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FTIR monitoring of oxazolidin-5-one formation and decomposition in a glycolaldehyde–phenylalanine model system by isotope labeling techniques

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ABSTRACT

Imines or Schiff bases formed through the interaction of reducing sugars with amino acids are known to play a critical role not only in initiating the Maillard reaction but also in its propagation through isomerization reactions initiated by the intermediate oxazolidin-5-one. FTIR spectroscopic evidence for the formation of this intermediate in a phenylalanine–glycolaldehyde model system was provided by taking advantage of a strong absorption band centered at 1778 cm^{-1}. The identity of this peak was confirmed by observing a shift to 1736 cm^{-1} when [^{13}C-1]phenylalanine was used. The intensity of this peak decreased over time with concomitant increase of two bands in the carbonyl absorption region, one centered at 1730 and the other at 1720 cm^{-1}. The former band was shifted to 1685 cm^{-1}, while the band at 1720 remained unchanged when [^{13}C-1]phenylalanine was used. The simultaneous formation of a carboxylic acid and a carbonyl band is consistent with the formation of an Amadori rearrangement product. Furthermore, time-dependent analysis of the formation and decomposition of the oxazolidin-5-one intermediate suggests that it is an important precursor of the Amadori rearrangement product. In addition, through the use of appropriate model systems, [^{15}N]phenylalanine and second-derivative spectral analysis, evidence was also provided for the formation of decarboxylated imines at 80 °C.

1. Introduction

Imines formed subsequent to carbonyl–amine reactions between amino acids and reducing sugars tend to undergo various chemical transformations depending on the nature of the carbonyl moiety. They can undergo Amadori rearrangement, the Strecker reaction, or cyclizations to generate N-containing heterocyclic compounds. Prior to these transformations, imines are also susceptible to isomerization reactions, further increasing the diversity of Maillard reaction products. Such isomerization reactions can proceed either through oxazolidin-5-one formation in dry systems to generate decarboxylated imine isomers or through transamination reactions (Fig. 1). The importance of oxazolidin-5-one formation lies in its ability to decarboxylate and form a non-stabilized azomethine ylide. This type of N-protonated azomethine ylide is prone to undergo a 1,2-prototropic shift and form two isomeric imines (B and C in Fig. 1), each of which is capable of producing distinct Maillard products. The formation of oxazolidin-5-one and subsequent generation of azomethine ylides have so far been verified only in model systems consisting of amino acids and simple aldehydes.[1,2] The chemistry of imine isomerization reactions through oxazolidin-5-one formation in amino acid–carbohydrate mixtures was first explored by Chu and Yaylayan[3] using a dry phenylalanine–glyceraldehyde model system. In this study, spectroscopic evidence was provided for the formation of a oxazolidin-5-one intermediate by the strong carbonyl absorption band centered at 1784 cm^{-1}. Spectroscopic studies using glyceraldehydes and various amino acids in toluene heated at 110 °C clearly indicated the formation of an intense peak in the range of 1780–1810 cm^{-1}, depending on the amino acid. The glyceraldehyde–asparagine model system,[4] for example, exhibited an absorption peak centered at 1778 cm^{-1}. The identity of the peaks was verified by observing the expected 40-cm^{-1} shift when [^{13}C-1]-labeled amino acids were used. Furthermore, evidence for the formation of the resulting azomethine ylide was also provided[3] using their specific ability to undergo 1,3-dipolar cycloadditions with dipolarophiles.[1] The addition of dipolarophiles, such as dimethyl fumarate, to the heated model systems led to a significant drop in intensity of the Maillard browning, indicating the importance of the resulting imines to the generation of color. In the previous studies we have examined imine isomerization reactions as a consequence of oxazolidin-5-one formation in the phenylalanine–glyceraldehyde model system using Py-GC/MS[3] and acrylamide generation through oxazolidin-5-one formation in the glucose–asparagine model system.[4] In this paper, we provide in-depth spectroscopic analysis of oxazolidin-5-one formation/decomposition in the phenylalanine–glycolaldehyde model system.

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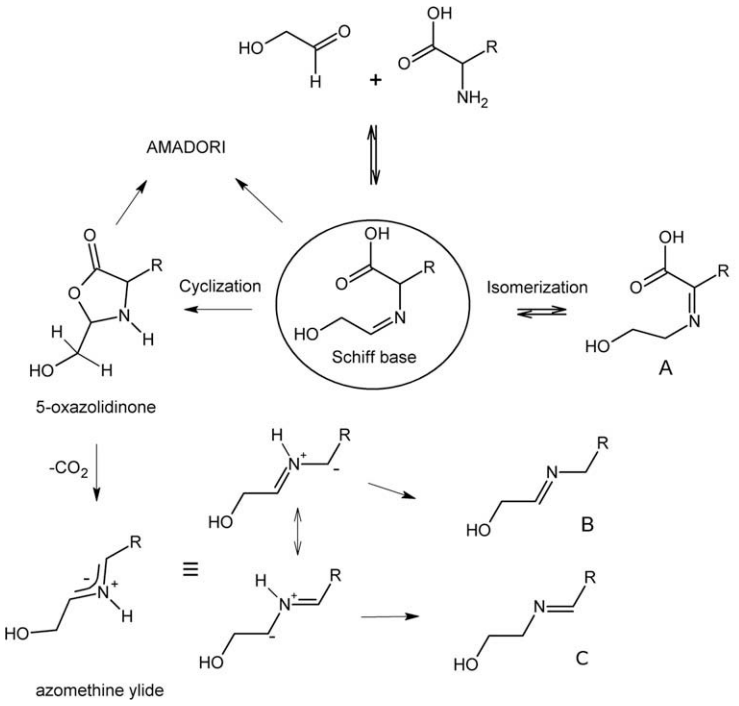

