Fourier transform near infrared spectroscopic study of dipeptides in chloroform

Mitsuhiro Miyazawa[a] and Masashi Sonoyama[b]

[a]*Laboratory of Chemical Prospecting, National Institute of Sericultural and Entomological Science, 1-2 Ohwashi, Tsukuba Science City, Ibaraki 305-8634, Japan.*

[b]*Department of Biotechnology, Faculty of Technology, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei City, Tokyo 184-8588, Japan.*

Introduction

Near infrared (NIR) measurement has become one of the most powerful techniques for non-destructive analysis in agricultural or industrial research and has easily offered quantitative and qualitative information for a wide range of samples. Nevertheless, NIR spectroscopy has rarely had the chance to be applied to the structural analysis of biological molecules. In our previous study,[1] NIR spectral analysis with the second derivative procedures indicated that the typical globular proteins in film state give only one NIR band near 4865 cm^{-1} (combination of amide A and II) in spite of a different relative amount of secondary structures. On the other hand, the broad feature centered at 4610 cm^{-1} consists of several overlapping components and each relative intensity is significantly changed with the proteins. Although this NIR band near 4610 cm^{-1} has been frequently used for the estimation of secondary structures in proteins,[2,3] precise interpretation is still under discussion.

N-Acetyl-N′ -methylamide derivatives of amino acids (Me-CO-NH-CHR-CO-NH-Me; R = side chain of amino acid) are often utilised as the simplest model peptides of proteins and investigated by means of various physicochemical methods.[4–22] In this work, NIR spectra of blocked dipeptides were investigated in order to obtain more detailed information on NIR bands between 4800 and 4500 cm^{-1}.

Methods

The dipeptides investigated in the present study were N-acetyl-N′ -methylamide derivatives of amino acids, alanine (Ala), glycine (Gly), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) and valine (Val); Ac-Ala-NHMe, Ac-Gly-NHMe, Ac-Ile-NHMe, Ac-Leu-NHMe, Ac-Phe-NHMe and Ac-Val-NHMe, where Ac = CH_3CO- and Me = CH_3. The methylester derivative, Ac-Ala-OMe, was also employed in order to discriminate the vibrational mode between peptide group and other functional group. All samples were obtained from Sigma Chemical Co and were used without further purification in all experiments. A CaF_2 cell with various pathlengths (2 cm–0.1 mm) were used in measurements of NIR and mid infrared spectra. All spectra were recorded on a Jasco *Herschel-350* Fourier-transform (FT) infrared spectrophotometer equipped with an NIR attachment at 4 cm^{-1} spectral resolution.

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Results and discussion

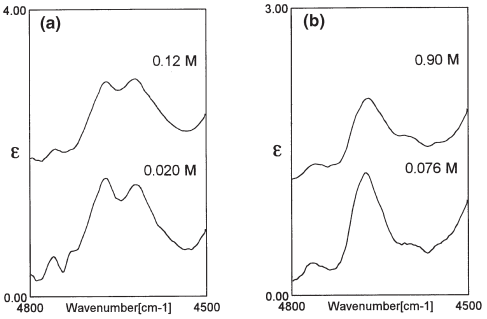

Figure 1. FT-NIR spectra of Ac-Ala-NHMe (a) and Ac-Ala-OMe (b).

NIR spectra of Ac-Ala-NHMe and Ac-Ala-OMe in chloroform solution are shown in Figure 1. Two distinct bands at 4621 and 4671 cm^{-1} were observed for Ac-Ala-NHMe, whose relative intensity is slightly changed at different concentrations. Several investigations suggested that Ac-Ala-NHMe molecules exist in equilibrium with an intermolecularly hydrogen-bonded association at high concentration.[11,14] Band assignments in the 5000–4500 cm^{-1} region of proteins or peptides have been investigated to some extent. According to the literature,[23–25] the band at 4621 cm^{-1} is due to the combination of amide I and III or amide B and II vibrational mode. Although the amide B absorption band exhibits near 3100 cm^{-1} at high concentrated solution (spectra not shown), it disappears at low concentration. However significant NIR intensity at 4621 cm^{-1} was observed in dilute solution. Thus, this NIR band is thought to be due to a combination of amide I and amide III mode. Spectral change in intensity is primarily expected to reflect molecular association. Ac-Ala-NHMe and Ac-Ala-OMe show a prominent peak at 4671 cm^{-1}. Holly *et al.*[24] carried out NIR measurements with alanine and N-deuterated alanine and their studies indicated that the band at 4670 cm^{-1} is due to the combination of the $-N^+H_3$ and $-CH_3$ group. However, the two alanine derivatives in this work do not contain the $-N^+H_3$ group. In our previous study [1] we observed a significant NIR band with the same frequency in globular proteins. Therefore, the band at 4670 cm^{-1} in alanine derivatives might be responsible for a peptide groups.

