Isothermal Fourier Transform Infrared Microspectrosopic Studies on the Stability Kinetics of Solid-State Intramolecular Cyclization of Aspartame Sweetener

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A novel Fourier transform infrared (FT-IR) microspectrophotometer equipped with differential scanning calorimetry (DSC) was used to investigate the kinetics of intramolecular cyclization of aspartame (APM) sweetener in the solid state under isothermal conditions. The thermal-dependent changes in the peak intensity of IR spectra at 1543, 1283, and 1259 cm^{-1} were examined to explore the reaction. The results support that the intramolecular cyclization process in APM proceeded in three steps: the methoxyl group of ester was first thermolyzed to release methanol, then an acyl cation was attacked by the lone pair of electrons available on nitrogen by an S_N1 pathway, and finally ring-closure occurred. The intramolecular cyclization of APM determined by this microscopic FT-IR/DSC system was found to follow zero-order kinetics after a brief induction period. The bond cleavage energy (259.38 kJ/mol) of thermolysis for the leaving group of $-OCH_3$, the bond conversion energy (328.88 kJ/mol) for the amide II NH band to DKP NH band, and the CN bond formation energy (326.93 kJ/mol) of cyclization for the DKP in the APM molecule were also calculated from the Arrhenius equation. The total activation energy of the DKP formation via intramolecular cyclization was 261.33 kJ/mol, calculated by the above summation of the bond energy of cleavage, conversion, and formation, which was near to the value determined by the DSC or TGA method. This indicates that the microscopic FT-IR/DSC system is useful as a potential tool not only to investigate the degradation mechanism of drugs in the solid state but also to directly predict the bond energy of the reaction.

Keywords: *Aspartame; isothermal stability; solid state; intramolecular cyclization; FT-IR/DSC system; bond energy; activation energy*

INTRODUCTION

Considerable interest has recently been directed to Fourier transform infrared (FT-IR) microspectroscopy for characterization of microscopic samples (Meesserschmidt and Harthcock, 1988; Wegmann 1998). Additionally, the thermal-dependent properties of samples have also been studied by combining the FT-IR microspectroscopy with a differential scanning calorimetry (DSC). This novel FT-IR/DSC microscopic system is a simple, rapid, and powerful tool for determining the thermal-dependent behavior of microsamples (Mirabella, 1988). It can measure the effect of temperature on the conformation of samples and also act as an accelerated stability testing method to predict product stability. This unique system has been extensively approached in our laboratory to investigate simultaneously the correlation between the structural change of the compound and the temperature applied, such as phase transition and desolvation of polymorphs or solvation of drugs (Lin, 1992; Tsai et al., 1993), thermotropic transition of skin lipid and protein with or without skin penetrating enhancers (Lin et al., 1994a, 1996a,b), kinetics of curing of silicon elastomers (Lin et al., 1994b), molecular interaction in Eudragits and poly(*N*-isopropylacrylamide) polymers (Lin et al., 1995, 1996c, 1999a,b), and thermal stability and reversibility of α-crystallin (Lin et al., 1998).

The stability of drugs in pharmaceutical products is a very critical issue in clinical drug therapy since it may influence the efficacy and safety of a drug. Before new methods are developed to stabilize the drugs, the solid-state stability of drugs has to be first characterized. The kinetics of the solid-state decomposition reactions of drugs should be studied to ensure and predict their shelf life (Byrn, 1982). Aspartame (APM) is a dipeptide consisting of the methyl ester of *N*-L-α-aspartyl-L-phenylalamine and always acts as a sweetener. With an indirect analytical method, it was found to undergo thermal-responsive intramolecular cyclization to form the cyclic compound 3-(carboxymethyl)-6-benzyl-2,5-diketopiperazine (DKP) (Leung et al., 1997, 1998). The kinetics of this solid-state reaction has been examined by the DSC method and thermogravimetric analysis (TGA) and best fitted the Prout−Tompkins equation (Leung et al., 1997; Prout and Tompkins, 1944). Our previous study has successfully used this FT-IR/DSC microscopic system to quickly and directly investigate the consecutive pathway of the dehydration process and intramolecular cyclization of APM dipeptides in the solid state (Cheng and Lin, 1999).