Figure 1. Summary of chemical transformations of the Schiff base formed between an amino acid and glycolaldehyde.

2. Results and discussion

The initiation of the Maillard reaction is mainly attributed to the successful formation of a Schiff base between the amino acid and the reducing sugar and its subsequent rearrangement into an Amadori product under appropriate moisture and pH conditions. However, in low-moisture systems, Schiff bases tend to prevail and subsequently undergo several transformations in addition to the Amadori rearrangement. Under basic pH and at room temperature, transamination[5] can generate its isomeric imine A shown in

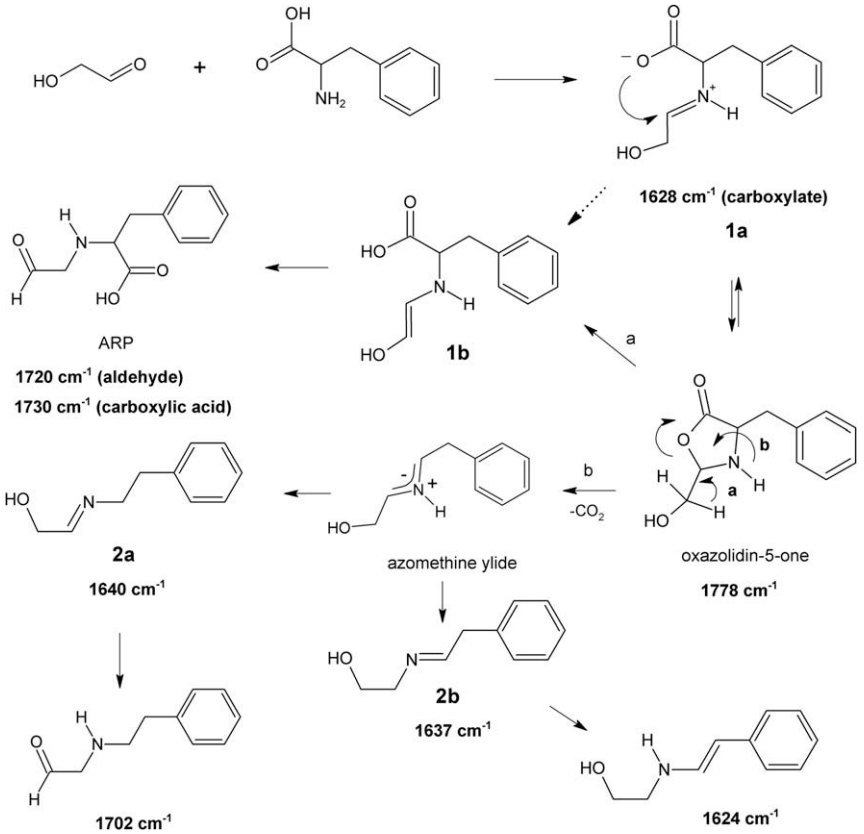

Figure 2. Band assignments of the intermediate compounds formed in the reaction between glycolaldehyde and phenylalanine.

Figure 1. Such isomeric imines can also be formed under pyrolytic conditions.[5,6] However, under dry and slightly acidic or neutral pH, they can undergo decarboxylation through the oxazolidin-5-one intermediate to produce decarboxylated isomeric imines (B and C in Fig. 1). The formation of an oxazolidin-5-one intermediate from Schiff bases, therefore, can provide an alternate pathway of degradation of sugars in addition to the Amadori pathway.

2.1. FTIR spectroscopy and isotope labeling studies

The oxazolidin-5-one intermediate can be conveniently studied using FTIR spectroscopy[1,2] due to the strong and characteristic absorption band ~1800 cm^{-1}. In the previous studies, FTIR spectroscopy was employed to indicate oxazolidin-5-one formation in both phenylalanine–glyceraldehyde[3] and asparagine–glyceraldehyde model systems.[4] Two sampling methods were developed to enhance the sensitivity of the detection. One method allows extraction of the oxazolidin-5-one from the heated reaction mixtures using toluene or methanol as solvents. The second method allows for monitoring the formation/degradation of oxazolidin-5-one in excess molten glycolaldehyde used as solvent (see Section 3).

