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Temperature-induced conformational changes in amyloid β(1-40) peptide investigated by simultaneous FT-IR microspectroscopy with thermal system

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Abstract

Temperature-dependent secondary structures of the amyloid β(1-40) peptide in the solid state were studied by simultaneous Fourier transform infrared/differential scanning calorimetry (FT-IR/DSC) microspectroscopic system with the heating-cooling-reheating cycle. The result indicates that a thermal transition temperature at 45°C was easily obtained from the three-dimensional plot of the transmission FT-IR spectra as a function of temperature. Furthermore, the thermal-dependent conformational transformations, due to denaturation and aggregation, of solid amyloid β(1-40) were mainly evidenced by reducing the compositions from 37 to 20–24% for α-helical and random coil structures but increasing the components from 27 to 45% for intermolecular β-sheet structures. Thermal-irreversible behavior and a poor thermal stability of solid amyloid β(1-40) were also observed from the poor restoration of the secondary conformational changes in the heated sample. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Amyloid β(1-40) peptide; Secondary structure; Stability; Fourier transform infrared/differential scanning calorimetry; Transition temperature

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease of the aged population that causes dementia. It is characterized by the deposition of amyloid which induces pathological brain lesions [1,2]. Extracelluar depositions consist of amyloid plaques and/or cerebrovascular amyloid, while intracellular depositions contain neurofibrillary tangles [3,4]. The major protein constituent of the depositions is amyloid β-peptide (Aβ), which is 39–43-residue peptide derived from

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the amyloid β-protein precursor. Aβ(1-40) and Aβ(1-42) are the predominant proteins in neuritic plaque while Aβ(1-43) is a minor component, but Aβ(1-39) is a major component in cerebrovascular deposits [3,5]. Three functional areas of the soluble Aβ peptide have been proposed: a lipophilic region in the middle of the peptide having an α-helical structure stabilized by intramolecular hydrogen-bonding forces, a lipophilic core at the end of peptide having a β-sheet structure to interact with other β-sheet conformation by hydrophobic forces, and polarized and charged, solvent-exposed areas to cross-link with membrane-bound receptors [3].

Freshly prepared solutions of Aβ(1-40) and Aβ(1-42) peptides are found to be non-toxic or less toxic, but neurotoxicity is observed after formation of a β-sheet structure induced by a suitable chemical medium [6,7] and can be associated with the degree of peptide aggregation and is enhanced with the increase of the aggregation [8]. This conformation-dependent biological activity of Aβ is very important and critical, since it might be related to the differences in aggregation state and types of solvent, although the aggregation state alone is not sufficient to predict the neurotoxicity [4,6,8,9]. Several investigations have focused on the study of conformational changes of Aβ in solution [10–12], but there was less attention given to the intact Aβ in solid state [13].

Fourier transform infrared (FT-IR) spectroscopy has been extensively used to investigate the secondary structure of diverse proteins in solution or in solid state [14–16]. The temperature-dependent conformation of proteins has also been studied by FT-IR but using several indirect determination methods. We have used a novel FT-IR microspectroscopy equipped with differential scanning calorimetry (DSC) to directly and simultaneously determine the thermo-dependent structural change of drugs, polymers, skin and proteins [17–21]. This one-step synchronous operation is fast, simple, sensitive, precise and reproducible. In the present study, we also used this microscopic FT-IR/DSC system with transmission mode to examine the intrinsic thermal-stability and conformational structure of Aβ(1-40) peptide in solid state by a heating-cooling-reheating cycle.

2. Materials and methods

2.1. Materials

The peptide Aβ(1-40) (A-1075, Lot No. 59H49551) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) without further purification. The commercially synthesized peptide was supplied as lyophilized white powder. Evaluated by the supplier, the peptide content and purity of Aβ(1-40) determined by amino acid analysis and HPLC were 75% and 99%, respectively. All samples were stored at −20°C, and allowed to warm to room temperature prior to use.