In the present study, this unique system was used to isothermally determine the kinetics of intramolecular cyclization in an APM molecule from thermal-responsive IR spectra. Moreover, the bond dissociation energy of

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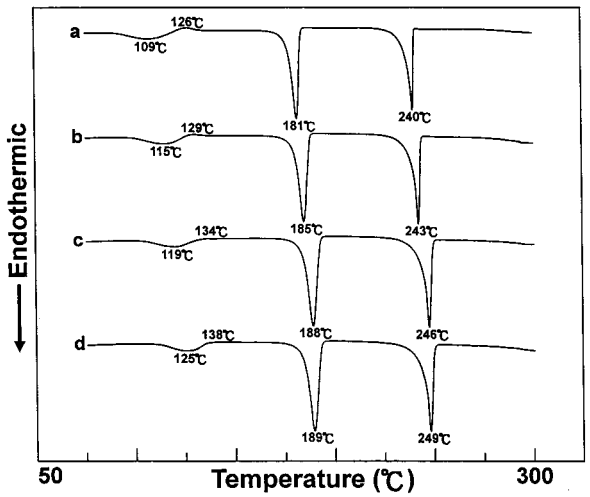

Figure 1. DSC thermograms of aspartame hemihydrate with four scanning heating rates. Key: a, 3 °C/min; b, 5 °C/min; c, 8 °C/min; d, 10 °C/min.

thermolysis and the bond formation energy of cyclization for DKP formation in the APM molecule were explored. The total activation energy of the DKP formation by this FT-IR/DSC microscopic technique was also calculated.

MATERIALS AND METHODS

Materials. Aspartame hemihydrate (APM) was obtained from Tokyo Kasei Indus. Co. Ltd (Tokyo, Japan) without further treatment. KBr crystals for pellets were purchased from JASCO Spectroscopic Co. Ltd (Tokyo, Japan).

Thermal Analysis of Aspartame. The DSC curves of APM crystals were determined by differential scanning calorimetry (DSC-910, TA Instruments Inc., New Castle, DE) with four heating rates of 3, 5, 8, and 10 °C/min in an open pan system under a stream of N_2 gas.

Transmission FT-IR/DSC Time-Scan Measurements (Lin et al., 1995, 1996). A small amount of APM crystals was sealed within two pieces of KBr pellets using a hydraulic press (200 kg/cm^2, 15 s). This compressed KBr disk was directly put in the DSC microscopy cell (FP 84, Mettler, Greifensee, Switzerland). The DSC microscopy cell was then placed on the stage of the microscope in the FT-IR microscopic spectrometer (Micro FTIR-200, JASCO, Tokyo, Japan) with an MCT detector. The system was operated in the transmission mode. The position and focus of the sample were adjusted through the microscope. The temperature of the DSC microscopy cell was monitored with a central processor (FT80HT, Mettler, Greifensee, Switzerland). The heating rate of DSC assembly was controlled at 3 °C/min in the isothermal condition. The isothermal procedure used a time-scan measurement program to control the DSC microscopy cell at 150, 155, 160, 165, and 170 °C, maintaining the sample at each temperature for 1 h. During the experiment, the sample disk was first equilibrated to the above prescribed temperature for about 3 min and then time-scanned. The thermal-responsive IR spectra were recorded while the sample disk was heated on a DSC microscope stage.