2.2. Infrared band assignments

In the phenylalanine–glycolaldehyde model system, the band assignments of the different intermediates incorporating the carboxylic acid moiety as depicted in Figure 2 were accomplished through the observation of a shift in the frequency of the absorption bands arising from C-1 of the carboxylic acid moiety, when phenylalanine was replaced with [^{13}C-1]phenylalanine. The specific bands of the imines **2a** and **2b** and their further reaction

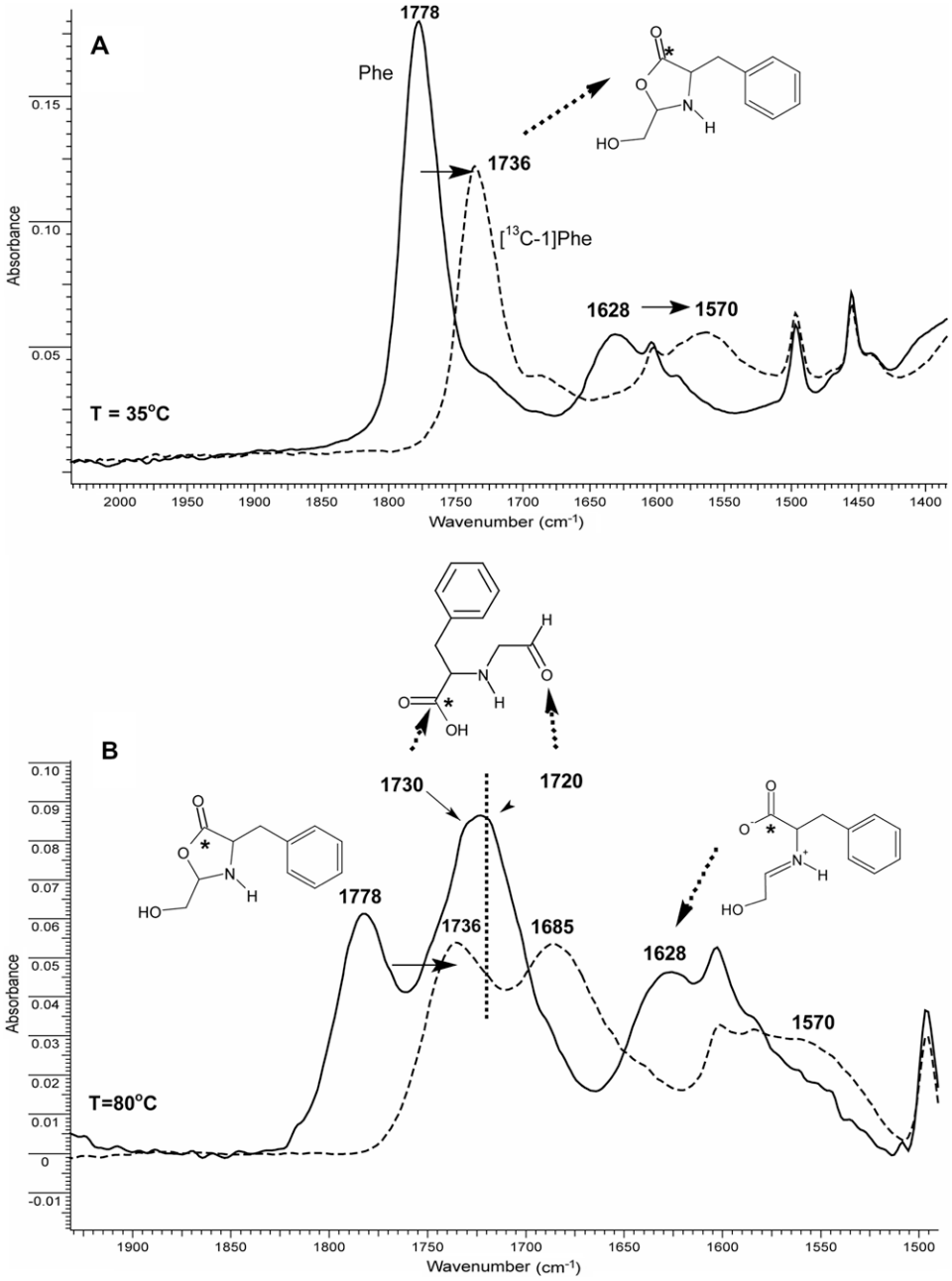

Figure 3. Absorption of the carbonyl and the imine regions (1900–1500 cm^{-1}) of the toluene extracts of phenylalanine–glycolaldehyde (solid lines) and [^{13}C-1]-phenylalanine–glycolaldehyde (dashed line) reaction mixtures acquired at (A) 35 °C and (B) 80 °C. An * indicates a ^{13}C-1 atom.