2.2. Transmission FT-IR microscopic determinations

The micro-samples of lyophilized Aβ(1-40) powder were adhered in part with cellophane tape and then directly determined by FT-IR microscopic spectrometer (Micro FTIR-200, Jasco Co., Japan) equipped with an MCT (mercury-cadmium telluride) detector using transmission technique [17,18]. The spectra of sample without compression with KBr were taken at 4 cm^{-1} resolution and at 200 scans. The position and focus of the sample were adjusted microscopically by means of an aperture through optical system (ATOS) for analysis. The aperture size employed was approximately 10 $\mu m \times 10$ μm.

2.3. Transmission FT-IR / DSC microscopic measurements

A very small amount of Aβ(1-40) powder was sealed into two pieces of KBr pellets (size: 2 mm × 2 mm, thickness: 0.5 mm) by a hydraulic press under 200 kg cm^{-2} for 15 s. The compressed sample was put directly into the DSC microscopy cell (FP 84, Mettler, Switzerland). This DSC cell was then settled on the stage of FT-IR microscopic spectrometer (Micro FTIR-200, Jasco, Japan) with an MCT detector. The system was operated in the transmission mode. The position and focus of the sample were adjusted microscopically through ATOS. The temperature of DSC system was monitored with a central processor (FP 80 HT, Mettler, Switzerland). The heating

rate of DSC assembly was controlled at 3° min^{-1} in the first or second-heating course from 25 to 120°C. The DSC heating program and IR spectra could be simultaneously recorded [18–21]. The temperature-dependent IR spectra at each point were determined with a resolution of 4 cm^{-1} and 20 scans.

2.4. Data acquisition and handling

An FT-IR thermal data analytical program of Jascoft for Window (Jasco Co., Japan) was used for data acquisition and handling. The protein secondary structure and composition of each component in amide I band of these IR spectra were estimated quantitatively by a least-squares fitting program. Second-derivative spectra were first used to verify the peak positions. The program iterates the curve-fitting process according to the Gaussian function. The fitting adjustment was performed until the synthetic curve matched the experimental one with a precision factor of $\leq 1\%$. The proportion of a component was computed to be the fractional area of the corresponding peak, divided by the sum of the areas of all the peaks. Three individual spectra were carried, and the mean was obtained. The error ($n = 3$) in the relative area is $\pm 1\%$ for area $> 20\%$ and $\pm 2\%$ for area $< 20\%$.

3. Results

Fig. 1 shows the transmission FT-IR spectra of solid Aβ(1-40) before and after compression with KBr pellet. Obviously, the peaks of both samples were at the same wavenumbers, indicating the compression process had little influence on the conformational change of the Aβ(1-40) peptide. Three-dimensional plot of the transmission FT-IR spectra of solid Aβ(1-40) within 3600–2800 and 1800–1000 cm^{-1}, as a function of temperature, is shown in Fig. 2. Once the solid Aβ(1-40) was first heated from 25 to 120°C, all the IR spectral frequencies and band shapes except at 1653 and 1541 cm^{-1} did not change with the increase of temperature. The peak at 3296 cm^{-1} was assigned to the NH stretching of amide A of protein. The peaks at 2966 and 2935 cm^{-1} were due to the asymmetric CH_3 and CH_2 stretching bands of protein, while the band at 2877 cm^{-1} was associated to the symmetric stretching mode of the CH_3 group which corresponded to the methyl groups of the side chains of proteins. The peaks at 1653 and 1541 cm^{-1} were assigned to the combination of major α-helix and minor random coil structures in amide I and II bands. The peak at 1400 cm^{-1} was due to the COO^- symmetric stretching band and/or the deformation of CH_2 and CH_3. The peaks at 1203 and 1138 cm^{-1} corresponded to the C–N and C–O stretching bands, respectively. In the first heating process, the peak at 1653 cm^{-1} assigned to the predominant α-helical structure gradually transformed to 1670 and 1631 cm^{-1} with the increase of temperature (Figs. 2 and 3a,b), but there appeared a thermal transition between 40 and 50°C. The peak at 1670 cm^{-1} was due to β-turn structure, but the peak at 1631 cm^{-1} was due to β-sheet structure [14–16]. Furthermore, the peak at 1541 cm^{-1} assigned to the predominant α-helix structure also shifted to 1537 (random coil) and 1523 (β-sheet) cm^{-1} with the increase of temperature. Fig. 3c reveals the temperature-induced alterations in peak intensity of four specified peaks at 1653, 1631, 1541 and 1523 cm^{-1} in amide I and II bands. The intensities of all these specified peaks kept constant from 25 to 45°C, then changed markedly to reach 75–80°C and finally maintained almost constant level beyond 80°C. Obviously, the marked change was found near 45°C, attributable to the thermal transition temperature of intact solid Aβ(1-40). This indicates that the transmission FT-IR/DSC microscopic system can easily and directly determine the intrinsic transitional temperature of Aβ(1-40).

