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Near-Infrared Spectrometric Determination of Di- and Tripeptides Synthesized by a Combinatorial Solid-Phase Method

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A new method based on near-infrared (NIR) spectrometry and partial least-squares analysis 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 from six amino acids with similar structures (Gly, Ala, Leu, Met, Phe, Val) on two different polymer beads (bead with and without a linker) using the solid-phase peptide synthetic method. The developed NIR method is capable of determining the identity of sequences of these di- and tripeptides (with and without the Fmoc protecting group) directly on the polymer beads. It can distinguish not only dipeptides from tripeptides but also peptides with very similar structures (e.g., bead-Gly-Ala-Ala, bead-Gly-Ala-Phe, bead-Gly-Ala-Leu, bead-Gly-Ala-Val, and bead-Gly-Ala-Met). More importantly, the method is capable of distinguishing di- and tripeptides with the same amino acid residues but different sequences (e.g., bead-Gly-Leu-Val from bead-Gly-Val-Leu).

Combinatorial synthesis has evolved significantly in the past few years to become the key component in almost every drug discovery program.[1−4] A vast number of compounds can be readily produced by the combinatorial synthetic method via solid-phase polymer substrates.[1−3] Novel, nondestructive analytical methods that have in situ capability are, therefore, required for the monitoring and analysis of this immense number of compounds. Techniques such as TLC, HPLC, GC, and MS are not suitable since they require cleavage of the compounds from the polymeric support.[1−3] The magic angle spinning NMR technique has been used. However, it suffers from low sensitivity and long acquisition time. The latter limitation makes it unsuitable for the analysis of high-sample throughput and the monitoring of reaction kinetics.[1−3] To date, near- and middle-infrared methods are probably the most promising technique.[4−8]

The near-IR (NIR) region covers the overtone and combination transitions of the C−H, O−H, and N−H groups, and since all organic compounds possess at least one or more of these groups, the NIR spectrometric method can, in principle, be used for the analysis of all organic compounds.[9] The extensive overlap among the NIR spectra of organic molecules does not impose a limitation but rather offers additional features to the NIR technique because NIR spectra of a mixture can be analyzed using multivariate methods (e.g., principal component analysis, partial least squares).[9] In fact, NIR spectrometry coupled with the multivariate methods has been successfully used for the simultaneous analysis of many compounds. However, their use for the in situ determination of organic compounds synthesized via a solid-phase method has not been realized. This is rather unfortunate considering the potential of the technique. The limitation is probably due to the fact that high-quality NIR spectra are required for the analysis. A NIR spectrometer that has high sensitivity, high light throughput, high stability, and no drift is required for the measurements of NIR spectra. Such a spectrometer can be constructed by using the recently available InGaAs photodiodes as the detector and the acousto-optic tunable filter (AOTF) as the dispersive element.

InGaAs photodiodes are known to have the lowest noise and highest sensitivity in the NIR spectral region. Recent advances in electronics and material science have made it possible to produce large-area (3-mm diameter) InGaAs photodiodes with extended wavelength response (from 1000 to 2600 nm). AOTF is an all-solid-state, electronic dispersive device which is based on the diffraction of light by acoustic wave in an anisotropic crystal.[10−12] The wavelength of the diffracted light is dependent on the frequency of the acoustic wave; only a monochromatic light will be diffracted from a crystal when a specific acoustic wave propagates through it. The scanning speed of the AOTF is, therefore, defined by the speed of the acoustic wave in the crystal, which is on the order of microseconds. As a consequence, compared to conventional gratings, the AOTFs have such advantages as being all solid state (contains no moving parts), having rapid scanning ability (μs), wide spectral tuning range, and high throughput, and giving high resolution (<1 nm).[10−12] The filters can also provide a unique means to maintain the intensity of the

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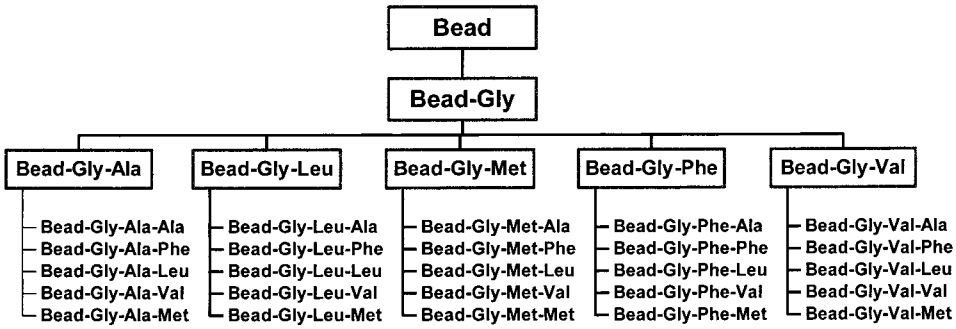

