Near-Infrared Multispectral Imaging Technique for Visualizing Sequences of Di- and Tripeptides Synthesized by Solid Phase Combinatorial Method

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A new method based on the near-infrared (NIR) multispectral imaging technique has been developed for the noninvasive and nondestructive determination of the identity and sequences of amino acid residues in di- and tripeptides. The di- and tripeptides were synthesized on polymer beads (i.e., Rink) using the solid phase peptide synthetic method. The developed method is capable of determining the identity of sequences of the peptides (with and without the Fmoc protecting group) directly on the polymer beads. It can distinguish not only dipeptides from tri-peptides but also peptides with very similar structures (e.g., Rink-Gly-Ala-Ala, Rink-Gly-Ala-Phe, Rink-Gly-Ala-Leu, Rink-Gly-Ala-Val, and Rink-Gly-Ala-Met). More importantly, it is capable of distinguishing peptides with the same amino acid residues but different sequences (e.g., Rink-Gly-Leu-Val from Rink-Gly-Val-Leu).

Index Headings: **Acousto-optic tunable filter; Combinatorial chemistry; Multispectral imaging; Peptides; Sequences.**

INTRODUCTION

Over the last two decades there has been an explosion of interest in solid-support based combinatorial chemistry techniques.[1–3] In fact, most drug discovery programs use such techniques as an integral part of their drug development procedures. The popularity stems from the increased drug screening efficiencies developed in the pharmaceutical industry.[1–3] The recent accessibility of relatively large target protein quantities (milligrams) produced through genetic engineering efforts is responsible for the increased screening efficiencies. It is now possible to screen more than 100 000 compounds in just a few months.[1–3] In addition, it is projected that this screening time could be reduced to just a single day. As a consequence of these high throughput screening approaches it is possible for an average pharmaceutical laboratory to screen its entire compound library in less than one week. Such efficient screening protocols increase the urgency of developing methods to produce much larger drug libraries.

Solid support based peptide synthesis techniques have demonstrated several distinct advantages over solution phase synthesis;[1–3] namely, ability to drive reactions to completion, easy product purification, as well as ease of automation. However, there remains one large hindrance to this synthetic technique, and that is the lack of a sensitive analytical method to follow solid-phase reactions and characterize resin-bound products.[4] Although several attempts have been made to address this obstacle, including FT-IR spectroscopy, mass spectroscopy, and chemical sensors, as well as FT Raman spectroscopy, none of these techniques have found widespread acceptance because they cannot be used for the direct determination of compounds attached to the resins and/or have rather low throughput.[5–10]

It is possible to characterize compounds synthesized through the combinatorial method directly when they are still attached to the resin by using a near-infrared (NIR) spectrometric technique. The possibility stems from the fact that the NIR light has relatively high penetrating power. It can penetrate the resins and hence facilitate the direct measurements of compounds attached to the resins.[11] Additionally, the NIR technique can generally be applied to all organic compounds because it is based on the measurements of the combination and overtone transitions of N–H, C–H, and O–H groups.[11] While the NIR spectrometric method has an advantage over other methods, it is not particularly suited as the detection method for combinatorial chemistry. The limitation is due to the low throughput of the NIR spectrometric method. Specifically, it is not possible to use the NIR spectrometric method for the determination of hundreds of compounds simultaneously. This limitation can be ameliorated by using not the NIR spectrometric method but rather an NIR multispectral imaging method.

Multispectral imaging is a technique that can simultaneously record spectral and spatial information of a sample, i.e., the recorded images contain signals that are generated by a sample and plotted as a function of spectral and spatial distribution.[12–17] Chemical information on a large area of a single sample or samples can be elucidated from recorded images. In these instruments, the spatial distribution of the sample is obtained by a camera, and the spectral information is gained by scanning a dispersive element. In order to be used for realtime and on-line monitoring (as in the combinatorial synthesis), the instrument must be able to sensitively and rapidly record spectral images of a sample. These requirements can be satisfactorily met with the instrument recently developed in our laboratory.[18,19] In this instrument, an acousto-optic tunable filter (AOTF) is used for spectral scanning.[20–22] AOTF is an electronic tunable filter that has rapid scanning ability, high diffraction efficiency, wide spectral tuning range, and high resolution.[20–22] Because of these advantages, the multispectral imaging instrument based on an AOTF is compact, has no moving parts, and can sensitively and rapidly record images of a sample or samples. Studies which to date were not possible can now be performed by using this newly developed instrument. These studies include the determination of the chemical

Received 17 January 2001; accepted 28 March 2001.
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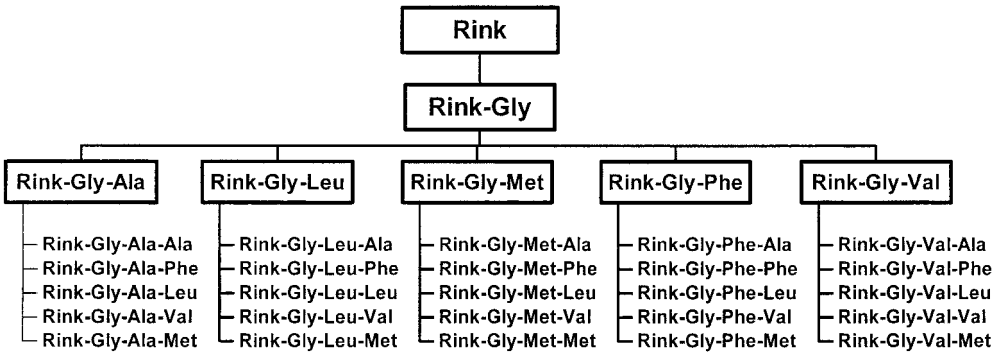

SCHEME I. Schematic diagram of the resin-bound peptide synthesis.

inhomogeneity of polymers,[18,19] the authentication of stock certificate and currency,[18] and the evidence of kinetic inhomogeneity.[19] We also successfully demonstrated in our preliminary study that the NIR multispectral imaging instrument can be used, for the first time, as the detector for the direct determination of several simple di- and tripeptides that were synthesized by using the combinatorial solid phase peptide synthetic method.[23] The aim of this study is to demonstrate that the NIR multispectral imaging technique can be successfully used to determine not only the identity but also the sequences of amino acid residues in peptides that are synthesized by solid phase combinatorial method.