When the first-heated Aβ(1-40) sample was reheated from 25 to 120°C, the frequency and shape of the three-dimensional transmission FT-IR spectra between 1800 and 1000 cm^{-1} almost did not change, as shown in Fig. 4. Moreover, the Aβ(1-40) sample first-heated to 120°C had the same IR spectra as the heated Aβ sample which did not restore to the original structure when the temperature returned to 25°C, suggesting the denaturation and irreversible properties of the solid

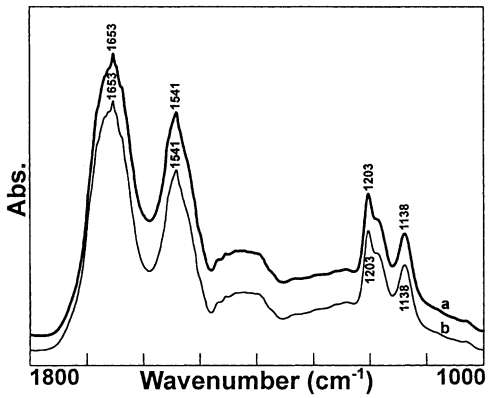

Fig. 1. The transmission FT-IR spectra of solid Aβ(1-40) before (a) and after (b) compression with a KBr pellet.

Aβ(1-40) after heating, particularly to amide I band. The temperature-induced variation of bands related to the amides I and II in the first and second heating courses is shown in Fig. 5. Clearly, both maximum peaks for amide I at 1653 cm^{-1} and amide II at 1541 cm^{-1} a had markedly decreased wavenumber from 45 to 50°C in the first heating process, in which the amide I band was more pronounced. During the reheating process, there was almost constant wavenumber maintained at 1631 cm^{-1} for the amide I band but the amide II band still shifted its wavenumber from 1537 to 1525 cm^{-1} and a transition temperature also showed at 45°C. It appears that the amide I band of Aβ(1-40) had a thermal-irreversible property but the amide II band was not confined to an irreversible structure. This strongly confirms that the amide I band is much more sensitive to the modification in the secondary conformation of the proteins and peptides than the amide II band after external change [14–16]. The thermal transition temperature was near 45°C for solid Aβ(1-40). Moreover, the thermal-irreversible property of Aβ(1-40) after heating treatment implies that Aβ has a poor thermal stability.

4. Discussion

Recently, a number of degenerative diseases are recognized to be related to abnormalities in protein conformation [22,23]. Thus, the relationship between the conformational change of protein and diseases has been considerably interested and critical. A proposed mechanism in the pathogenesis of these diseases is the conversion of soluble normal protein into its insoluble and aggregated form with rich β-sheet structure [1,2]. Amyloid aggregates have been recognized to be a pathological hallmark of several fatal diseases, such as AD, the prion-related diseases, Down's syndrome and type II diabetes, due to the conversion of the normal soluble amyloid to aggregated amyloid within neuritic plaques and cerebral vessels [22,23].