products were assigned through the use of glycolaldehyde–phenethylamine and ethanolamine–phenylacetaldehyde reaction systems, respectively. Figure 3a shows the FTIR spectra of the toluene extracts of the [^{13}C-1]phenylalanine–glycolaldehyde and phenylalanine–glycolaldehyde systems acquired at 35 °C. Figure 3a indicates a 42-cm^{-1} shift for the band centered at 1778 cm^{-1} and ~58-cm^{-1} shift for the band centered at 1628 cm^{-1}. These bands, because of their shifts when [^{13}C-1]phenylalanine was used, must be due to the C-1 atom of phenylalanine. The 1778 cm^{-1} band can be assigned to the oxazolidin-5-one,[1–4] and the band centered at 1628 cm^{-1} can be assigned to the carboxylate moiety. It is interesting to note the absence of the free carboxylic acid absorption band that appears at 1732 cm^{-1} in the spectrum of phenylalanine hydrochloride salt and was confirmed by its shift to 1690 cm^{-1} when [^{13}C-1]phenylalanine·HCl was used (see Fig. 4). However, when the temperature of the freshly prepared toluene extract was increased from 35 °C to 80 °C (see Fig. 5) and monitored over a 10-min period, the oxazolidin-5-one band centered at 1778 cm^{-1} sharply decreased, and a new band appeared and increased over time (see Fig. 5). The new band was composed of two peaks, one centered around 1730 and the other around 1720 cm^{-1} (see Fig. 5). The 1730 cm^{-1} band was assigned to the carboxylic acid of the Amadori rearrangement product as confirmed by its shift to 1685 cm^{-1} when [^{13}C-1]phenylalanine was used (see Fig. 3b). The Amadori product should also exhibit a carbonyl absorption band that was logically assigned to the band at

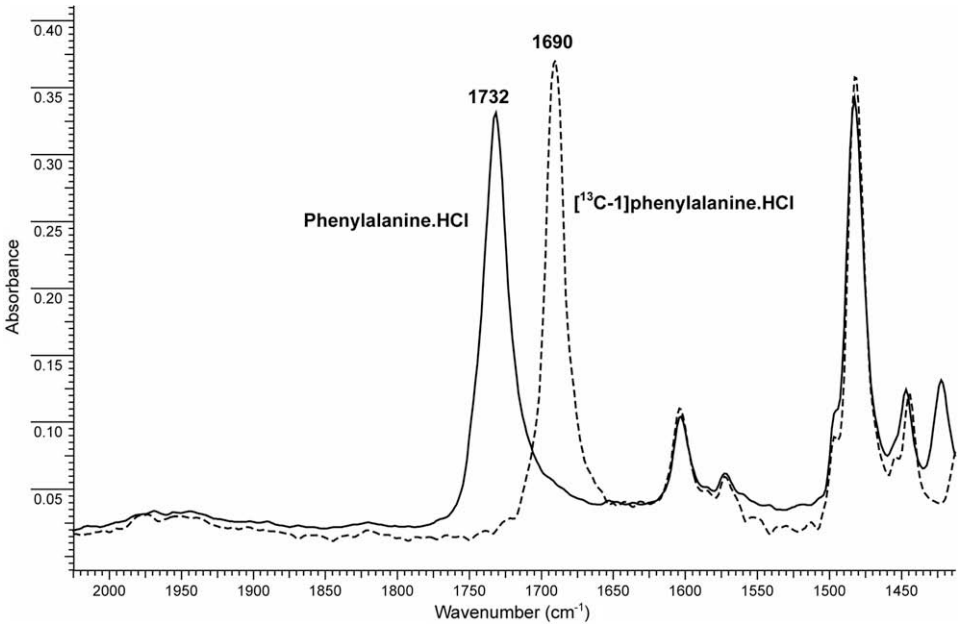

Figure 4. Infrared spectra (1900–1450 cm^{-1} region) of phenylalanine·HCl (solid line) and [^{13}C-1]phenylalanine·HCl (dashed line).

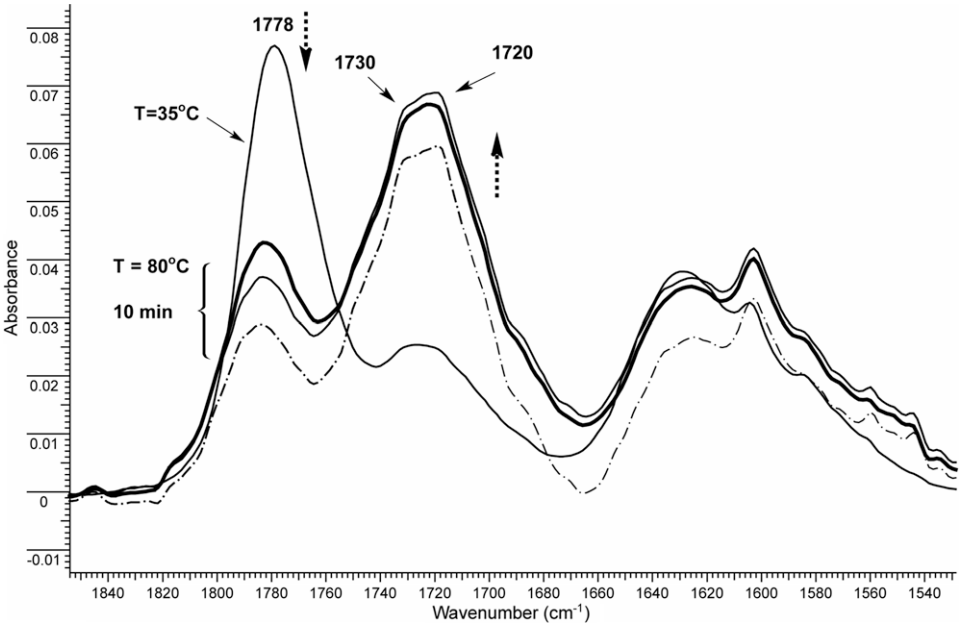

Figure 5. Absorption of the carbonyl and the imine regions (1800–1540 cm^{-1}) of the toluene extracts of phenylalanine–glycolaldehyde reaction mixtures acquired at 80 °C over a period of 10 min showing the intensity of the band at 1778 cm^{-1} acquired at 35 °C as a reference.