EXPERIMENTAL

Instrumentation. The NIR multispectral imaging instrument used in this study is similar to the instrument used in our previous study.[18,19,23] Essentially, in this instrument, a 250-W, 12 V halogen tungsten lamp was used as the light source. A 2.5 cm focal length CaF_2 lens was used to collimate the incident white light from the lamp into a non-collinear AOTF (Crystal Technologies). Light diffracted by the AOTF was transmitted through the sample and recorded by an NIR camera. In our previous study, an InGaAs NIR camera was used to record images of the samples. In this study, that camera was replaced by a liquid N_2 cooled, 320 × 256 pixel Indium Antimonide (InSb) focal plane array camera (Santa Barbara Focal Plane, Goleta, CA). The replacement was made because the InGaAs camera is sensitive only in a relatively narrow spectral region (from 1.0 to 1.7 μm), whereas the InSb camera can cover a relatively larger region (from 1.0 to 5.0 μm) and that peptides are known to have stronger absorption in the longer wavelength NIR region (>1.7 μm).[24–26] Images recorded by the InSb camera were grabbed and transferred to a personal computer by a frame grabber (Dipix Technologies Model XPG-1000). The Unscrambler Version 7.5 (Camo® ASA) chemometric software package was purchased from Applied Chemometrics (Sharon, MA).

Chemicals. Fmoc Rink Amide resin was obtained, as dry beads, from Polymer Laboratories (Amherst, MA). According to the manufacturer, Fmoc Rink Amide (Rink) resin has a loading of 0.91 mmol/g and a nominal particle size of 150–300 μm. The Rink resin is supplied with an attached 9-Fluorenylmethoxycarbonyl (Fmoc) protecting group. Prior to use, the Fmoc group was removed from the resin.

Fmoc-Alanine, Fmoc-Glycine, Fmoc-Leucine, Fmoc-Methionine, Fmoc-Phenylalanine, Fmoc-Valine, and N-Hydroxybenzotriazole (HOBT) were purchased from Calbiochem-Novabiochem Corporation (La Jolla, CA). All amino acids were specified by the manufacture to have purities greater than 99% and to contain less than 0.5% of the D-enantiomer.

Solid-Phase Peptide Synthesis. Fmoc Rink Amide (Rink) resin was used as the solid support during the batch-wise solid-phase peptide synthesis. Six different Fmoc-amino acids were used for this study; namely, Fmoc-Gly, Fmoc-Ala, Fmoc-Leu, Fmoc-Met, Fmoc-Phe, and Fmoc-Val. A total of thirty-one peptides (a monopeptide, 5 dipeptides, and 25 tripeptides) were synthesized on this resin. The peptide products were synthesized according to the schematic diagram shown in Scheme I. As illustrated, for all peptides the initial residue was Gly. Following attachment of Gly to the resin, five dipeptides were synthesized by coupling Fmoc-Ala, Fmoc-Leu, Fmoc-Met, Fmoc-Phe, and Fmoc-Val to the resin-bound Gly residue (Rink-Gly). Subsequently, a third series of reactions were performed to elongate the resin-bound dipeptide to three residues. The tripeptides were synthesized by coupling Fmoc-Ala, Fmoc-Leu, Fmoc-Met, Fmoc-Phe, and Fmoc-Val to the resin-bound dipeptides. Prior to removing the Fmoc protecting group on the peptides, a portion of monopeptide, dipeptide, and tripeptide resins were removed and archived for measurement. The batch-wise peptide synthesis was carried out in three steps: (1) activation of the amino acid, (2) coupling reaction, and (3) deprotection.

(1) Activation. Three equivalents of the amino acid and six equivalents of N-hydroxybenzotriazole (HOBT), relative to the solid-support loading, were dissolved in a minimum amount of DMF. The mixture was cooled to 0 °C and three equivalents of 1,3-diisopropylcarbodiimide (DIC) were then added. This mixture was then allowed to equilibrate for 2 min to completely activate the carboxylic acid group.

(2) Coupling. The activated amino acid was then added to a round-bottom flask containing the solid-support in a minimum amount of DMF. Following reaction for 2 min, two equivalents of diisopropylethylamine (DIPEA), relative to the resin loading, were added (i.e., Resin: Amino Acid: HOBT: DIC: DIPEA = 1:3: 6: 3: 2). The reaction

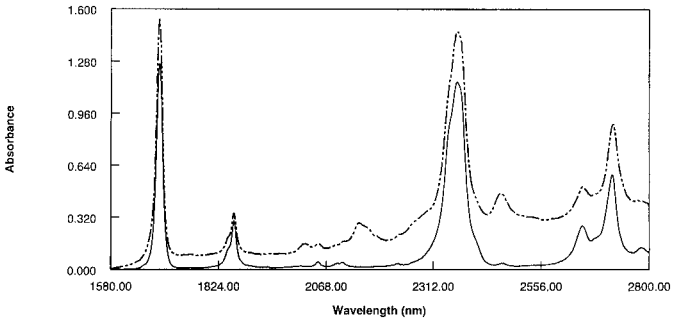

FIG. 1. Near-infrared spectra of the Rink resin in chloroform (dotted line) and chloroform alone (solid line).

was allowed to proceed for 2 h. Subsequently, a small amount of resin (10–20 mg) was removed and the presence of any unreacted amino groups was determined by using the ninhydrin test.[27] Coupling reaction was repeated if more than 10% of unreacted amino acid was detected.