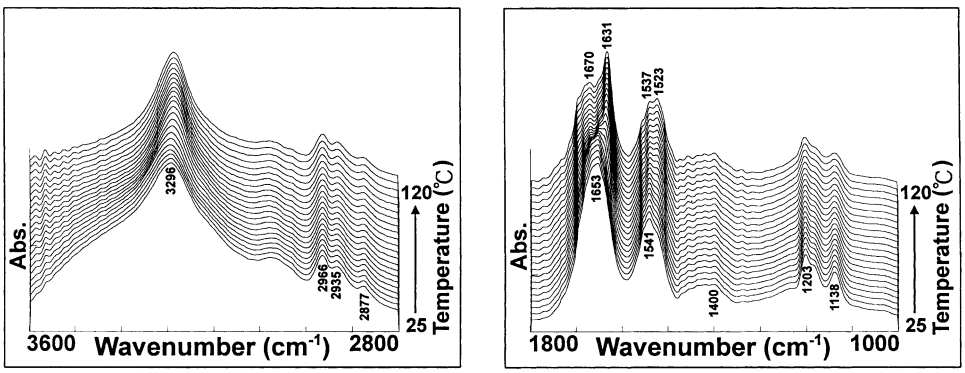

Fig. 2. Three-dimensional plot of the transmission FT-IR spectra of a solid Aβ(1-40) peptide within 3600–2800 and 1800–1000 cm^{-1} with respect to the first-heating process.

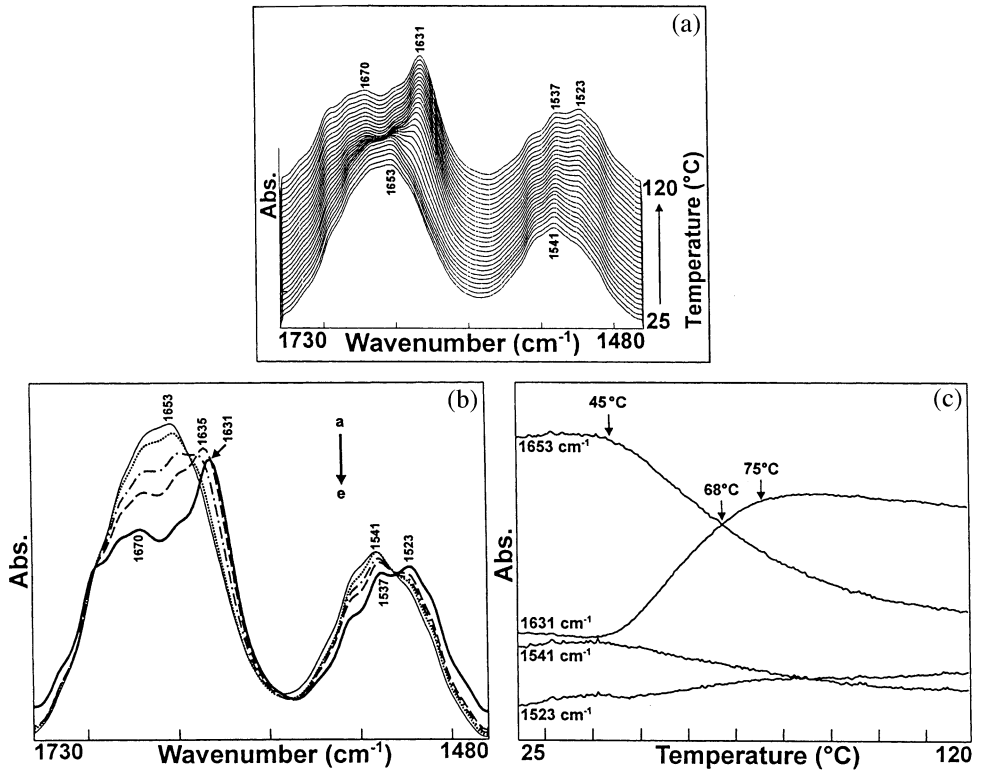

Fig. 3. Temperature-dependent changes in amide I and II bands (a,b) and in four specified peak intensities of solid Aβ(1-40) peptide (c). Sample at a, 25°C; b, 50°C; c, 60°C; d, 70°C; e, 120°C.

