Kinetic Study of Thermally Stimulated Dissociation of Inclusion Complex of 1-Methylcyclopropene with α-Cyclodextrin by Thermal Analysis

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The thermally stimulated dissociation of the inclusion complex of 1-methylcyclopropene (1-MCP) with α-cyclodextrin (α-CD) in solid state was studied by means of thermogravimetry (TG) and differential scanning calorimetry (DSC). The mass loss of 1-MCP/α-CD inclusion complex occurs in four separated phases with the thermal dissociation of the inclusion complex and release of 1-MCP taking place in the second phase between 90 and 230 °C. The kinetic parameters of the dissociation reaction (the apparent activation energy of dissociation, E_D, the reaction order of thermal dissociation, n, and the pre-exponential factor, k_0) were evaluated. The dissociation reaction was satisfactorily described by the unimolecular decay law, where the reaction order, $n = 1$. The effect of the molar ratio of 1-MCP to α-CD (inclusion ratio) in the inclusion complex on the temperature dependence of the dissociation reaction was also studied. The E_D decreased with increasing inclusion ratio indicating higher complex stability at lower inclusion ratios. The extrapolation of the E_D of the inclusion complexes with different inclusion ratios to 1 mol 1-MCP/mol α-CD yielded the "true" E_D of 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol for TG and DSC, respectively. The "true" ln k_{0TG} and the "true" ln k_{0DSC} were also determined by extrapolation, yielding values of +4.5 ± 1.0 and −0.3 ± 0.3, respectively.

Introduction

Olefin compounds that chemically inhibit ethylene perception in plants and thereby counteract ethylene in inducing physiological responses namely senescence, abscission, color change, and ripening,[1] have been attracting a great deal of attention in the horticultural field. As an ethylene inhibitor, 1-methylcyclopropene (1-MCP) is effective and commercially available, and thus of considerable interest.[2] Since discovery, 1-MCP has resulted in an explosion of research on its effects on fruits, vegetables, and ornamental crops.[3,4] Food use registration for 1-MCP has been obtained in countries like Australia, Austria, Brazil, Canada, Israel, The Netherlands, New Zealand, South Africa, the U.K., the U.S.A., etc.,,[1] and registration in Japan is still underway. The commercialization of 1-MCP was followed by rapid adoption by many apple industries around the world.[3] 1-MCP effectively inhibits ethylene perception at extremely low concentrations over an extended period of time[2] via a nontoxic mode of action.[5] For the purpose of speculating its potential applications, considerably extensive studies that cover a wide variety of produce and ornamentals including apple, apricot, banana, mango, pear, broccoli, lettuce, carnation, rose, etc., have consequently been carried out, in which details of the action, application, and effects of 1-MCP are reported.[6−21]

In addition to the aforementioned applications, 1-MCP is also a valuable substance in fundamental scientific researches, especially as a tool that helps scientists make major advances in understanding the role of ethylene in plants and gain insight into the fundamental processes that are involved in ripening and senescence of fresh produce.[3] In a study by Nakatsuka et al., 1-MCP was used to examine the regulation of ethylene synthesis in tomato fruit and their findings suggested that a strong positive feedback regulation was involved in ethylene biosynthesis at the gene transcriptional level.[22] Mwaniki et al. also utilized 1-MCP for elucidation of the role of ethylene in the regulation of genes encoding β-galactosidase and hence the softening process in "La France" pear (*Pyrus communis* L.).[23] Both ethylene and 1-MCP were used by Mainardi et al. to artificially induce or delay the ripening of banana (*Musa accuminata* AAA cv. Nanicão) so as to investigate the gene expression and activity profile of α-1,4-glucan-phosphorylase during the ripening process.[24]

Cyclodextrins (CD) are water-soluble cyclic oligosaccharides made up of 6, 7, and 8 α-1,4-linked glucopyranose units designated respectively as α-, β-, and γ-cyclodextrin. The truncated molecular structure of CD gives rise to a unique torus-shaped cavity which is capable of including a great variety of substances at molecular level to form a stable inclusion complex (host−guest complex) in both solid and aqueous phases. The so-called guest molecules encompass substances of solid (menthol,[25,26] imidazole derivatives[27]), liquid (flavors[28,29]), and gaseous states (1-MCP,[30] CO_2[31,32]).

1-MCP is currently being commercialized as a water-soluble powder that comprises its inclusion complex with α-CD, the form in which it is stable under dry condition with greatly diminished explosiveness.[33] With the solid-state inclusion complex being mostly the form in which 1-MCP is handled at present, studying the solid-state thermal dissociation kinetics would provide an insight into the heat stability of the inclusion complex. Instead of being limited to batch application in closed environments, a better understanding of the heat stability of the inclusion complex may help develop new ways of application especially those that require some pretreatment with heat. For instance, in the study on 1-MCP release from packaging films incorporated with 1-MCP/α-CD inclusion complex by Hotchkiss et al.,[34] the data on the heat stability of the inclusion complex would be a useful piece of information particularly for the preparation of the films by heat-pressing.

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Besides analytical methods such as IR, NMR, UV spectroscopy, X-ray diffractometry, and chromatography, thermal analysis has also been applied in the characterization of CD and their inclusion complexes.[35−40] A review on the applications of thermal methods to CD and their inclusion complexes was published by Giordano et al.[41] In most of the reported works, however, thermoanalytical data were meant only for qualitative analyses. There are rather few papers being published on the kinetic study of either the inclusion or the dissociation reaction of CD inclusion complexes based on the thermoanalytical data. Despite the fact that it is of utter importance to study the dissociation of 1-MCP/α-CD inclusion complex in response to heat to evaluate the heat stability of the inclusion complex, the kinetics of thermal dissociation of the inclusion complex still remains unexplored to the best of our knowledge. We devoted this work to studying the thermally stimulated dissociation kinetics of 1-MCP/α-CD inclusion complex in solid state. The dissociation reaction was investigated under nonisothermal conditions by thermogravimetry (TG) and differential scanning calorimetry (DSC). Furthermore, the molar ratio of 1-MCP to α-CD (inclusion ratio) of the inclusion complex was varied for investigation of the effect on the dissociation reaction. Our study leads to the establishment of kinetics parameters namely the "true" apparent activation energy of thermal dissociation, E_D, the dissociation reaction order, n, and the "true" pre-exponential factor, k_0 that account for the dissociation reaction. The "true" E_D, the "true" k_{0TG}, and the "true" k_{0DSC} were defined as the respective kinetic parameters estimated at the inclusion ratio of 1 mol 1-MCP/mol α-CD.