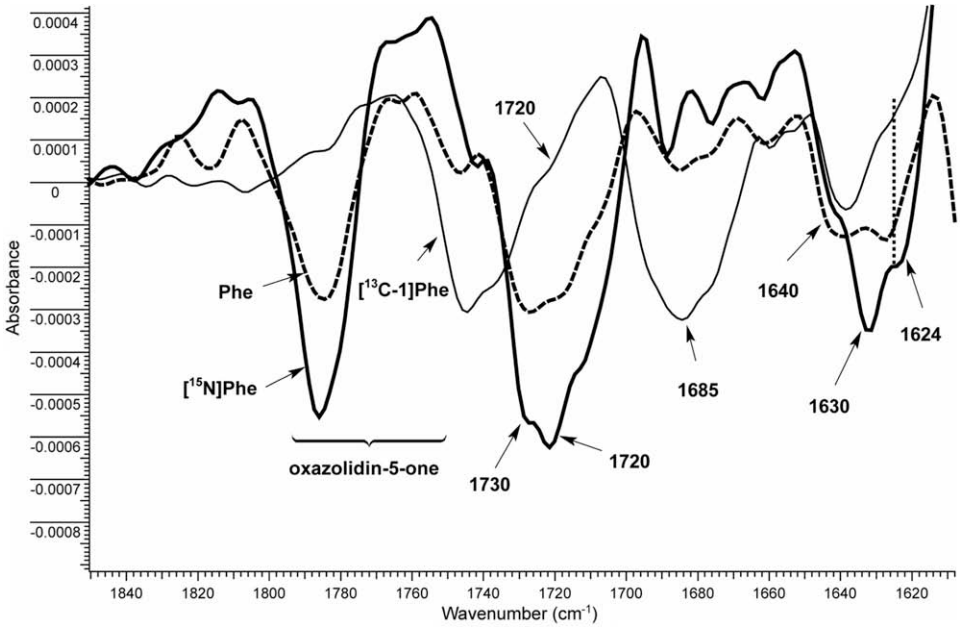

Figure 6. Second-derivative spectra (Savitsky Golay polynomial 2, points 15) of toluene extracts of phenylalanine–glycolaldehyde, [^{13}C-1]phenylalanine–glycolaldehyde, and [^{15}N]phenylalanine–glycolaldehyde reaction mixtures heated at 80 °C for 10 min.

1720 cm^{-1}. Both peaks appeared and increased simultaneously with temperature. Contrary to the peak at 1730 cm^{-1}, the peak at 1720 cm^{-1} did not shift when [^{13}C-1]phenylalanine was used as shown in Figure 3b, and it was confirmed by a second-derivative spectrum (Fig. 6).

Furthermore, to assign the bands of the two imines formed after decarboxylation of the oxazolidin-5-one intermediate, the glycolaldehyde–phenethylamine and glycolaldehyde–phenethylamine·HCl were analyzed by FTIR to assign the absorption band of imine **2a** in Figure 2. Figure 7 shows the formation of a band centered around 1640 cm^{-1} when phenethylamine was used, as well as the appearance of two additional bands at 1666 and 1702 cm^{-1} when phenethylamine was replaced with phenethylamine·HCl. Under the strong basic conditions of phenylethylamine, Amadori rearrangement is not encouraged and only initial imine is formed at 1640 cm^{-1} as shown in Figure 7. However, under the acidic conditions of phenetylamine·HCl, it is expected that the imine will rearrange into the Amadori product. Figure 7 shows the appearance of two bands one at 1666 and the other at 1702 cm^{-1}. The former was tentatively assigned as an enaminol band, and the latter as the aldehyde of the Amadori compound. Similar experiments were performed with ethanolamine–phenylacetaldehyde and ethanolamine·HCl–phenylacetaldehyde to identify the absorption band of imine **2b** in Figure 2. Figure 8 indicates the formation of a band centered at 1637 cm^{-1} with a shoulder at 1624 cm^{-1}. The peak at 1637 cm^{-1} completely