The present study reveals that the amide I band of Aβ(1-40) had an irreversible property but the amide II band was not confined to this irreversible structure during reheating process (Fig. 5). The reason might be due to the amide I band was more sensitive to modify the secondary conformation of the proteins and peptides than the amide II band after external change [14–16]. Due to the thermal-irreversibility and thermal-sensitivity of amide I band, furthermore, the Aβ(1-40) peptide after first-heating process could not restore to original structure but transformed to another compound. This compound maintained a constant peak position at 1631 cm^{-1} for amide I band but changed in peak position from 1537 cm^{-1} to 1525 cm^{-1} for amide II band with the increase of temperature. The changes in amide II band is not related to the original Aβ(1-40) peptide during second-heating process.

It is also evident that the amide I band of the intact solid Aβ(1-40) peptide had a maximum

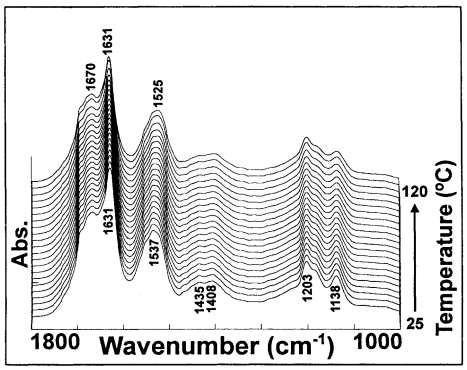

Fig. 4. Three-dimensional plots of transmission IR spectra of the heated Aβ(1-40) peptide between 1800 and 1000 cm^{-1} with respect to the reheating process.

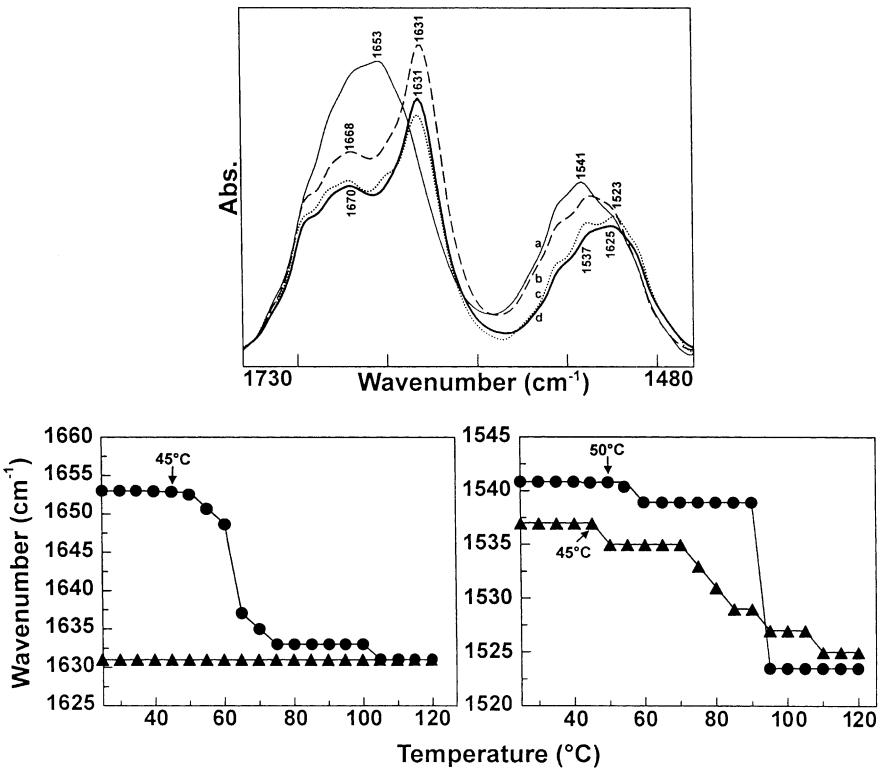

Fig. 5. The shift of amides I and II bands of solid Aβ(1-40) peptide in the first (b)and second (c) heating processes. a, native solid Aβ(1-40) peptide without heating (25°C); b, first-heated sample after cooling to 25°C; c, native solid Aβ(1-40) peptide after first-heating to 120°C; d, first-heated sample after reheating to 120°C.