Materials and Methods

Materials. α-CD ($(C_6H_{10}O_5)_6$, $M_w = 973$, purity ≥99%) was obtained from Ensuiko Sugar Refining Co., Ltd. (Tokyo, Japan). The α-CD crystals were dried in-vacuo at 90 °C for 24 h before use. All the chemicals used were of reagent grade unless otherwise indicated and the chemicals were used without additional purification. 3-Chloro-2-methylpropene (98%) and lithium diisopropylamide (30 wt % suspension in mineral oil) were purchased from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Nitrogen was purchased from Iwatani Industrial Gases Corporation (Osaka, Japan) in gas cylinders. Distilled water was used throughout the entire experiment.

1-MCP Synthesis. 1-MCP was synthesized according to the method reported by Sisler and Serek[42] with some modifications as has been described in detail in our previous study.[30] Briefly, 2.4 mL of 3-chloro-2-methylpropene (98%) was reacted with 21.42 g of lithium diisopropylamide (30 wt % suspension in mineral oil) and 1-MCP was formed as lithium salt suspended in mineral oil. After reaction, high vacuum was pulled on the suspension liquid to eliminate volatile impurities particularly the remaining 3-chloro-2-methylpropene. The collected suspension liquid was then stored at −25 °C until use. During the encapsulation procedure, the frozen suspension liquid was first thawed and then reacted with distilled water to produce 1-MCP gas, which subsequently complexes with α-CD.

Preparation of 1-MCP/α-CD Inclusion Complex. As has been previously reported,[30] inclusion complexation of 1-MCP with α-CD was carried out in a hermetic agitated vessel. An α-CD solution of 50 mM at 20 °C was prepared on the basis of 100 g of distilled water and filled into a 500 mL SCHOTT DURAN laboratory glass bottle (DURAN Produktions GmbH and Co. KG, Mainz, Germany) with a modified screw cap. This bottle, designated as the encapsulation vessel, was immersed in a 20 °C water bath for temperature equilibration prior to the encapsulation process.

Meanwhile, vacuum was pulled to about 2.7 kPa in another SCHOTT DURAN 500 mL laboratory glass bottle, designated as the reaction vessel, which contained 100 g of distilled water using the PTFE diaphragm vacuum pump V-700 connected to the vacuum controller V-850 (BÜCHI Labotechnik AG, Flawil, Switzerland). Roughly 3 g of previously thawed suspension liquid of lithium salt of 1-MCP was injected into the reaction vessel through a rubber septum (Shimadzu Corp., Kyoto, Japan) fitted on the screw cap. Agitation was performed for about 15 min to promote complete reaction and evaporation of 1-MCP gas into the head space.

Next, vacuum was also pulled to about 2.7 kPa in the encapsulation vessel containing temperature-equilibrated α-CD solution. Transfer of 1-MCP from the reaction vessel to the encapsulation vessel was carried out by connecting the two vessels to one another with a Teflon tube of 6 mm i.d. through the BVLM 20-0808 bulkhead union elbows (Pisco USA, Inc.) installed on the screw caps of each vessel. Nitrogen was filled into the reaction vessel to create atmospheric pressure to promote 1-MCP transfer until the pressure of both vessels reached the atmospheric value. Encapsulation was carried out at 20 °C and promoted by agitation of the α-CD solution at an agitation rate of 200 rpm for 6 h. At the end of the encapsulation process, the 1-MCP/α-CD inclusion complex was collected as a precipitate by centrifugation (3000 rpm, 15 min). The recovered wet precipitate was then dried in-vacuo at ambient temperature for 24 h.

Preparation of Inclusion Complex Varying in Inclusion Ratio. The inclusion ratio of 1-MCP/α-CD inclusion complex is defined as the molar ratio of 1-MCP to α-CD in the inclusion complex itself. The inclusion ratio of the inclusion complex was varied to investigate the effect of inclusion ratio on the thermal dissociation of 1-MCP from α-CD. The inclusion complex powder collected after vacuum drying was subjected to heat treatment at 120 °C in the Advantec VR-320 vacuum drying oven (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) for treatment times of 0, 30, 90, and 240 min. With treatment time, the inclusion ratio was reduced progressively from 0.72 to 0.66, 0.53, and 0.39 mol 1-MCP/mol α-CD.

Thermogravimetry (TG). Mass loss in 1-MCP/α-CD inclusion complex as a function of temperature was recorded on the EXSTAR 6000 TG/DTA (TG/DTA 6200, SII Nano Technology Inc., Tokyo, Japan) equipped with the Muse Measurement software, version 3.7 (SII Nano Technology Inc.). The equipment was calibrated prior to measurement using a 20 mg weight. Measurements were performed by nonisothermal method on samples of masses within 5 ± 0.5 mg in open aluminum pans. The samples were analyzed at three different heating rates of 5, 7, and 10 °C/min from 25 to 300 °C under a nitrogen flow at 300 mL/min. An open empty aluminum pan was used as the reference. Triplicate measurements were made for each treatment. The data were analyzed using the Muse Measurement software, version 3.7.