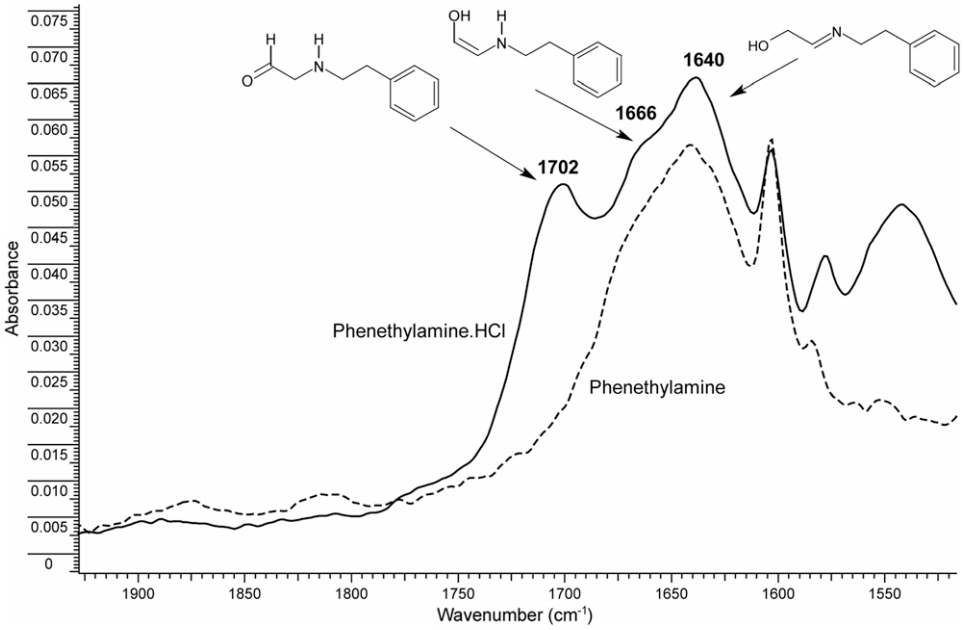

Figure 7. Absorption of the carbonyl and the imine regions (1800–1550 cm^{-1}) of the phenethylamine–glycolaldehyde (dashed line) and phenethylamine·HCl–glycolaldehyde (solid line) reaction mixtures heated at 70 °C for 2 min.

collapsed into the 1624 cm^{-1} band when ethanolamine·HCl was used, indicating the acid-catalyzed isomerization of the imine **2b** into a conjugated system as shown in Figure 8. The results of the above band-assignment studies are summarized in Figure 2.

2.3. FTIR monitoring of oxazolidin-5-one formation and decomposition in the glycolaldehyde–phenylalanine model system

The FTIR spectrum (see Fig. 3a) of the toluene extract of a heated (1 min at 110 °C) mixture of glycolaldehyde–phenylalanine indicated the presence of mainly oxazolidin-5-one intermediate in this extract. Figure 3a also shows the absorption band of the carboxylate anion at 1628 cm^{-1} of the Schiff base **1a** in Figure 2, indicating the formation of oxazolidin-5-one from precursor **1a**. After 24 h of storage at room temperature, the toluene extract did not show any decomposition as opposed to the methanol extract (prepared in a similar fashion at 65 °C) that showed complete disappearance of the oxazolidin-5-one band and formation of a dark-brown solution. Monitoring of the toluene extract on the ATR crystal at 80 °C indicated a fast decomposition over a period of 10 min and its conversion into the Amadori product as indicated by the concomitant appearance of a carboxylic acid band centered at 1730 cm^{-1} and a carbonyl band centered at 1720 cm^{-1} (see Fig. 5). Furthermore, examination of the second-derivative spectra of the toluene extracts generated from phenylalanine–

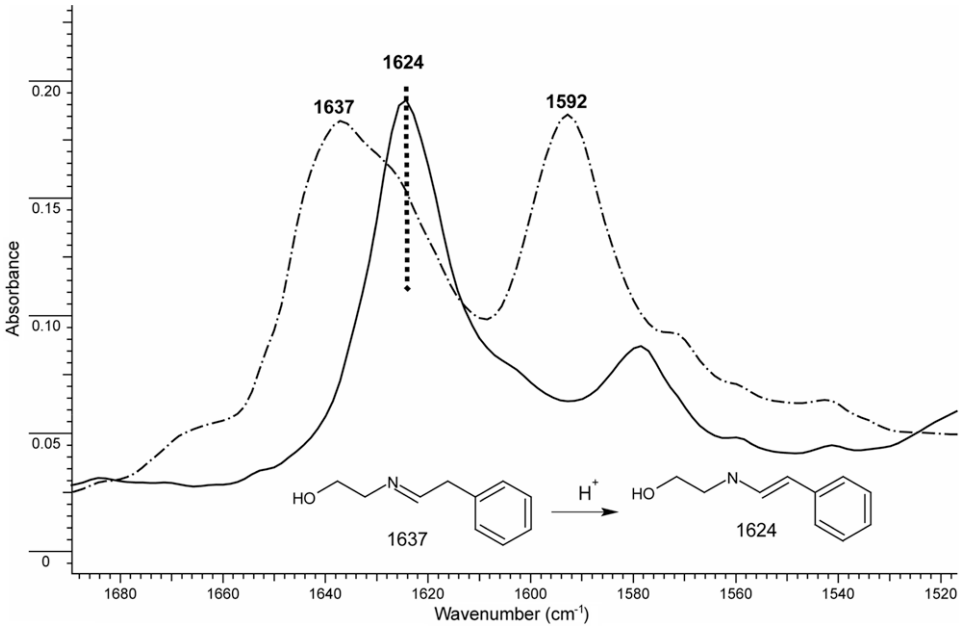

Figure 8. Absorption of the carbonyl and the imine regions (1800–1550 cm^{-1}) of the ethanolamine–phenylacetaldehyde (dash-dot) and ethanolamine·HCl–phenylacetaldehyde (solid line) reaction mixtures heated at 35 °C for 2 min.