(3) Deprotection. Removal of the Fmoc protecting group was accomplished by washing the resin twice (for 15 min each time) with 55% piperidine in DMF. This step was taken because it is known that the free amino group of the deprotected resin-bound peptides may attack the peptide resin linkage to produce a cyclic diketopiperazine compound. This intramolecular aminolysis is amino acid-residue specific and is known to occur readily in peptides containing Gly residue (which is present in all peptides used in this study). Washing with 55% piperidine/DMF for 5 min is known to minimize the dipeptide cleavage.[28,29] The resin was then washed three times with DMF followed by decarboxylation with 2:1 (v/v) dioxane: water. Finally, the resin was washed with DMF and chloroform five times each to give a free amino group.

Rink resin and Rink-peptides were suspended in chloroform. The swollen resin was placed in a 2.0 mm pathlength cell and the NIR multispectral images were measured from 1580 to 2800 nm using the multispectral imaging instrument. The absorption spectra were calculated by taking the average intensity of 101 × 101 pixels in each picture (a total of 610 images were recorded from 1580 to 2800 nm). Each spectrum shown is an average of 6 recordings.

RESULTS AND DISCUSSION

Thirty-one resin-bound peptides were synthesized on a polystyrene-based solid support, Fmoc Rink Amide (Rink) resin. Because the polystyrene core of this support is specified to be lightly cross-linked (~1%), it is expected that polar solvents can permeate freely into its core. As described above, solid phase method was used to synthesize peptides that are attached to this Rink resin. In this resin, the reactive amino group is separated from the polystyrene core by the acid-labile linker. This linker facilitates the liberation of the synthesized peptides from the resin by simply treating the peptide bound resin with 50% trifluoroacetic acid in DCM for 15 min at room temperature.[30]

Near-Infrared Spectra of Peptides Attached to Rink Resin. Figure 1 shows the NIR spectra of pure chloroform and of the Fmoc-protected Rink resin suspended in chloroform. As illustrated, differences between the two spectra are well-resolved bands at 2020, 2142, and 2464 nm. The band at 2020 nm can be assigned to the N–H of the Fmoc-protected aliphatic amino group. The complex band centered at 2142 nm and the band at 2464 nm may be attributed to the aromatic C–H stretch of the polystyrene backbone.[24–26] Aliphatic C–H combination transition may be responsible for the shoulder at 2300 nm.[24–26] This band can also be traced to methylene groups in the resin. Since the amino group is involved in all solid-phase syntheses, the 2020 nm band can be used to monitor amide bond formation during coupling reactions. Moreover, the aliphatic C–H combination band at 2300 nm may facilitate identification of amino acid residues through absorption of their side groups. This is due to the fact that each residue contains a certain number of C–H bonds (in the methylene groups as well as the methyl groups). Combination absorption of these aliphatic groups makes it possible to distinguish closely related compounds.

The NIR spectra of the Fmoc protected dipeptides on Rink resin (i.e., Rink-Gly-Ala-Fmoc, Rink-Gly-Leu-Fmoc, Rink-Gly-Met-Fmoc, Rink-Gly-Phe-Fmoc, and Rink Gly-Val-Fmoc) are shown in Fig. 2. The spectra in this figure were corrected for influence of chloroform. It was found that Rink resin absorbs a significant amount of chloroform (~6.0 ml/g). Correction was made by subtracting the chloroform spectrum by using an empirical weighting factor of 0.85. The correction factor of 0.85 was selected because, as illustrated in Fig. 1, the band at 1857 nm is due primarily to the absorption of chloroform and that the absorbance of the Rink resin in chloroform at this wavelength is about 85% as intense as that of the chloroform spectrum. Therefore, it is assumed that a large proportion (~85%) of the resin volume is due to chloroform.

As illustrated in the figure, the N–H band at 2020 nm (in Fig. 1) is slightly shifted to 2026 nm. This seems to indicate that an amide bond formed during coupling.[24–26] The 2290 nm band of the amino acid residue side group can be used to differentiate dipetides that have different amino acid residues (i.e, side groups). As an example, in this region, the bands for Rink-Gly-Met-Fmoc and Rink-Gly-Leu-Fmoc are centered at 2284 nm and 2290 nm, respectively. Expectedly, these bands demonstrate much greater variation when compared to the other bands in the spectrum. Specifically, the corresponding bands of Rink-Gly-Ala, Rink-Gly-Leu, Rink-Gly-Met, Rink-Gly-Phe, and Rink-Gly-Val are different not only in size and shape but also in the peak positions among one another.

A significant intensity variation in the aliphatic C–H band at 2350 nm seems to indicate that the light beam is strongly attenuated by the resin. In order to increase intensity of the light incident to the sample (and hence to increase the the signal-to-noise ratio of the measurement), the power of the RF signal that was applied to the AOTF was increased from 2.0 W to 2.5 W in the subsequent measurements of resin bound deprotected peptides. This step was taken because it is known that increasing the RF power leads to an increase in the intensity of the light diffracted from the AOTF.[20–22]

Spectra for deprotected dipeptides on Rink resin are shown in Fig. 3. Bands at 2020, 2142, and 2290 nm occur in positions similar to those observed for Fmoc protected dipeptides on Rink resin. Following deprotection, addi-