Differential Scanning Calorimetry (DSC). A preliminary study was carried out to determine the thermal lag of the calorimeter cell based on the melting of high purity indium. An arbitrary weight of indium chip was sandwiched in the middle between two layers of 2.5 mg of inclusion complex, in the way that it was insulated by the inclusion complex from the aluminum pan wall, so as to obtain reliable data on the thermal lag of the system. The samples were subjected to DSC measurements at heating rates of 2, 5, 10, and 20 °C/min within

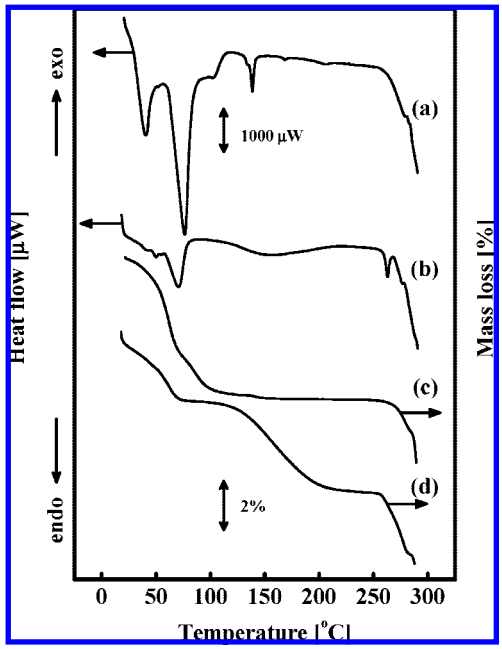

Figure 1. Typical DSC and TG curves of 5 mg samples of the uncomplexed α-CD (a and c) and the 1-MCP/α-CD inclusion complex (b and d), respectively. The thermal analysis was carried out at 5 °C/min.

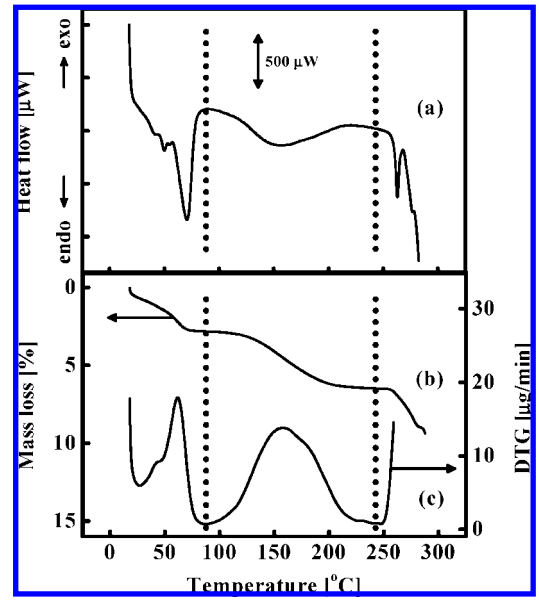

Figure 2. Typical DSC (a), TG (b), and DTG (c) curves of 5 mg 1-MCP/α-CD inclusion complex recorded at 5 °C/min. The dashed lines define the temperature region of thermal dissociation.

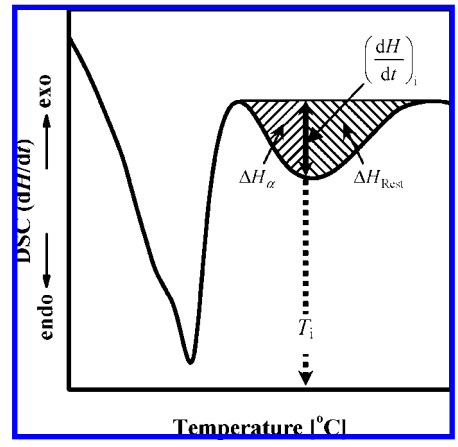

Figure 3. Definition of ΔH_α, ΔH_{Rest}, and $(dH/dt)_i$ used for determination of kinetic parameters from the DSC curve.

25−300 °C. The thermal lag was determined as the shift of the endothermic melting peak of indium from the reference value of 156.6 °C as a result of the insulation. The maximum thermal lag was 2.1 °C recorded at 20 °C/min. Because the highest heating rate employed in this study was 10 °C/min, which revealed a lag of only 1.2 °C, the thermal lag was regarded as negligible.

Heat flow in the inclusion complex during temperature increment was measured using the EXSTAR 6000 DSC (DSC 6220, SII Nano Technology Inc.) equipped with the Muse Measurement software, version 3.7 (SII Nano Technology Inc.). Temperature and sensitivity calibration of the equipment was performed prior to measurement using high purity indium (Mp, 156.6 °C; ΔH, 28.59 mJ/mg) and stannum (Mp, 232.0 °C; ΔH, 60.62 mJ/mg). All the measurement conditions—the range of sample size, heating rate, and temperature, and the reference material—are identical to that applied in the TG analysis, except for the nitrogen flow rate, which was set at 30 mL/min. The aluminum pans were also left open to obtain results that are comparable with that from the TG analysis. Measurements were made in triplicate for each treatment and the data were analyzed using the Muse Measurement software, version 3.7.

Results and Discussion

Thermal Characterization of 1-MCP/α-CD Inclusion Complex. Figure 1 represents the typical DSC and TG curves of 5 mg samples of the uncomplexed α-CD (a and c) and the 1-MCP/α-CD inclusion complex (b and d), respectively, recorded at the heating rate of 5 °C/min. CD are known to crystallize as hydrates, with water molecules in part included in the cavity of the cyclic macromolecule and in part localized in the interstices between macromolecules within the crystal lattice.[43] The uncomplexed α-CD demonstrated a wide dehydration phase, which occurred from 20 to 120 °C as endothermic processes with two sharp peaks at 40.4 and 76.2 °C, and a shallow peak at 102.7 °C in the DSC curve (Figure 1a). The correspondence of the endothermic peaks with dehydration has earlier been reported by researchers who have studied on the dehydration of α-CD of different polymorphs by thermal analysis.[44−46] Bettinetti et al.[43] observed a two-step dehydration DSC patterns with a slight shoulder after the second peak for one of their α-CD samples, which they claimed to be characteristic for form I α-CD hexahydrate polymorph. Meanwhile, Bettinetti et al.[46] reported a fairly similar dehydration profile of form I α-CD hexahydrate to ours, which was composed of two sharp endothermic peaks at 44 and 72 °C, and a shallow one at 98 °C. The dehydration phase could also be observed in the inclusion complex within a narrower temperature range between 25 and 90 °C (Figure 1b).