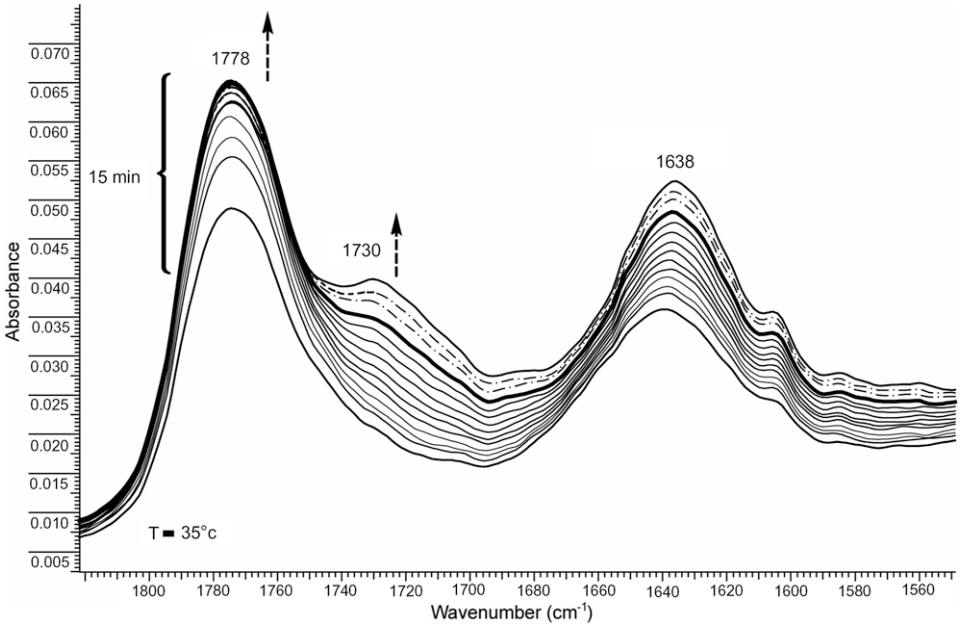

Figure 9. Time-dependent spectra of glycolaldehyde–phenylalanine mixture heated at 35 °C for 55 min showing the initial 15 min.

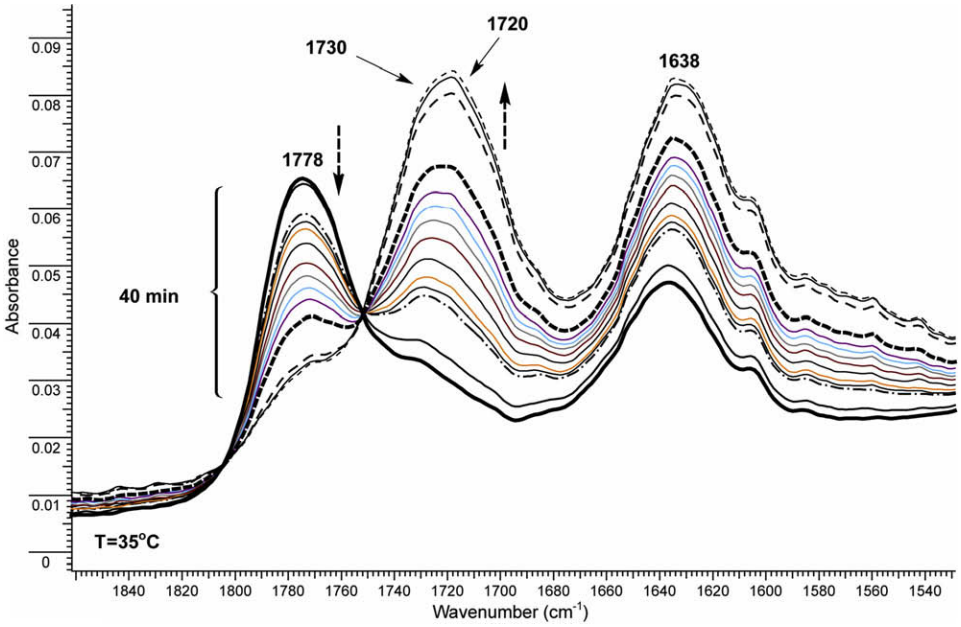

Figure 10. Time-dependent spectra of glycolaldehyde–phenylalanine mixture heated at 35 °C for 55 min showing the final 40 min.