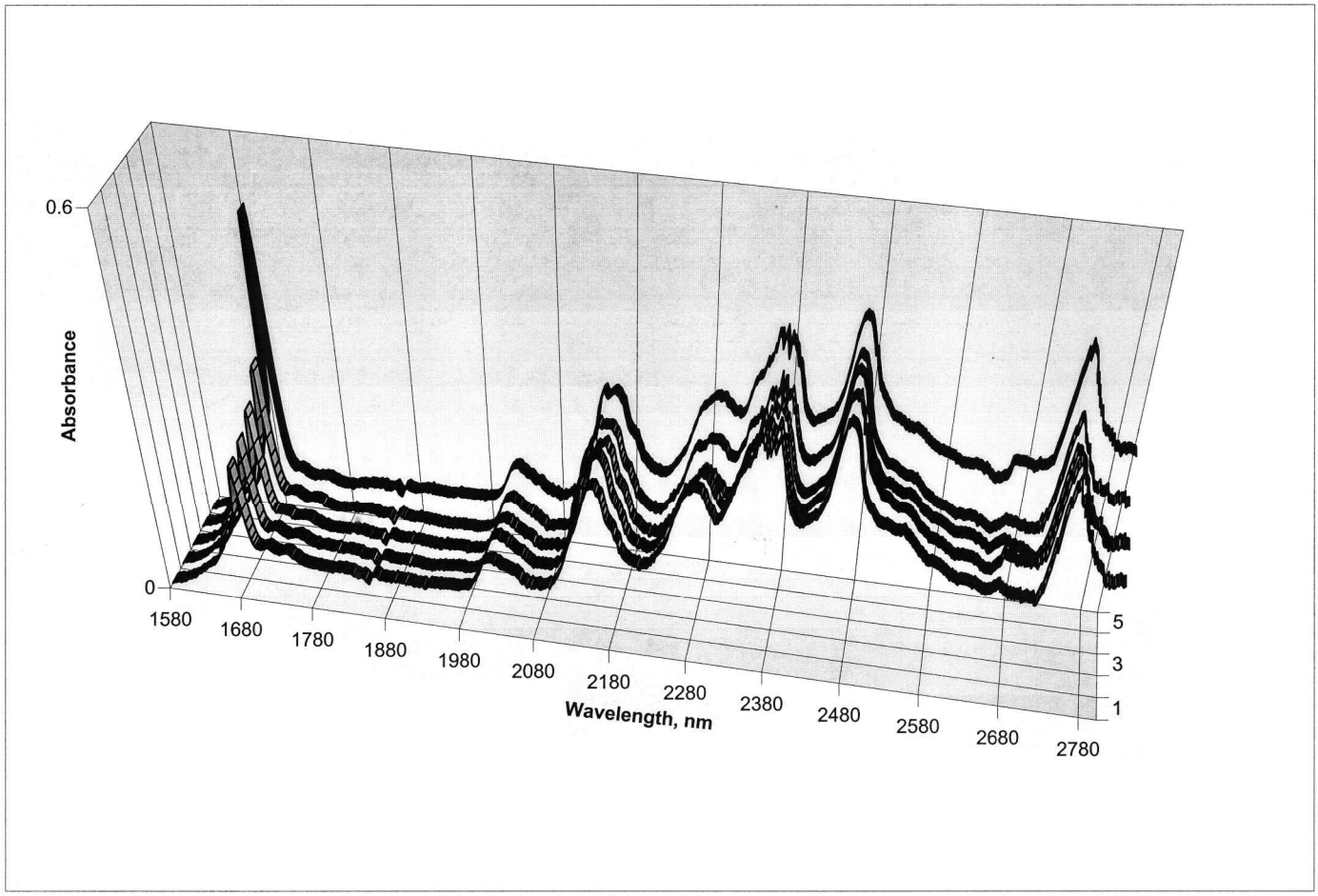

FIG. 2. Near-infrared spectra of Fmoc protected dipeptides on Rink resin: from front to back: Rink-Gly-Met-Fmoc, Rink-Gly-Ala-Fmoc, Rink-Gly-Phe-Fmoc, Rink-Gly-Val-Fmoc, and Rink-Gly-Leu-Fmoc.

tional shoulders at 2042 and 2200 nm that can be attributed to the deprotected amino group are observed. In the present figure, combination bands due to the amino acid residue side groups occur at 2290 nm. The peak position wavelength spread in this region is not as broad as that observed for Fmoc protected dipeptides. Conceivably, the amino acid residue side group bands are coalesced by the more polar amino group generated following deprotection.

Heavily overlapped bands throughout the NIR region make it impossible to use a single wavelength for the determination of any resin-bound peptides on Rink resin. However, it may be possible to use the partial least-squares regression (PLSR) method, a full spectrum chemometric technique, for the determination of resin-bound residues.

Analysis of Resin Bound Peptides. It is evident from Figs. 2 and 3 that in the region between 1972 and 2320 nm, resin bound peptides exhibit the largest spectral variations. Accordingly, partial least-squares regression (PLSR) models were developed by using this wavelength range. The calibrations were performed by using the PLS1 algorithm and the cross validation method with the Unscrambler chemometric software package. Prior to calibration, the solvent corrected resin-bound peptide absorption spectra were corrected for variations due to light scattered by the insoluble support. The multiplicative scatter correction (MSC) pretreatment was performed using the wavelength range from 1580 to 1655 nm. This region was chosen because it contains little absorption information related to the peptide identity.