RESULTS AND DISCUSSION

Thermal Stability of APM Determined by DSC Methods. Thermal analysis techniques such as DSC and TGA have proven useful in evaluating the kinetic parameters of various reactions and materials. Figure 1 shows the DSC curves of APM determined by a series of heating rates between 3 and 10 °C/min. Generally, one broad and two sharp endothermic peaks and one exothermic peak were observed in DSC curves, although peak temperature varied with different heating rates. With the increase of heating rates, all of the peak temperatures shifted to the higher temperature range. Different heating rates might change the peak shape and peak temperature of a sample, thus the choice of a correct heating rate is of vital importance in ascribing the correct temperature to a transition. The broad endothermic peaks within 109−125 °C were due to the dehydration of APM hemihydrate, but the exothermic peaks at 126−138 °C might be attributed to the recrystallization of APM to form anhydrous APM. Moreover, the endothermic peaks ranging from 181 to 189 °C were due to the intramolecular cyclization of APM molecules to form DKP (Leung et al., 1998). Since APM proceeded in the solid-state reaction before the melting point of anhydrous APM, the melting point of anhydrous APM could not be found on the thermogram. The endothermic peaks within 240−249 °C should be assigned to the melting of solid products of DKP.

According to the Kissinger equation in which the reaction rate varied with temperature and then the peak temperature also varied with heating rate (Kissinger, 1957), the activation energy of intramolecular cyclization of APM can be calculated.

$$\frac{\mathrm{d}(\ln \beta/T^2)}{\mathrm{d}(1/T)} = \frac{-E_a}{R}$$

where β is the heating rate (°C/min), R is the gas constant (8.314 J/(mol K)), E_a is the activation energy (J/mol), and T is the peak temperature (K). From the slope of a plot of $\ln \beta/T^2$) and $1/T$, the E_a was calculated as 250.3 kJ/mol for the intramolecular cyclization of APM. This value of E_a was close to 265 ± 6 kJ/mol by a DSC method and 268 ± 8 kJ/mol determined by a TGA method (Leung et al., 1997, 1998). It should be noted that the solid-state degradation is usually slower than solution degradation, producing a greater activation energy.

Evidence of Intramolecular Cyclization in APM Molecules. Parts A−D of Figure 2 represent the three-dimensional plots of FT-IR spectra of APM between 1800 and 1160 cm^{-1} with four heating temperatures (155, 160, 165, and 170 °C) as a function of heating time. Moreover, the critical changes in IR spectra for each heating temperature are also superimposed in Figure 2A-1−D-1. It is evident that within the critical heating intervals the peak intensity of IR spectra of APM at certain wavenumbers changed markedly with temperature. The critical heating intervals for conversion were 46−60 min for the 150 °C heated sample (figure not shown), 26−40 min for the 155 °C heated sample, 16−30 min for the 160 °C heated sample, 2−9 min for the 165 °C heated sample, and 0−2 min for the 170 °C heated sample, respectively. The changes in IR spectral wavenumber occurred at 1736−1718, 1543, 1377−1362, 1283−1259, and 1225 cm^{-1}. The peak at 1736 cm^{-1} due to the carbonyl stretching vibration of ester disappeared gradually, but a new peak at 1718 cm^{-1} assigned to the carbonyl C═O of carboxylic acid increased gradually via a critical heating time. The amide II-related NH peak at 1543 cm^{-1} disappeared stepwise. The peaks at 1377 and 1225 cm^{-1} assigned to the bending of methyl group and C—O stretching of ester disappeared also. Moreover, the peak at 1283 cm^{-1} assigned to the CN bond of DKP gradually appeared but the peak at 1259 cm^{-1} corresponding to the methoxyl group disappeared stepwise.