The first dehydration phase in the uncomplexed α-CD involved a 5.5 ± 0.5% mass loss (Figure 1c). The dehydration processes occurred through a wider temperature range from 20 to 170 °C as compared to the DSC analysis in which the mentioned processes ended at approximately 120 °C. The total mass loss incurred in these stages of dehydration is equivalent to about 3.2 water molecules that might possibly be bonded interstitially to an α-CD molecule. This amount is slightly lower than the reported 4 interstitially bonded water molecules in α-CD hexahydrate,[45,47,48] which are readily liberated in the first

phase dehydration, presumably due to the pretreatment of vacuum drying that might have led to a mixture of α-CD hexahydrate and anhydrous α-CD. Meanwhile, the first phase dehydration resulted in a 2.6 ± 0.3% mass loss in the inclusion complex, which is equivalent to about 1.5 water molecules that might possibly be bonded interstitially to the α-CD (Figure 1d). This may plausibly be partly resulted from the changes in crystalline structure of α-CD triggered by the inclusion complexation with 1-MCP that might have given rise to narrower interstitial space.[30] Manor and Saenger also concluded from their diffraction study that the macrocyclic conformation of α-CD ring are different when a water dimer is the guest molecule from that observed with other guest molecules such as 1-propanol, methanol, potassium acetate, and iodine.[48]

A small endothermic reaction that peaked at about 139 °C was recorded in the DSC curve of the uncomplexed α-CD. The similar endothermic peak was related by several groups of researchers to the phase transition of anhydrous α-CD to another form because there was no corresponding mass loss.[43,46] Nevertheless, a slight corresponding mass loss (0.2 ± 0%) was found in the TG curve (Figure 1c) of our sample.

Thermal analysis methods can satisfactorily recognize the inclusion complexation of 1-MCP in α-CD. A broad shallow endothermic peak accompanied by a mass loss that took place between 90 and 230 °C was detected in the 1-MCP/α-CD inclusion complex. This phenomenon was deduced to represent the dissociation of the inclusion complex. The spread of the endothermic dissociation reaction over a great range of temperature may imply the wide difference in the interaction between the 1-MCP molecule and the α-CD cavity in terms of interaction strength. Small mass losses accompanied by small endothermic peaks were recorded at approximately 250−285 °C for both the uncomplexed α-CD and the inclusion complex. The similar phenomenon was reported by Kohata et al.[49] in their study on the thermal dehydration of α- and γ-CD, according to which, the mass loss was attributed to the loss of very tightly bound water. Berbenni et al.[47] also confirmed by FT-IR analysis of the evolved gas that the mass loss was due to the release of water molecules. According to Lindner and Saenger, these water molecules were possibly held within the α-CD cavity.[45] Kohata et al.[49] and Berbenni et al.[47] reported respective losses of 0.3 and 0.7 water molecule in their uncomplexed α-CD sample during this dehydration phase, but 0.8 water molecule (a mass loss of 1.3 ± 0%) was liberated from our samples. About 1.6 water molecules (a mass loss of 2.7 ± 0.2%) were lost over this dehydration phase in the inclusion complex. These water molecules could possibly possess as strong interactions with the macromolecules of the inclusion complex as those of the included water molecules with uncomplexed α-CD. However, the orientations of these particular water molecules in the crystalline structure are still unknown.

The typical DSC, TG and derivative thermogravimetry (DTG) curves of 5 mg of 1-MCP/α-CD inclusion complex recorded at 5 °C/min are shown in Figure 2. The inclusion ratio of the inclusion complex was calculated from the TG data as 0.72 ± 0.01 mol 1-MCP/mol α-CD. The area bounded by the dashed lines represents the temperature range where the dissociation of the inclusion complex occurs, within which the kinetic study on the thermal dissociation of 1-MCP/α-CD inclusion complex was performed. The kinetic parameters of the dissociation of the inclusion complex were determined calorimetrically as well as gravimetrically based on the DSC and DTG curves, respectively.

Computation of Kinetic Parameters. There are two groups of methods used to analyze nonisothermal solid-state kinetic data—model fitting and model-free methods.[50] In this study, we employed one of the nonisothermal model-fitting methods—the Freeman and Carroll method that was originally developed assuming a reaction-order model ($f(\alpha) = (1 - \alpha)^n$) as elaborated below.

1-MCP/α-CD inclusion complex thermally dissociates into its respective solid and gaseous constituents:

$$\text{1-MCP/α-CD} \xrightarrow[\text{temp}]{} \text{1-MCP} + \text{α-CD} \tag{1}$$

Assuming that the above reaction follows an *n*th-order kinetics and obey the general rate equation[51]

$$\frac{d\alpha}{dt} = k(T)(1 - \alpha)^n \tag{2}$$

where $(d\alpha)/(dt)$ (1/s) represents the reaction rate, α the fractional dissociation, k (1/s) the specific dissociation rate constant at temperature T (K), and n the reaction order. As discussed earlier, the patterned peak in Figure 3 represents the dissociation of the inclusion complex. The peak area before an arbitrary temperature, T_i, patterned by the diagonally right up strokes, corresponds proportionally to the amount of dissociated complex at T_i. Meanwhile, the undissociated portion is illustrated by the area after T_i, which is patterned by the diagonally right down strokes. Thus, the dissociated and undissociated fractions of 1-MCP/α-CD inclusion complex could be described respectively by

$$\alpha = \frac{\Delta H_\alpha}{\Delta H_{\text{Total}}} \tag{3}$$

and

$$1 - \alpha = \frac{\Delta H_{\text{Rest}}}{\Delta H_{\text{Total}}} \tag{4}$$

where ΔH_{Total} is the total peak area obtained by integration of the DSC curve between the commencement and the completion of the dissociation reaction. ΔH_α is the integrated area up to T_i, and ΔH_{Rest} is the area from T_i until the end of dissociation. Substituting (3) and (4) into (2), and rearranging give the below equation, which enables the computation of the specific dissociation rate constant, k at T_i from the DSC curves:

$$k = \frac{1}{\Delta H_{\text{Total}}\left(\frac{\Delta H_{\text{Rest}}}{\Delta H_{\text{Total}}}\right)^n} \cdot \left(\frac{dH}{dt}\right)_i \tag{5}$$

where $(dH/dt)_i$ is the height of the DSC curve at T_i.