PLS1 calibrations were developed with the use of the solvent corrected NIR spectra of a single monopeptide, 5 dipeptides, and 25 tripeptides on Rink resin as well as unreacted Rink resin. Moreover, calibrations were developed for Fmoc protected as well as deprotected peptides. As described in the previous section (e.g., in Scheme I), the initial residue in all resin-bound peptides was Gly while the second residue (in dipeptides and tripeptides) and third residue (in tripeptides) were composed of Ala, Leu, Met, Phe, and Val. It is known that chain length (i.e., number of residues) and the residue position along the peptide chain affect the NIR spectra. Effects due to the chain length (i.e., monopeptide, dipeptide, or tripeptide) are expected because the number of C–H, N–H, and amide bonds increases with chain length. This facilitates the differentiation of dipeptides from tripeptides as well as monopeptides. Additionally, absorption due to the amino acid side groups is known to be sensitive to peptide chain length. This may be due to the fact that residues along the peptide exist in slightly different chemical environments. Residues are, therefore, expected to exhibit different absorption characteristics dependent on the local environment (i.e., adjacent residues). As a consequence,

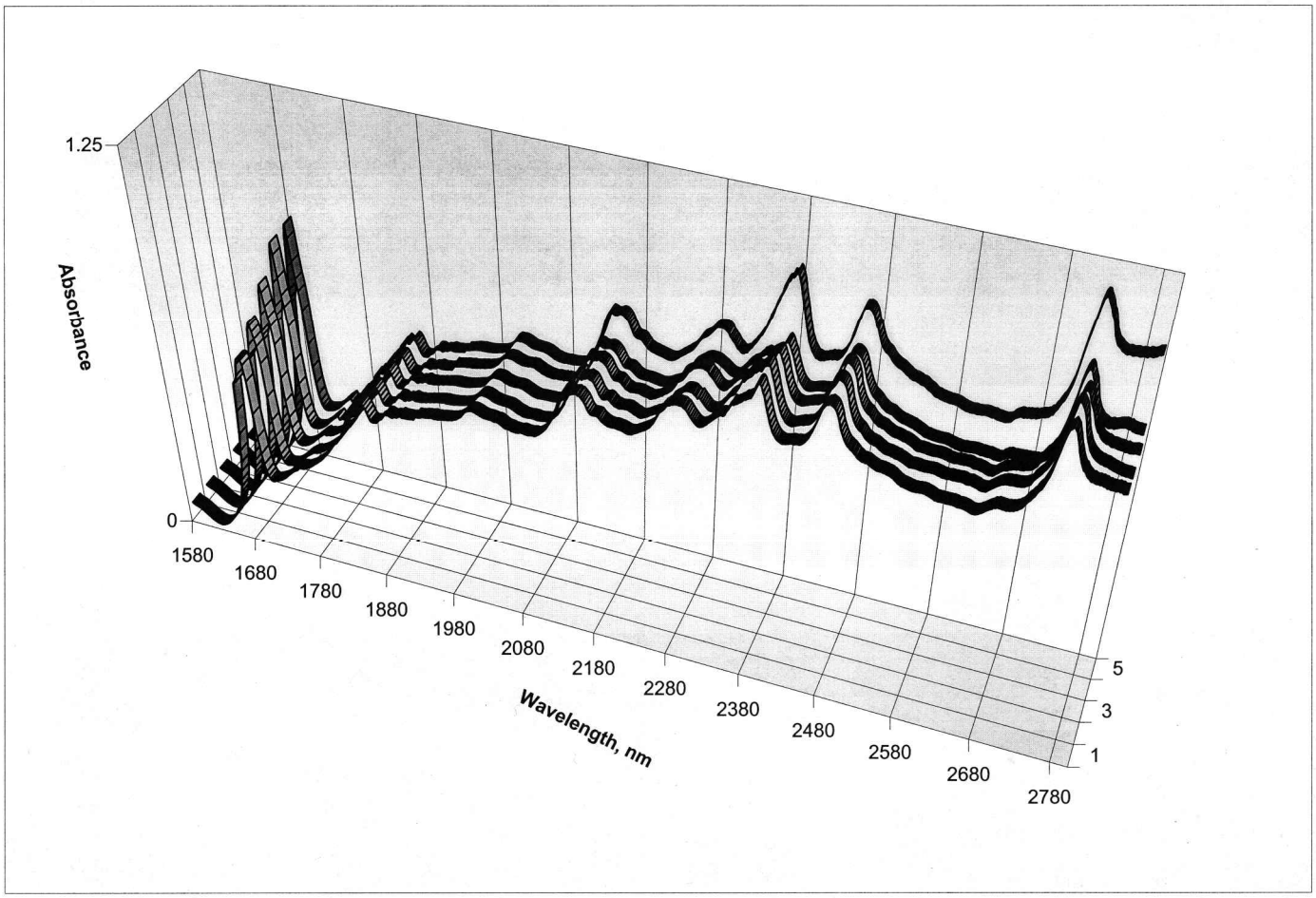

FIG. 3. Near-infrared spectra of deprotected dipeptides on Rink resin: from front to back: Rink-Gly-Met-NH_2, Rink-Gly-Phe-NH_2, Rink-Gly-Val-NH_2, Rink-Gly-Leu-NH_2, and Rink-Gly-Ala-NH_2.

residues may be broadly classified into two groups depending on their position along the peptide chain; namely, terminal residues and internal residues. Terminal residues (Term-) occur at the N-terminus of the peptide and are directly attached to the Fmoc protection group (in protected peptides) or the amino group (in deprotected peptides). Conversely, internal residues (Int-) are flanked by two residues and/or attached to the insoluble support and a second residue. For example, in the tripeptide Rink-Gly-Ala-Leu-Fmoc, the Gly and Ala are internal residues, while the Leu is the terminal residue.

Twelve PLS1 calibrations were developed to model the influence of internal residues (i.e., Int-Gly, Int-Ala, Int-Leu, Int-Met, Int-Phe, and Int-Val) and terminal residues (i.e., Term-Gly, Term-Ala, Term-Leu, Term-Met, Term-Phe, and Term-Val) on the NIR spectra of resin-bound peptides. Additionally, a PLS1 calibration was also developed to account for the absorption due solely to the unreacted resins.