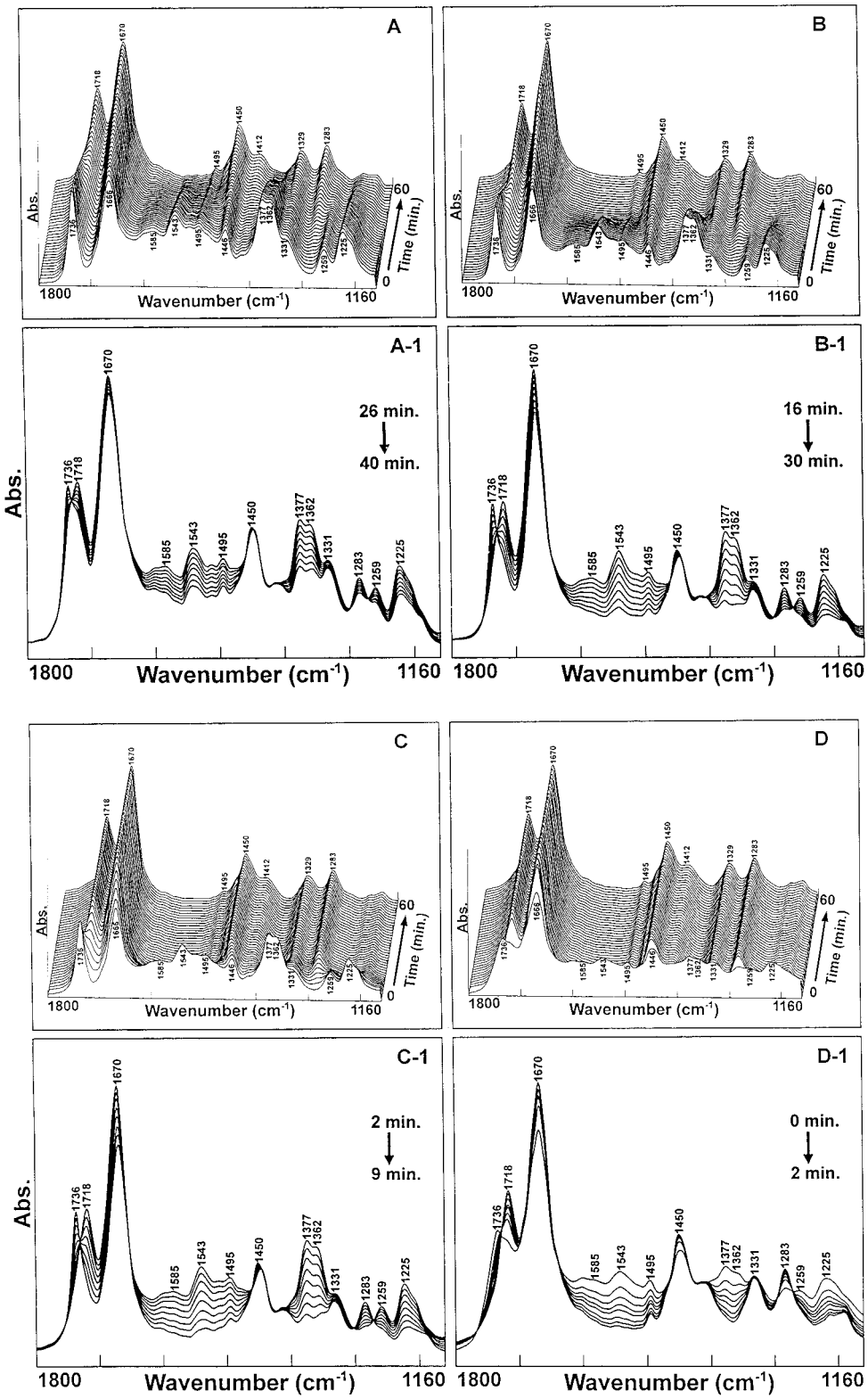

Figure 2. Three-dimensional plots of FT-IR spectra of aspartame hemihydrate with four heating temperatures as a function of heating time (A−D) and the critical changes in IR spectra for each heating temperature (A-1−D-1) Isothermal heating temperature: (A, A-1) 155 °C; (B, B-1) 160 °C; (C, C-1) 165 °C; (D, D-1) 170 °C.