NIR spectra of Ac-Gly-NHMe, Ac-Ile-NHMe and Ac-Val-NHMe at different concentration are shown in Figure 2. In the case of three dipeptides, the peaks around 4620 cm^{-1} are assigned to the amide I–amide III combination modes and high-wavenumber components near 4670 cm^{-1} are due to the peptide groups. NIR spectra of Ac-Ile-NHMe and Ac-Val-NHMe exhibit small band at 4620 cm^{-1}, differing from other dipeptides, which are indicative of the presence of a formation other than the associ-

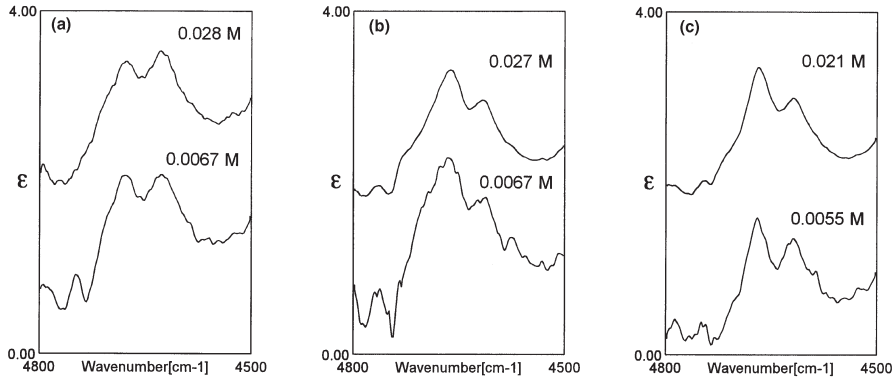

Figure 2. FT-NIR spectra of Ac-Gly-NHMe (a), Ac-Ile-NHMe (b) and Ac-Val-NHMe (c).

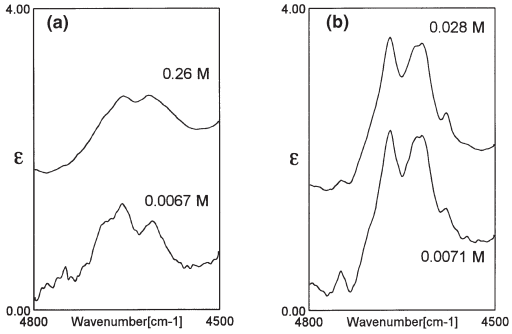

Figure 3. FT-NIR spectra of Ac-Leu-NHMe (a) and Ac-Phe-NHMe (b).

ated structure. No obvious changes in this NIR region could be observed because of the low solubility of the test compounds.

Figure 3 shows NIR spectra of Ac-Leu-NHMe and Ac-Phe-NHMe. The high solubility of Ac-Leu-NHMe enables experimentation over a wide concentration range. The band at 4609 cm^{-1} exhibits a peak position shift toward the high wavenumber side as well as an increase in intensity when the concentration is increased. Both shift and intensity of the band may be depend on equilibrium between associated and monomer species. In addition, a characteristic shoulder band near 4690 cm^{-1} was observed. Leucine residue contains several kinds of CH vibration in the side chain. Therefore, this significant shoulder band would be expected in the combination mode involving CH vibration. The NIR spectra of Ac-Phe-NHMe displays different features, which give rise to four bands at

Table 1. Observed frequencies in chloroform solutions.

Dipeptides	Frequency (cm^{-1})	
Ac-Ala-NHMe	(0.020 M) 4671 4621	(0.12 M) 4671 4621
Ac-Ala-OMe	(0.076 M) 4675 4609	(0.90 M) 4671 4609
Ac-Gly-NHMe	(0.0067 M) 4679 4626	(0.028 M) 4679 4628
Ac-Ile-NHMe	(0.0067 M) 4665 4613	(0.027 M) 4663 4615
Ac-Val-NHMe	(0.0055 M) 4670 4619	(0.021 M) 4669 4617
Ac-Leu-NHMe	(0.0067 M) 4686 4657 4609	(0.26 M) 4686 4655 4615
Ac-Phe-NHMe	(0.0071 M) 4671 4626 4619 4580	(0.028 M) 4671 4628 4619 4578

4671, 4628, 4619 and 4578 cm^{-1}. Wang *et al*.[25] suggested that aromatic polypeptides such as poly-Phe, poly-Tyr and poly-Trp show peaks due to aryl CH stretch– aryl CH bend combinations in the 4400–4700 cm^{-1} region. Hence, the bands below 4630 cm^{-1} of Ac-Phe-NHMe are assignable to the aryl CH combinations and amide I and III combinational mode. The highest frequency band at 4671 cm^{-1} is due to the peptide group.

All the observed band frequencies are listed in Table 1.

Conclusions

The present study investigated the NIR spectra of dipeptides as the model compound of proteins. These observations indicated that several kinds of combination bands lie close the 4800–4500 cm^{-1}. Although it is very difficult to make unambiguous assignments in NIR spectra, the measurement of dipeptides provides new insight into the interpretation of NIR data. In addition, the absorption coefficient in the NIR region is extremely small in comparison to the mid-infrared region. The reduced absorption intensity in the NIR region allows much longer path lengths of samples. Therefore, NIR spectroscopy would be valuable technique for *in vivo* measurements with a high absorption coefficient such as those observed in proteins.

Acknowledgements

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