Results from the PLS cross validation show that calibrations for thirteen models require a relatively small number of factors for optimal performance (from 9 to 38). The root mean standard error of prediction (RMSEP), standard error of prediction (SEP) values given by this method are on the order of 10^{-5} mmol/g. In both groups of models (i.e., Fmoc protected and deprotected peptides), the SEP are ~11% of the resin loading (0.91 mmol/g). Both groups show good linearity performance. Correlation coefficients for all calibrations are greater than 0.90.

A total of eighteen resin-bound peptides (nine Fmoc protected and nine deprotected) were randomly selected to validate the performance of these calibrations. The selected peptides were Rink-Gly, Rink-Gly-Ala, Rink-Gly-Ala-Met, Rink-Gly-Leu-Val, Rink-Gly-Met, Rink-Gly-Met-Leu, Rink-Gly-Phe-Leu, Rink-Gly-Phe-Phe, and Rink-Gly-Val-Leu. The NIR spectra of these peptides were remeasured, in a single compartment cell, using the imaging spectrometer. The spectra were subsequently corrected for scattering (using the multiplicative scatter correction). The PLS calibrations were then used to predict the amino acid residue concentrations in these peptides. Plotted in Figs. 4A and 4B are accuracy data for Fmoc protected peptides and deprotected peptides on Rink resin, respectively. In these figures, the average concentration predicted by the PLS model is plotted against the amino acid residue (arranged in increasing mass). The loading on Rink resin is 0.91 mmol/g and therefore the residue concentration predicted by the PLS model is expected to be identical to this value. As illustrated in these figures, the average PLS predicted residue concentration is in good agreement with the expected residue loading.

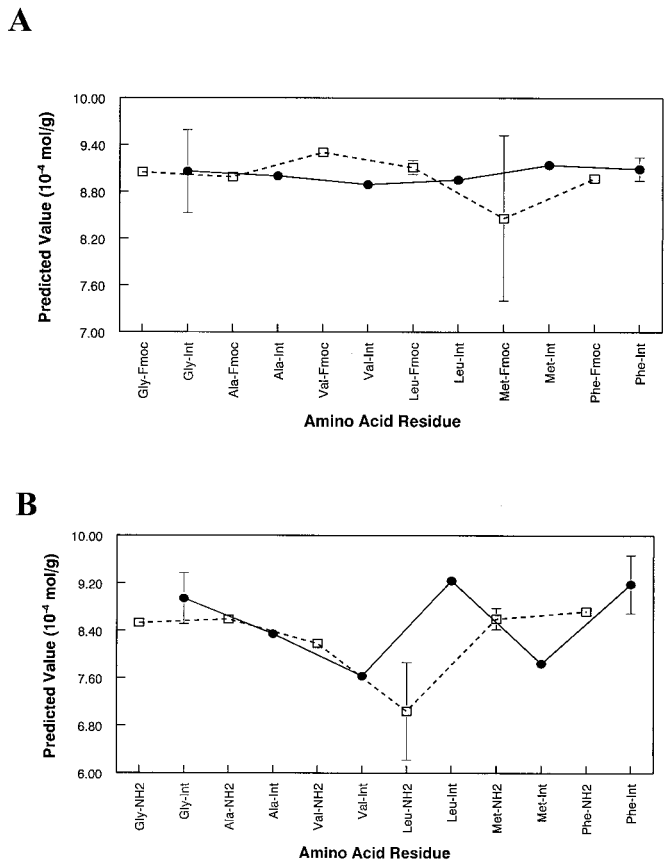

FIG. 4. Plot of accuracy of predicted concentration of amino acid internal residues (solid line) and terminal residues (dashed line) for (**A**) Fmoc protected peptides, and (**B**) for deprotected peptides on RinkAMS resin.

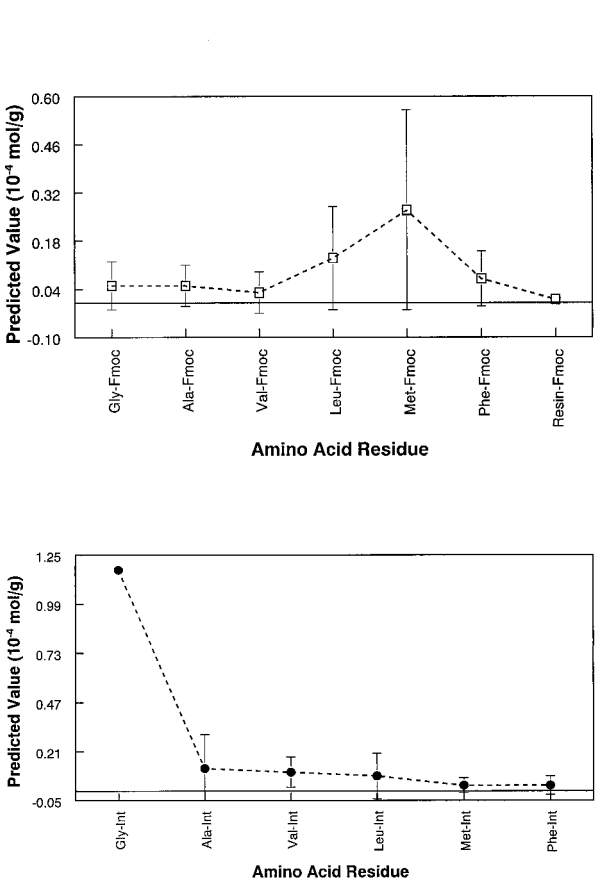

FIG. 5. Plot of precision of predicted concentration of amino acid terminal residues (**A**) and internal residues (**B**) for Fmoc protected peptides on Rink resin.