glycolaldehyde, [^{13}C-1]phenylalanine–glycolaldehyde, and [^{15}N]-phenylalanine–glycolaldehyde heated at 80 °C for 10 min (see Fig. 6) indicated the formation of imines (1620–1650 cm^{-1} region). Although the intensities of these bands were weak, there is clear indication for the formation of imines **2a** and **2b** that absorb very close to each other (1640 vs 1637 cm^{-1}) and appear centered around 1640 cm^{-1} in the phenylalanine and [^{13}C-1]phenylalanine model systems, but, as expected, shifted to 1630 cm^{-1} in the [^{15}N]phenylalanine system (Fig. 6). The presence of a peak at 1624 cm^{-1} further confirms the formation of imine **2b** (see Fig. 8). There was no evidence for the presence of a peak at 1702 cm^{-1} (decarboxylated Amadori product), which was expected from the results of the model studies with glycolaldehyde–phenethylamine·HCl. However, considering the fact that the 1702 cm^{-1} peak in this model system quickly degraded when the sample was monitored over a 10-min period at 70 °C, it is, therefore, not expected to be detected at 80 °C.

To monitor the formation and decomposition of the oxazolidin-5-one intermediate, excess glycolaldehyde was melted by heating in methanol and evaporating the solvent. The melt was applied onto the ATR crystal, followed by addition of powdered phenylalanine on the surface of the melt (see Section 3). Even at 35 °C, the rate of oxazolidin-5-one formation was very fast. By the time the first spectrum was acquired, the peak at 1778 cm^{-1} was already formed (see Fig. 9). Within 15 min of the acquisition of the first spectrum, the intensity of the peak started to decrease, and it completely disappeared in the next 40 min with the concomitant formation and increase of Amadori product as indicated by the appearance of carboxylic acid and aldehyde peaks at 1730 and 1720 cm^{-1}, respectively (see Fig. 10). Attempts to isolate the Amadori product from the mixture were not successful due to the instability of the product.

2.4. Implications of formation of an oxazolidin-5-one intermediate in the mechanism of the Amadori rearrangement

The fact that the Amadori product appeared and increased in intensity only after the collapse and decrease of the oxazolidin-5-one intermediate (see Figs. 3, 5, and 10) indicates that this intermediate also undergoes Amadori rearrangement similar to the Schiff base. Consequently, ring opening initiated by the nitrogen atom leads to decarboxylation of the oxazolidin-5-one and formation of the azomethine ylide, and ring opening through a proton shift of the C-2 hydrogen atom of the sugar moiety leads to the formation of enaminol **1b**, followed by an Amadori rearrangement as indicated in Figure 2. This observation may explain why the formation of the Amadori rearrangement product of phenethylamine with glycolaldehyde required a temperature of 70 °C (see Fig. 7), whereas Amadori product formation with phenylalanine occurred even at 35 °C (see Fig. 9). Although it can be argued that the oxazolidin-5-one intermediate can revert to **1a** and then undergo Amadori rearrangement, if this were the case, the peaks associated with the Amadori product would have appeared before the appearance of oxazolidin-5-one peak in Figure 9.

In conclusion, the detection of an oxazolidin-5-one intermediate as an immediate precursor of the Amadori rearrangement product not only contributes to our detailed understanding of this important reaction, but also can explain why amino acids are more reactive than their amine counterparts in undergoing the Amadori rearrangement process.

3. Experimental

All reagents and chemicals were purchased from Sigma–Aldrich (Milwaukee, WI) and were used without further purification. The labeled [^{13}C-1]phenylalanine and [^{15}N]phenylalanine were purchased from Cambridge Isotope Laboratories (Andover, MA).

3.1. Extraction of oxazolidin-5-one 'toluene extract' and FTIR analysis

An equimolar mixture of glycolaldehyde (10 mg) and phenylalanine (6 mg) was heated in toluene (200 μL) for 1 min (the solution turns light yellow with the formation of a brown residue) at 110 °C in an open vial (1 mL). Samples (1 μL) of the supernatant solution above the residue were repeatedly applied and evaporated onto an ATR crystal and immediately scanned at specified temperatures. FTIR spectra were recorded on a Bruker Alpha-P FTIR spectrometer (Bruker Optic GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector, a temperature-controlled

single-bounce diamond attenuated total reflectance (ATR) crystal, and a pressure application device for solid samples. At a fixed temperature, the spectra were acquired every 60 s for 20 min or 40 min. A total of 32 scans at 4-cm^{-1} resolution were co-added. Processing of the FTIR data was performed using Bruker OPUS software.

3.2. FTIR monitoring of oxazolidin-5-one and imine formation

Glycolaldehyde dimer (10 mg) was heated in MeOH (100 μL) for 5 min, and the solvent was evaporated to yield a colorless melt. The melt was applied onto the ATR crystal, and L-phenylalanine powder (6 mg) or phenethylamine or phenethylamine·HCl was spread over the melt. The infrared spectra were immediately recorded at the indicated temperatures every 60 s over a 20-min period on a Bruker Alpha-P spectrometer (Bruker Optic GmbH, Ettlingen, Germany) described above. Similarly, phenylacetaldehyde was applied onto the ATR crystal, followed by ethanolamine or ethanolamine·HCl, and immediately the infrared spectra were recorded at indicated temperatures.

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