Similar procedures were applied for determination of k from the TG data for reciprocal validation. The DTG curves were used for analyses instead of the DSC curves and the specific dissociation rate constant, k could be described by

$$k = \frac{1}{\Delta m_{\text{Total}}\left(\frac{\Delta m_{\text{Rest}}}{\Delta m_{\text{Total}}}\right)^n} \cdot \left(\frac{dm}{dt}\right)_i \quad (6)$$

where Δm_{Total} is the total peak area obtained by integration of the DTG curve between the commencement and the completion of the mass loss of sample which corresponds to the release of 1-MCP gas, Δm_{Rest} the area from T_i until the end of dissociation, $(dm/dt)_i$ the height of the DTG curve at T_i.

The specific dissociation rate constant, k is a function of temperature, T and can be expressed by the Arrhenius relation as

$$k = k_0 \cdot \exp\left(-\frac{E_D}{RT}\right) \quad (7)$$

where k_0 is the pre-exponential factor, E_D the apparent activation energy of dissociation, R the universal gas constant and T the absolute temperature.

The k values were determined between 30% ($\alpha = 0.3$) and 70% ($\alpha = 0.7$) of the total peak area. An Arrhenius plot of the logarithmized k ($\ln k$) versus the inversed temperature ($1/T$) was employed as a tool for determination of the reaction order, n. The n value was varied in the range 0.5−3 by an interval of 0.5, and the one that gave the most linear plot was determined as the reaction order. The apparent activation energy of thermal dissociation, E_D for the 1-MCP/α-CD inclusion complex analyzed at 5 °C/min and the coefficient of determination, R^2 of the linear regression of Arrhenius plot were tabulated in Table 1. The data represent the mean values of three independent runs calculated for different n values. Except for the case when $n = 0.5$, no alteration of R^2 was observed down to the second digit after the decimal point with all R^2 values greater than 0.99, indicating equally satisfactory linear regression of the Arrhenius plots. Because no reactant other than 1-MCP/α-CD inclusion complex is present in the nonisothermal dissociation reaction, it could reasonably be regarded as a unimolecular reaction which depends only on the concentration of the inclusion complex. Thus, the mechanism of the solid-state dissociation reaction would plausibly be best described by the unimolecular decay law ($f(\alpha) = (1 - \alpha)$) with $n = 1$. Generally, rather close values of E_D were obtained by both the TG and the DSC methods. At $n = 1$, the E_D were calculated to be 42.3 ± 0.6 and 39.8 ± 1.0 kJ/mol from the respective methods. These close values prove that both calculation methods for the determination of E_D are reliable. As shown in Figure 4, although the k values calculated from the TG and the DSC methods differ from one another, the inclination of the Arrhenius plots was almost similar thus yielding close E_D values.

Heat Treatment of Inclusion Complex. The inclusion complex was subjected to heat treatment to vary its inclusion ratio. After being heated at 120 °C for 30, 90 and 240 min, the inclusion ratio of the inclusion complex was reduced to values as tabulated in Table 2. For each treatment time, the data represent the mean value of nine independent measurements, which consist of triplicate measurements at three heating rates of 5, 7, and 10 °C/min. The inclusion ratio decreased with heat treatment time in a quadratic manner. Meanwhile, a surprising phenomenon was observed where the moisture content of the inclusion complex increased with heat treatment time, in inverse proportion to the inclusion ratio. The well linear correlation between the moisture content and inclusion ratio with a coefficient of determination of 1.00, as demonstrated in Figure 5, plausibly indicated the displacement of a released 1-MCP molecule from the α-CD cavity by approximately 2.8 water molecules. This result is in rather good agreement with the reported 2.5 water molecules possibly residing in the cavity of uncomplexed α-CD.[45,47]

TABLE 1: Variation of Apparent Activation Energy of Dissociation with Dissociation Reaction Order, n, and Coefficient of Determination, R^2 of Linear Regression of the Arrhenius Plot[a]

	TG		DSC	
n	E_D [kJ/mol]	R^2 [−]	E_D [kJ/mol]	R^2 [−]
0.5	18.5 ± 0.7	0.95 ± 0.01	15.1 ± 0.9	0.91 ± 0.02
1.0	42.3 ± 0.6	1.00 ± 0	39.8 ± 1.0	1.00 ± 0
1.5	66.1 ± 0.7	1.00 ± 0	64.5 ± 1.2	1.00 ± 0
2.0	89.9 ± 0.8	1.00 ± 0	89.2 ± 1.3	1.00 ± 0
2.5	113.7 ± 0.9	1.00 ± 0	133.9 ± 1.5	1.00 ± 0
3.0	137.5 ± 1.1	1.00 ± 0	138.6 ± 1.7	1.00 ± 0

[a] Sample size is 5 mg, heating rate is 5 °C/min.