Additionally, the average PLS predicted concentrations are accurate within ~20% of the actual residue concentration (0.91 mmol/g on Rink resin). Shown in Figs. 5A and 5B and 6A and 6B are the precision data of PLS calibration models for the Fmoc protected peptides and deprotected peptides on Rink resin, respectively. Figures 5A and 6A represent the precision of the terminal residue models while figures 5B and 6B depict the precision of the internal residue calibrations. As shown in these figures, the plots are reasonably flat and close to the theoretical value of 0.0 mmol/g (no residue).

Taken together, Figs. 4–6 clearly demonstrate that the PLS1 calibrations can model the spectral variation in resin-bound peptides well. The method provides an effective means for the determination of not only the identity but also the sequences of the resin-bound peptides. As an example, Table I lists the PLS predicted concentrations of the closely related resin-bound peptides Rink-Gly-Leu-Val and Rink-Gly-Val-Leu. For the Fmoc protected peptide, Rink-Gly-Val-Leu-Fmoc, a concentration of 9.10×10^{-4} mol/g is expected for the Int-Gly, Int-Val, and Term-Leu residues. Further, the Int-Leu and Term-Val concentrations are expected to be 0.0 mmol/g (no residue). As illustrated in the table, the PLS models predict 9.14, 9.01, and 8.89×10^{-4} mol/g for the Int-Gly, Term-Leu, and Int-Val residues, respectively. In addition, the Int-Leu and Term-Val models predict concentrations of 0.00 and 0.18×10^{-4} mol/g, respectively. Clearly, the PLS models can distinguish terminal residues from internal residues (i.e., Term-Val vs. Int-Val) as well as residues with different side groups (i.e., Term-Val vs. Term-Leu). Discrimination of these closely related compounds demonstrates the effectiveness of the PLS calibration models. These results are significant since Leucine is different from Valine by only a methylene group.

Experiments were subsequently designed to demonstrate that the developed PLS calibration models can be used to predict the concentrations of several resin-bound peptides measured simultaneously. A total of nine peptides on Rink resin (i.e., Rink-Gly, Rink-Gly-Ala, Rink-Gly-Ala-Met, Rink-Gly-Leu-Val, Rink-Gly-Met, Gly-Met-Leu, Rink-Gly-Phe-Leu, Rink-Gly-Phe-Phe, and Rink-Gly-Val-Leu) were measured in three groups by using a home-built multicompartment cell. This cell was constructed of aluminum with microscope slides as windows and has three independent compartments. Each compartment has a clear optical aperture of 0.45 cm × 1.30 cm and a 2.0 mm pathlength. Similar to measurements made with the single compartment cell, the NIR absorption spectra were calculated by taking the average intensity over a square pixel area in each picture. However, the pixel area used to calculate the absorption spectra is not 101 × 101 pixels as in measurements made with the single compartment cell but only 31 × 31 pixels. Corrections were made to compensate for small wavelength differences between the center of the image and

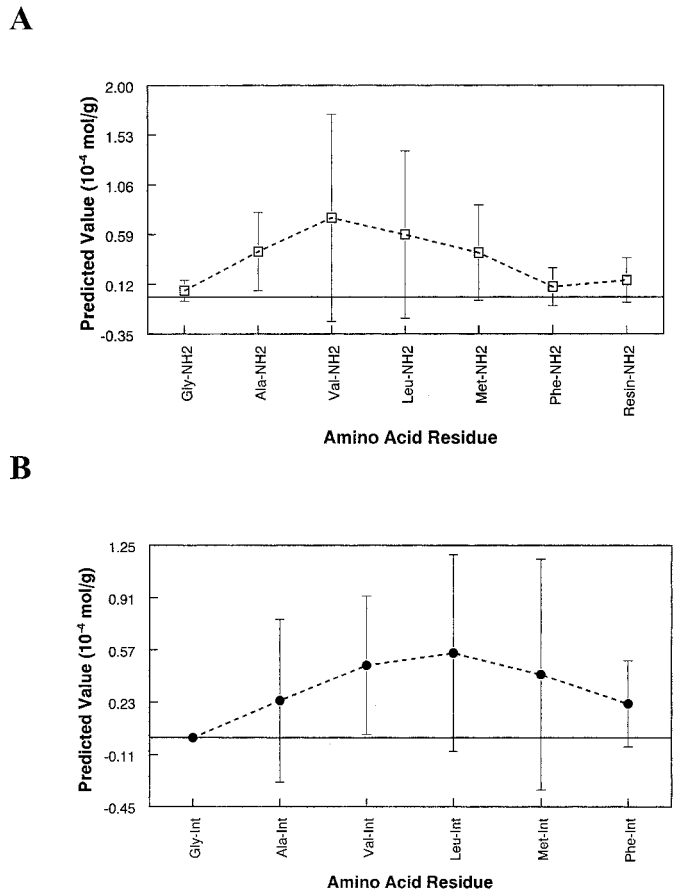

FIG. 6. Plot of precision of predicted concentration of amino acid terminal residues (**A**) and internal residues (**B**) for deprotected peptides on Rink resin.

the image edge. Additionally, corrections were made to compensate for light intensity variations across the focal plane array. Following these corrections, the Rink-Gly-Val-Leu peptide measured in the three compartment cells is predicted to contain 8.96, 9.02, and 8.61 $\times$ 10^{-4} mol/g for Int-Gly, Term-Leu, and Int-Val, respectively. These values are in good agreement with the actual value of 9.10 $\times$ 10^{-4} mol/g. As expected, the method is also effective for the determination of residues that are not there. Concentrations of Int-Leu and Term-Val, were calculated to be 0.00 and 0.39 $\times$ 10^{-4} mol/g, respectively.

TABLE I. PLS predicted concentrations of peptides on rink resin.