This indicates that the DKP was formed in APM via intramolecular cyclization. Although the critical heating time was different from each prescribed heating temperature, the main alteration in the above IR spectral wavenumbers was similar, suggesting the same intramolecular cyclization process in the APM molecule. The step of intramolecular cyclization process based on the changes in IR peak intensity is diagramed in Figure 3. This process was supposed to proceed by three steps: The methoxyl group of ester was first thermolyzed during the heating process to release methanol, then an acyl cation was attacked by a lone pair of electrons available on nitrogen by an S_N1 pathway, and finally ring-closure occurred.

Kinetics and Bond Energy of Cyclization of APM by Micro FT-IR/DSC System. The changes of

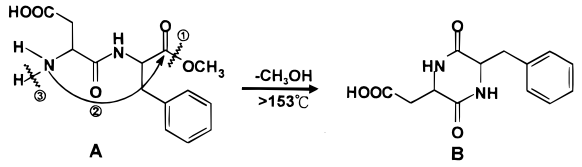

Figure 3. Intramolecular cyclization process of aspartame.

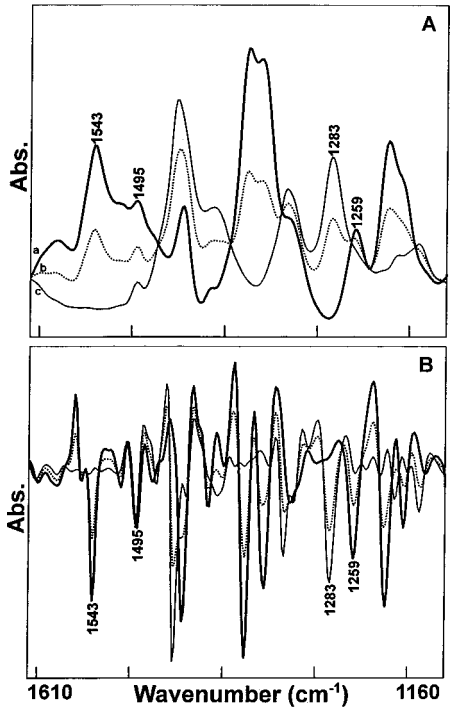

Figure 4. Comparison of the original FT-IR and its second-derivative spectra determined at isothermal 160 °C heating temperature as a function of heating time. Heating time: a, before heating; b, 22 min; c, 60 min.

four IR peaks at 1543, 1495, 1283, and 1259 cm^{-1} were studied to investigate the intramolecular cyclization of APM, as shown in Figure 4. The peaks at 1543 and 1259 cm^{-1}, assigned respectively to the amide II N−H band and the C−O of ester, disappeared gradually with an increase of heating time, but the peak at 1283 cm^{-1} corresponding to the C−N band in the DKP was formed stepwise. The peak at 1495 cm^{-1} due to the C=C stretching of the benzene ring maintained a constant intensity with heating and could be used as an internal marker. Thus the kinetics of intramolecular cyclization of APM to form DKP were studied by plotting the peak area ratio of 1543, 1259, or 1283 cm^{-1} to 1495 cm^{-1} against the heating time, as indicated in Figure 5. This clearly indicates that the peak area ratios for 1543 cm^{-1}/1495 cm^{-1} or 1259 cm^{-1}/1495 cm^{-1} decreased with heating, but the peak area ratios for 1283 cm^{-1}/1495 cm^{-1} increased. DKP formation was apparently heat-dependent, increasing between 150 and 170 °C. The values of peak area ratio reached a constant plateau (0 or constant), except for the 150 °C treated sample, suggesting the reaction was almost complete throughout the heating course.