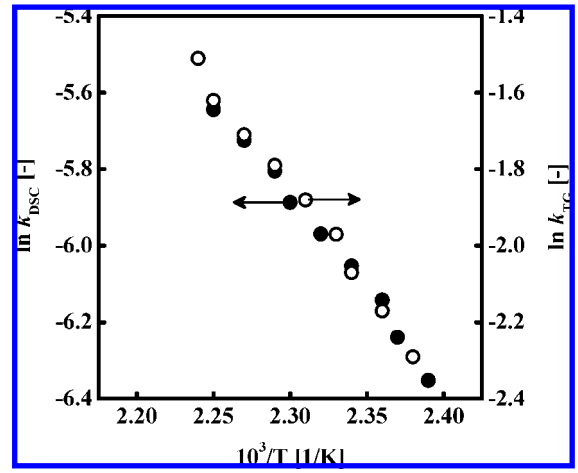

Figure 4. Arrhenius plots of the specific dissociation rate constant, k calculated using (5) and (6) from DSC and TG data, respectively. DSC and TG were performed on 5 mg samples at 5 °C/min.

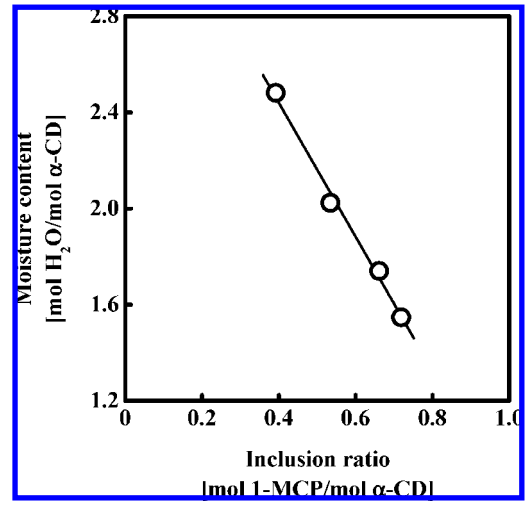

Figure 5. Moisture content as a function of inclusion ratio of inclusion complexes after heat treatment at 120 °C.

Effect of Inclusion Ratio on Dissociation. Five milligrams of the heat-treated inclusion complexes with inclusion ratios ranging from 0.39 to 0.66 mol 1-MCP/mol α-CD and the untreated inclusion complex were subjected to TG and DSC analyses at heating rates of 5, 7, and 10 °C/min. The apparent activation energy of dissociation, E_D obtained from the TG and

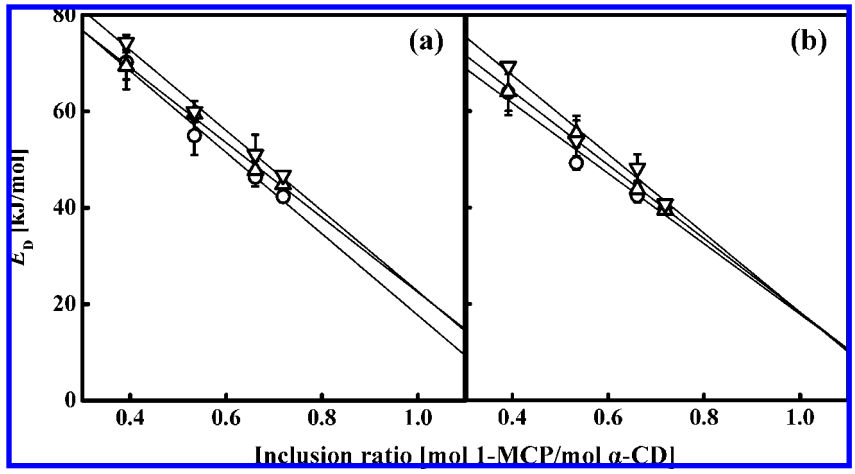

Figure 6. Apparent activation energy of dissociation calculated from TG (a) and DSC (b) data for inclusion complexes of various inclusion ratios. The solid lines are the linear regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α-CD for estimation of the "true" E_D. Heating rate: ○, 5 °C/min; Δ, 7 °C/min; ▽, 10 °C/min.

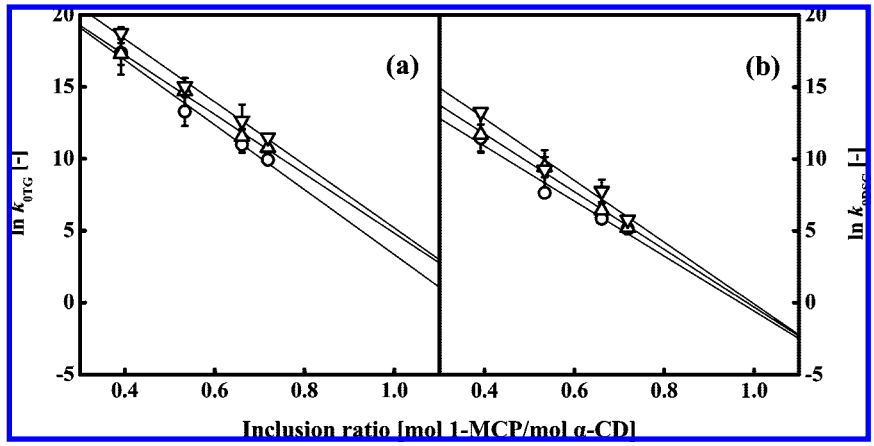

Figure 7. Logarithmized pre-exponential factor calculated from TG (a) and DSC (b) data for inclusion complexes of various inclusion ratios. The solid lines are the linear regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α-CD for estimation of the "true" ln k_{0TG} and the "true" ln k_{0DSC}. Heating rate: ○, 5 °C/min; Δ 7 °C/min; ▽, 10 °C/min.

TABLE 2: Inclusion Ratio and Moisture Content of 1-MCP/α-CD Inclusion Complex after Heat Treatment at 120 °C

treatment time [min]	inclusion ratio [mol 1-MCP/mol α-CD]	moisture content [mol H_2O/mol α-CD]
0	0.72 ± 0.01	1.55 ± 0.15
30	0.66 ± 0.01	1.74 ± 0.27
90	0.53 ± 0.01	2.02 ± 0.27
240	0.39 ± 0.02	2.48 ± 0.34

the DSC data collected at the prescribed heating rates was plotted as a function of the inclusion ratio of the inclusion complexes as illustrated in Figure 6a,b, respectively. Both the TG and the DSC data revealed a generally similar trend, where E_D heightened as the inclusion ratio diminished, implying higher complex stability and as well a growing dependency of the specific dissociation rate constant, k on temperature at lower inclusion ratios. The possible explanation for the phenomenon would be that the heat treatment might have forcefully expelled 1-MCP molecules from the α-CD cavities in a selective manner dependent on the strength of the interaction and thus the remaining 1-MCP molecules needed progressively higher energy for dissociation. The ln k_{0TG} and ln k_{0DSC}, when plotted against the inclusion ratio, revealed a trend comparable to that of E_D (Figure 7).