	PLS predicted concentrations (mmol/g)				
Sample	Internal Gly	Internal Leu	Terminal Leu	Internal Val	Terminal Val
Single cell					
Rink-Gly-Leu-Val-Fmoc	8.79	8.95	0.00	0.00	**9.30**
Rink-Gly-Val-Leu-Fmoc	**9.14**	0.00	**9.01**	**8.89**	0.18
Rink-Gly-Leu-Val-NH_2	**9.25**	**9.24**	0.83	0.40	**8.18**
Rink-Gly-Val-Leu-NH_2	**9.16**	1.64	**6.16**	**7.63**	2.12
Three-compartment cell					
Rink-Gly-Leu-Val-Fmoc	**8.79**	**8.95**	0.00	0.00	**9.30**
Rink-Gly-Val-Leu-Fmoc	**8.96**	0.00	**9.02**	**8.61**	0.39
Rink-Gly-Leu-Val-NH_2	**9.05**	**9.01**	0.40	0.91	**8.91**
Rink-Gly-Val-Leu-NH_2	**9.09**	0.36	**9.05**	**8.33**	0.00

In summary, it has been demonstrated that the NIR multispectral imaging technique is capable of determining the identity and sequences of amino acid residues of the peptides (with and without the protecting groups) directly on the polymer beads. These peptides were synthesized on polymer beads using the solid phase peptide synthetic method. The method can distinguish not only dipeptides and tripeptides but also peptides with very similar structures (e.g., Rink-Gly-Ala-Ala, Rink-Gly-Ala-Phe, Rink-Gly-Ala-Leu, Rink-Gly-Ala-Val, and Rink-Gly-Ala-Met). More importantly, it is capable of distinguishing peptides with the same amino acid residues but different sequences (e.g., Rink-Gly-Leu-Val from Rink-Gly-Val-Leu). Experiments are currently in progress to expand the applications of the method to larger peptides.

ACKNOWLEDGMENT

Acknowledgment is made to the National Institutes of Health, National Center for Research Resource, Biomedical Technology Program for financial support of this work.

1. A. Czarnik, Anal. Chem. **70,** 378A (1998).
2. A. W. Czarnik and S. H. DeWitt, *A Practical Guide to Combinatorial Chemistry* (American Chemical Society, Washington, DC, 1996).
3. S. R. Wilson and A. W. Czarnik, *Combinatorial Chemistry, Synthesis and Application* (John Wiley, New York, 1997).
4. E. Atherton and R. C. Sheppard, *Solid Phase Synthesis: A Practical Approach* (IRL Press, New York, 1989).
5. K. Russell, D. C. Cole, F. M. McLaren, and D. E. Pivonka, J. Am. Chem. Soc. **118,** 7941 (1996).
6. D. E. Pivonka and T. R. Simpson, Anal. Chem. **69,** 3851 (1997).
7. B. Yan, G. Kumaravel, H. Anjaria, A. Wu, R. C. Petter, C. F. Jewell, and J. R. Wareing, J. Org. Chem. **60,** 5736 (1995).
8. B. Yan and Q. Sun, J. Org. Chem. **63,** 55 (1998).
9. H. U. Gremlich and S. L. Berets, Appl. Spectrosc. **50,** 532 (1996).
10. D. E. Pivonka and R. B. Sparks, Appl. Spectrosc. **54,** 1584 (2000).
11. *Handbook of Near-Infrared Analysis,* D. A. Burns and E. W. Ciurczak, Eds. (Marcel Dekker, New York, 1992).
12. M. D. Morris, *Microscopic and Spectroscopic Imaging of the Chemical State* (Marcel Dekker, New York, 1993).
13. P. J. Treado, I. W. Levin, and E. N. Lewis, Appl. Spectrosc. **48,** 607 (1994).
14. H. R. Morris, C. C. Hoyt, and P. J. Treado, Appl. Spectrosc. **48,** 857 (1994).
15. E. N. Lewis, P. J. Treado, R. C. Reeder, G. M. Story, A. E. Dowrey, C. Marcott, and I. W. Levin, Anal. Chem. **67,** 3377 (1995).
16. E. N. Lewis, A. M. Gorbach, C. Marcott, and I. W. Levin, Appl. Spectrosc. **50,** 263 (1996).
17. P. Colarusso, L. H. Kidder, I. W. Levin, J. C. Fraser, J. F. Arens, and E. N. Lewis, Appl. Spectrosc. **52,** 106A (1998).
18. C. D. Tran, Y. Cui, and S. Smirnov, Anal. Chem. **70,** 4701 (1998).
19. M. Fischer and C. D. Tran, Anal. Chem. **71,** 953 (1999).
20. C. D. Tran, Anal. Chem. **64,** 971A (1992).
21. C. D. Tran, Talanta **45,** 237 (1997).
22. C. D. Tran, Anal. Lett. **33,** 1711 (2000).
23. M. Fischer and C. D. Tran, Anal. Chem. **71,** 2255 (1999).
24. S. Holly, O. Egyed, and G. Jalsovszky, Spectrochim. Acta **48A,** 101 (1992).
25. K. Wael, C. Bruynee, and Th. Zeegers-Huyskens, Spectrosc. Lett. **31,** 283 (1998).
26. R. T. Conley, *Infrared Spectroscopy* (Allyn and Bacon, Boston, 1966), Chap. 7.
27. E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, Anal. Biochem. **34,** 595 (1970).
28. G. B. Fields and R. L. Noble, Int. J. Peptide Protein Res. **35,** 161 (1990).
29. J. Jones, *The Chemical Synthesis of Peptides* (Claredon Press, Oxford, 1991), p. 24.
30. H. Rink, Tet. Let. **28,** 3787 (1987).