peak at 1653 cm^{-1} associated with the predominant α-helix and less random coil structures, suggesting a higher proportion of α-helix conformation existing in the intact solid Aβ(1-40) [14–16]. With the increase of first-heating temperature, however, the maximum peak at 1653 cm^{-1} gradually transformed to 1670 and 1631 cm^{-1}, particularly from 45°C. Both peaks were assigned to β-structure, and the transformation of α-helix to β-structure in first heating process occurred at approximately 45°C, as indicated in Figs. 6 and 7. Fig. 6 shows the curve-fitted component bands in four representative amide I spectra of Aβ(1-40), in which the peaks at 1695, 1685, 1628 and 1618 cm^{-1} were due to the intermolecular β-sheet structures, the peak at 1668 cm^{-1} was assigned to β-turn structure, the peak at 1649 cm^{-1} corresponded to the combination of α-helix and random coil conformations, the peaks at 1637 and 1632 cm^{-1} were attributable to intramolecular β-sheet structures, and the peak at 1609 cm^{-1} is associated to the side chain of amino acids [14–16], respectively. The changes in the secondary structural components of solid Aβ(1-40) with the first-heating temperature are plotted in Fig. 7. It is evident that within the range of 45–50°C there appeared an obvious reflection point. Beyond this range, the components of secondary structures changed from 31 to 28% for β-turn, from 37 to 20–24% for α-helix and random coil, from 27 to 45% for intermolecular β-sheet, and from 5 to 0% for intramolecular β-sheet structures, respectively. In the this heating process, the main change in composition was 15%, which was due to the transformation of α-helix and random coil structures to the inter-

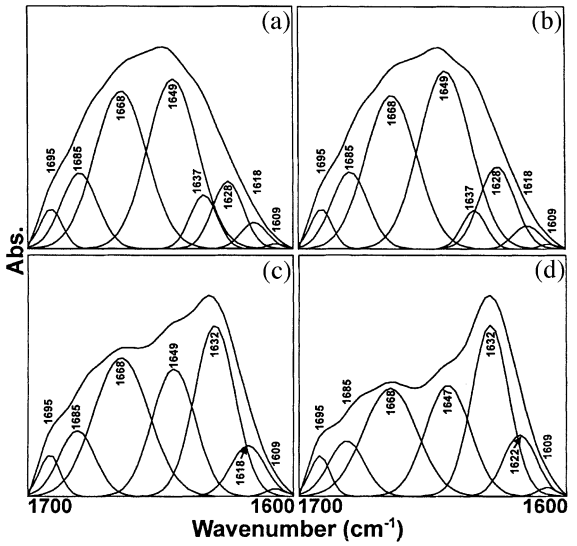

Fig. 6. Transmission IR spectra and the best-fitting individual component bands within the 1700–1600 cm^{-1} region of amide I band for solid Aβ(1-40) peptide in the first-heating process. Sample at a, 25°C; b, 50°C; c, 70°C; d, 120°C.

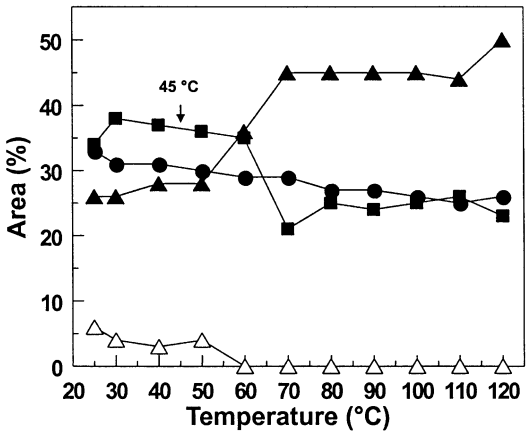

Fig. 7. Temperature-induced changes in secondary structures of solid Aβ(1-40) peptide in the first heating process. ▲, intermolecular β-sheet; ●, β-turn; ■, α-helix and random coil; △, intramolecular β-sheet.

molecular β-sheet structure. The thermal-dependent denaturation and aggregation of solid Aβ(1-40) were clearly evidenced by this increase in intermolecular β-sheet structures.