The calculated values of E_D, ln k_{0TG}, and ln k_{0DSC} became less clustered around the mean values with decreasing inclusion ratio, which are shown by the diverging patterns of the data, especially from DSC analysis (Figures 6b and 7b). The increasingly scattering results at lower inclusion ratios indicated the increasing susceptibility of dissociation to the effect of heating rate with decreasing inclusion ratio, which might possibly be related to the progressive increase of energetically diversified complex molecule species during the heat treatment process, to which the inclusion complex was subjected for varying its inclusion ratio.

By noting that the E_D at 0.72 mol 1-MCP/mol α-CD were least scattered around the respective mean values of 44.6 ± 2.0 and 40.0 ± 0.9 kJ/mol for TG and DSC, it is reasonable to deduce that the E_D will continue to converge until the inclusion ratio reaches 1 mol 1-MCP/mol α-CD, at which the unimolecular decay law is anticipated to best describe the dissociation reaction. The inclusion complex with an inclusion ratio of 1 mol 1-MCP/mol α-CD is an ideal inclusion complex, in which every α-CD cavity is occupied by a molecule of 1-MCP. We speculate that this imaginary inclusion complex could possibly be almost energetically uniform, which would account for the converging patterns of E_D toward 1 mol 1-MCP/mol α-CD. Thus, the estimated E_D by regression and extrapolation to the inclusion ratio of 1 mol 1-MCP/mol α-CD would plausibly represent the "true" E_D. On the basis of the assumption, the "true" E_D was calculated by averaging the E_D values determined

by regression analysis and extrapolation to 1 mol 1-MCP/mol α-CD for different heating rates (solid lines in Figure 6). Both the TG and DSC methods revealed rather close values of "true" E_D of 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol, respectively. The "true" ln k_{0TG} and the "true" ln k_{0DSC} were also determined by the same method (solid lines in Figure 7), unveiling their respective values of +4.5 ± 1.0 and −0.3 ± 0.3.

It is commonly known that despite the comparatively higher resolution of reaction phases by low heating rates, the obtained results tend to deviate largely, most probably, for instance in the case of heat stimulated dissociation, due to the readsorption of the dissociated gas. Nevertheless, high heating rates also pose a similar problem of scattering results owing to higher possibility for the establishment of a high temperature gradient in the sample. However, in our case, the latter is not likely as the thermal lag for the heating rate of 10 °C/min was recorded at a mere 1.2 °C.

Conclusions

The thermal dissociation kinetics of 1-MCP/α-CD inclusion complex was analyzed by a nonisothermal model-fitting method—the Freeman and Carroll method. The dissociation of every 1-MCP molecule by heat treatment at 120 °C was accompanied by an adsorption of 2.8 water molecules. This may imply the important role played by water molecules in the dissociation of 1-MCP/α-CD inclusion complex. Because temperature and humidity are the two main factors that affect the stability of an inclusion complex during storage, the dissociation of 1-MCP/α-CD inclusion complex in response to storage humidity is also of interest. The research study on dissociation with respect to storage humidity is currently underway. Both TG and DSC were fairly reliable for the determination of E_D although the k values were significantly different between the two methods. The temperature dependency of the thermal dissociation reaction of 1-MCP/α-CD inclusion complex exhibits highly reproducible consistency, regardless of the thermal analysis method employed. The "true" E_D was determined to be 20.9 ± 2.8 and 18.1 ± 0.2 kJ/mol for TG and DSC, respectively. The stability of the 1-MCP/α-CD inclusion complex against dissociation is dependent to a great extent upon the initial inclusion ratio of the inclusion complex. The information on the heat stability of the 1-MCP/α-CD inclusion complex is expected to be useful for the viability assessment of unit processes that involve heat treatment during the development of new ways of application for the inclusion complex.