The solid-state reactions of drugs have been extensively analyzed but are usually found not clearly zero-order or first-order, although the Prout−Tompkins equation has also been used (Byrn, 1982; Leung et al., 1997). Although the kinetics of the solid-state reaction in the APM molecule via intramolecular cyclization can best fit the Prout−Tompkins equation determined by

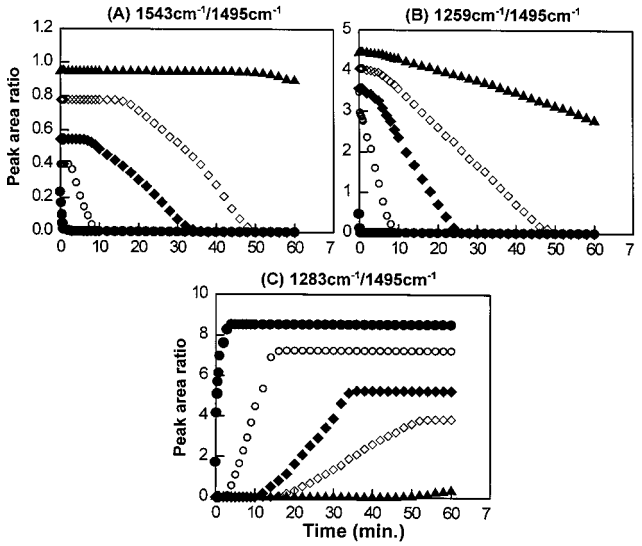

Figure 5. Kinetics of the temperature-dependent intramolecular cyclization in aspartame. Key: ●, 170 °C; ○, 165 °C; ◆, 160 °C; ◇, 155 °C; ▲, 150 °C.

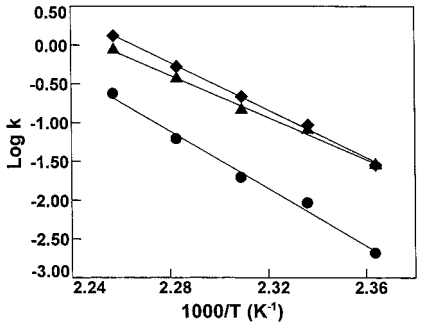

Figure 6. Arrhenius plot for intramolecular cyclization of aspartame for each specific spectrum. Key: ●, 1543 cm^{-1}; ◆, 1283 cm^{-1}; ▲, 1259 cm^{-1}.

the DSC method or TGA technique (Leung et al., 1997, 1998), the present study found that the intramolecular cyclization of APM still obeyed zero-order kinetics after a brief induction period, since the plot of the fraction decomposed or formed versus time was nearly linear ($R > 0.99$). Thus the rate constants for the ester cleavage of $-OCH_3$, the disappearance of the amide II NH band, and the CN bond formation of the DKP in APM molecule at different temperatures were obtained from the slope of linear plots.

The rate constants (k) are then plotted versus temperature (T) according to the Arrhenius equation

$$k = A \exp(-E_a/RT)$$

where A is a constant known as the frequency factor and R is the gas constant. A plot of ln k against $1/T$ was obtained as indicated in Figure 6. A good linear relationship for each of three plots was obtained ($R > 0.99$). According to our proposed scheme (Figure 3) for the intramolecular cyclization in APM, three steps have been delineated. Thus, the energy for the representative step was calculated. The bond dissociation energy of thermolysis for the leaving group of $-OCH_3$ was 259.38 kJ/mol, the bond conversion energy for the amide II NH band was 328.88 kJ/mol to DKP NH band, and the CN bond formation energy of cyclization for DKP was 326.93 kJ/mol, respectively. The total activation energy of the DKP formation via intramolecular cyclization was 261.33

kJ/mol, calculated by the summation of bond conversion and bond formation energies. The 261.33 kJ/mol for the intramolecular cyclization of APM determined by micro FT-IR/DSC system was near the value of 265 ± 6 kJ/mol by the DSC method and 268 ± 8 kJ/mol by the TGA method (Leung et al., 1997, 1998). This strongly demonstrates that the microscopic FT-IR/DSC system not only is a potential tool to investigate the degradation mechanism of drug or sweetener in the solid state but also can directly predict the bond energy of the reaction site.

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