Many scientists believe that the protein conformational change is critical to the disease process. Furthermore, the progression of the disease may

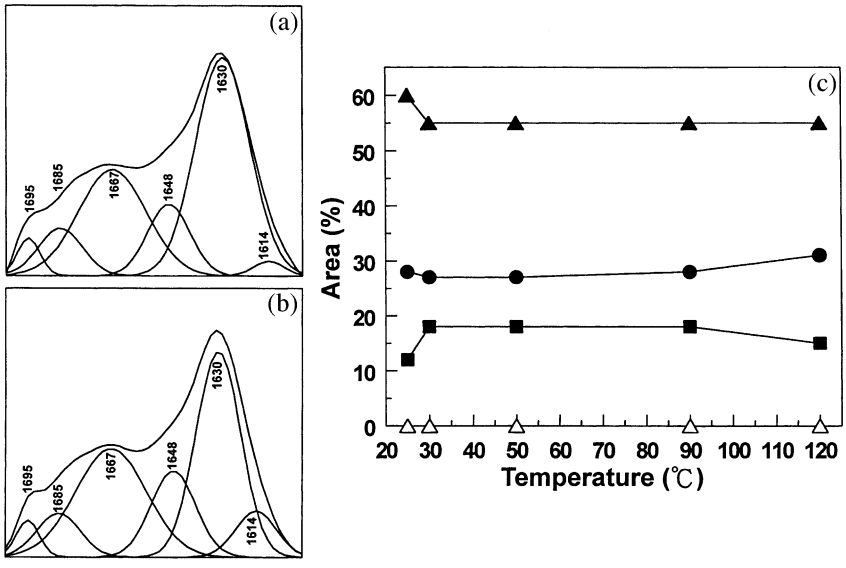

Fig. 8. Temperature-induced changes in secondary structures of the heated Aβ(1-40) peptide in the second heating process. (a) first-heated sample after cooling to 25°C; (b) first-heated sample after reheating to 120°C; (c) ▲, intermolecular β-sheet; ●, β-turn; ■, α-helix and random coil; △, intramolecular β-sheet.

be related to the changes in protein secondary structure so as to form insoluble β-sheet plaques [3,12,24]. The transformation of α-helix to β-sheet structure for common protein or peptide after external treatments (such as pH, temperature, peptide concentration, pressure, etc.) has been widely investigated and conformed by the order of progress from α-helix, random coil and then to β-sheet structure [9,10,25–28]. In this study, much formation of β-sheet structure by heating the Aβ(1-40) peptide was also evident in the same process. The mechanism should be more studied, but Aβ(1-40) fibril formation from aggregation has been proposed by a first-order kinetic model in solution, in which this fibrilization was consecutively associated with the binding of Aβ(1-40) monomers onto the ends of existing fibrils [29,30].

Once the heated Aβ(1-40) sample was reheated from 25 to 120°C, the composition of each component kept almost constant independent of the temperature (Fig. 8). There was no significant change in the secondary structures for the heated solid Aβ(1-40) during second-heating process. The obvious difference in the secondary structural compositions observed for the solid Aβ(1-40) between first- and second-heating processes implied the thermal denaturation and irreversible property of solid Aβ(1-40). This was markedly contrast to the thermal behavior of α-crystallin, in which α-crystallin had high thermal stability and reversibility via the heating process [20]. The difference in constitution, construction and types of protein might be responsible for this contrary results.

In conclusion, the solid Aβ(1-40) had a thermal-irreversible property and a poor thermal stability. The thermal-dependent denaturation and aggregation of solid Aβ(1-40) were clearly evidenced by the increase of intermolecular β-sheet structures. Moreover, a thermal transition temperature near 45°C was found for solid Aβ(1-40).

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