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References and Notes

(1) Watkins, C. B. *Int. J. Postharv. Technol. Innov.* **2006**, *1*, 62.
(2) Sisler, E. C. *Biotechnol. Adv.* **2006**, *24*, 357.
(3) Watkins, C. B. *Biotechnol. Adv.* **2006**, *24*, 389.
(4) Serek, M.; Woltering, E. J.; Sisler, E. C.; Frello, S.; Sriskandarajah, S. *Biotechnol. Adv.* **2006**, *24*, 368.
(5) Environmental Protection Agency. Federal Register, July 26, 2002. Vol. 67, No. 144, p 48796.
(6) Watkins, C. B.; Nock, J. F.; Whitaker, B. D. *Postharvest Biol. Technol.* **2000**, *19*, 17.
(7) Moran, R. E.; McManus, P. *HortScience* **2005**, *40*, 161.
(8) Fan, X.; Argenta, L.; Mattheis, J. P. *Postharvest Biol. Technol.* **2000**, *20*, 135.
(9) Bagnato, N.; Barrett, R.; Sedgley, M.; Klieber, A. *Int. J. Food Sci. Technol.* **2003**, *38*, 745. 2003.
(10) Lohani, S.; Trivedi, P. K.; Nath, P. *Postharvest Biol. Technol.* **2004**, *31*, 119.
(11) Hofman, P. J.; Jobin-Décor, M.; Meiburg, G. F.; Macnish, A. J.; Joyce, D. C. *Aust. J. Exp. Agric.* **2001**, *41*, 567.
(12) Jiang, Y.; Joyce, D. C. *Ann. Appl. Biol.* **2000**, *137*, 321.
(13) Ekman, J. H.; Clayton, M.; Biasi, W. V.; Mitcham, E. J. *Postharvest Biol. Technol.* **2004**, *31*, 127.
(14) Argenta, L. C.; Fan, X. T.; Mattheis, J. P. *J. Agric. Food Chem.* **2003**, *51*, 3858.
(15) Fan, X.; Mattheis, J. P. *HortScience* **2000**, *35*, 885.
(16) Able, A. J.; Wong, L. S.; Prasad, A.; O'Hare, T. J. *Postharvest Biol. Technol.* **2002**, *26*, 147.
(17) Wills, R. B. H.; Ku, V. V. V.; Warton, M. A. *J. Sci. Food Agric.* **2002**, *82*, 1253.
(18) Fan, X.; Mattheis, J. P. *HortScience* **2000**, *35*, 1312.
(19) Hassan, F. A. S.; Gerzson, L. *Int. J. Hortic. Sci* **2002**, *8*, 29.
(20) Skog, L. J.; Blom, T.; Schaefer, B.; Digweed, B.; Fraser, H.; Brown, W. *Acta Hortic.* **2001**, *543*, 55.
(21) Müller, R.; Sisler, E. C.; Serek, M. *Sci. Hortic.* **2000**, *83*, 51.
(22) Nakatsuka, A.; Shiomi, S.; Kubo, Y.; Inaba, A. *Plant Cell Physiol.* **1997**, *38*, 1103.
(23) Mwaniki, M. W.; Mathooko, F. M.; Matsuzaki, M.; Hiwasa, K.; Tateishi, A.; Ushijima, K.; Nakano, R.; Inaba, A.; Kubo, Y. *Postharvest Biol. Technol.* **2005**, *36*, 253.
(24) Mainardi, J. A.; Purgatto, E.; Vieira, A., Jr.; Bastos, W. A.; Cordenunsi, B. R.; Nascimento, J. R. O.; Lajolo, F. M. *J. Agric. Food Chem.* **2006**, *54*, 7294.
(25) Yoshii, H.; Sakane, A.; Kawamura, D.; Neoh, T. L.; Kajiwara, H.; Furuta, T. *J. Inclusion Phenom. Macrocycl. Chem.* **2007**, *57*, 591.
(26) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. *J. Food Sci.* **2003**, *68*, 1234.
(27) Morin, N.; Guillaume, Y. C.; Peyrin, E.; Rouland, J.-C. *Anal. Chem.* **1998**, *70*, 2819.
(28) Kant, A.; Linforth, R. S. T.; Hort, J.; Taylor, A. J. *J. Agric. Food Chem.* **2004**, *52*, 2028.
(29) Reineccius, T. A.; Reineccius, G. A.; Peppard, T. L. *J. Food Sci.* **2004**, *69*, 58.
(30) Neoh, T. L.; Yamauchi, K.; Yoshii, H.; Furuta, T. *J. Agric. Food Chem.* **2007**, *55*, 11020.
(31) Neoh, T. L.; Yoshii, H.; Furuta, T. *J. Inclusion Phenom. Macrocycl. Chem.* **2006**, *56*, 125.
(32) Cramer, F.; Henglein, F. M. *Chem. Ber.* **1957**, *90*, 2572.
(33) Pest Management Regulatory Agency, 2004. 1-Methylcyclopropene, Regulatory note REG 2004-07, PMRA, Health Canada, Ottawa, Ont., 50 pp, www.pmra-arla.gc.ca.
(34) Hotchkiss, J. H.; Watkins, C. B.; Sanchez, D. G. *J. Food Sci.* **2007**, *72*, E330.
(35) Zhou, S.; Wang, L.; Zhang, A.; Lin, K.; Liu, W. *J. Agric. Food Chem.* **2008**, *56*, 2708.
(36) Bergamasco, R. C.; Zanin, G. M.; Faria de Moraes, F. *J. Agric. Food Chem.* **2005**, *53*, 1139.
(37) Zhang, A.; Liu, W.; Wang, L.; Wen, Y. *J. Agric. Food Chem.* **2005**, *53*, 7193.
(38) Yu, S. Z.; Li, X. T.; Li, J. H.; Wang, J. Y.; Tian, S. J. *J. Therm. Anal.* **1997**, *49*, 1517.
(39) Yilmaz, V. T.; Karadağ, A.; İçbudak, H. *Thermochim. Acta* **1995**, *261*, 107.
(40) Szafranek, A. *J. Therm. Anal.* **1988**, *34*, 917.
(41) Giordano, F.; Novak, C.; Moyano, J. R. *Thermochim. Acta* **2001**, *380*, 123.
(42) Sisler, E. C.; Serek, M. *Physiol. Plant.* **1997**, *100*, 577.
(43) Bettinetti, G.; Novák, Cs.; Sorrenti, M. *J. Therm. Anal. Calorim.* **2002**, *68*, 517.
(44) Klar, B.; Hingerty, B.; Saenger, W. *Acta Crystallogr.* **1980**, *B36*, 1154.
(45) Lindner, K.; Saenger, W. *Acta Crystallogr.* **1982**, *B38*, 203.
(46) Bettinetti, G. P.; Novak, C.; Rillosi, M.; Giordano, F.; Mura, P. In *Proceedings of the Eighth International Symposium on Cyclodextrins*; Szejtli, J., Szente, L., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 29−32.
(47) Berbenni, V.; Marini, A.; Bruni, G. *Thermochim. Acta* **1998**, *322*, 137.
(48) Manor, P. C.; Saenger, W. *J. Am. Chem. Soc.* **1974**, *96*, 3630.
(49) Kohata, S.; Jyodoi, K.; Ohyoshi, A. *Thermochim. Acta* **1993**, *217*, 187.
(50) Khawam, A.; Flanagan, D. R. *J. Pharm. Sci.* **2006**, *95*, 472.
(51) Borchardt, H. J.; Daniels, F. *J. Am. Chem. Soc.* **1957**, *79*, 41.

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