiactive molecule. Int. J. Biol. Macromol. **40**, 182–192 (2007).
14. Dong, N., Xue, S.-F., Zhu, Q.-J., Tao, Z., Zhang, B., Peng, Z.-B., In: Thomson (ed.) Supramol. Chem. Taylor & Francis, London England Wales (2007).
15. Zhao Y.-J., Xue S.-F., Zhu Q.-J., Tao Z., Zhang J.-X., Wei Z.-B., Long L.-S., Hu M.-L., Xiao H.-P., Day A.I.: Synthesis of a symmetrical tetrasubstituted cucurbit[6]uril and its host–guest compound with 2,2'-bipyridine. Chin. Sci. Bull. **49**, 1111–1116 (2004).
16. Lin, J.-X., Zhang, Y.-Q., Zhang, J.-X., Xue, S.-F., Zhu, Q.-J., Tao, Z.: Dig. J. Mol. Struct. (2007). doi: 10.1016/j.molstruc.2007.05.017.
17. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965).
18. Hou, S.-S., Tan, Y.-B., Xu, J., Zhou, J.-F.: Synthesis and study of novel pseudorotaxane. supramolecular self-assemblies of cucurbituril[6] and butyl viologen. Chin. J. Org. Chem. **25**, 934–939(2005).