Figure 1. Schematic diagram of the resin-bound peptide synthesis.

light source (by controlling either the frequency or the power of the applied rF signal through a feedback loop).[13,14] As a consequence of this development, a NIR spectrophotometer based on the AOTF should be very sensitive, should have high light throughput, high stability, and no drift. In fact, we recently developed an AOTF-based NIR spectrometer and successfully used it for a variety of spectroscopic and analytical measurements including the determination of trace amounts of monomethylhydrazine and the study of inclusion complex formation between cyclodextrins and aromatic compounds, as well as the detector for flow injection analysis.[15−18]

The information presented is indeed provocative and clearly indicates that it is possible to use this AOTF-based NIR spectrometer for the in situ determination of compounds synthesized using the solid-phase method. Such consideration prompted us to initiate this study, which aims to use the AOTF-NIR spectrometer for the noninvasive determination of the identity and sequence of amino acid residues in peptides that are synthesized using the solid-phase method. Preliminary results on the analysis of di- and tripeptides are reported.

MATERIALS AND METHODS

Instrumentation. The NIR spectrophotometer used in this work was the same as that used in our previous studies.[15−18] The only difference between the spectrometer used in this work and that used previously is that two identical 1-mm core optical fibers were used to direct the light from the sample beam to the sample and from the sample to the InGaAs detector. This modification was made to facilitate measurements of peptides directly in the reaction chamber cell. Software written in C++ language was used to control the instrument, to acquire data, and to save the data in ASCII format for subsequent analysis using other software packages (e.g., the Unscrambler version 7.5 (Camo ASA) chemometric software package was purchased from Applied Chemometrics (Sharon, MA)).

Chemicals. Aminomethylpolystyrene (AMS) and Fmoc Rink Amide (Rink) resins were purchased from Polymer Laboratories (Amherst, MA). Both resins were received as dry beads. The AMS resin has a loading of 1.83 mmol/g and a nominal particle size of 400−500 μm. The 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) protection group. Prior to use, the Fmoc protection group was removed from the resin.

Fmoc-Ala, Fmoc-Gly, Fmoc-Leu, Fmoc-Met, Fmoc-Phe, Fmoc-Val, and *N*-hydroxybenzotriazole (HOBT) were purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA). The Fmoc-amino acids were specified by the manufacture to have purities greater than 99% and contain less than 0.5% of the D-enantiomer.

Solid-Phase Peptide Synthesis. AMS and Rink resins were used as solid supports for the batch-wise solid-phase peptide synthesis. A total of 31 (1 monopeptide, 5 dipeptides, and 25 tripeptides) peptides were synthesized from 6 *N*-Fmoc-amino acids on each resin. The peptides were synthesized according to the schematic diagram shown in Figure 1. As illustrated, for all peptides the initial residue was glycine. 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 glycine residue. Subsequently, a third series of reactions was performed to elongate the resin-bound dipeptide to three residues. That is, 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 deprotecting group on the peptide, a portion of monopeptide, dipeptide, and tripeptide resins was removed and archived for measurement. The batch-wise peptide synthesis was carried out in three steps: (a) activation of the amino acid, (b) coupling reaction, and (c) deprotection.

a. Activation. Three equivalents of the amino acid and 6 equiv of HOBT, relative to the solid-support loading, were dissolved in a minimum amount of DMF. The mixture was cooled to 0 °C, and 3 equiv of 1,3-diisopropylcarbodiimide (DIC) was then added. The mixture was allowed to equilibrate for 2 min to completely activate the carboxylic acid group.

b. Coupling. The activated amino acid was then added to a round-bottom flask containing the solid support in a minimum amount of DMF, and the reaction was allowed to proceed for 2 min before 2 equiv of diisopropylethylamine (DIPEA) (relative to the resin loading) was added (this would give resin/amino acid: HOBT:DIC:DIPEA = 1:3:6:3:2). The mixture was then allowed to react 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 Kaiser (ninhydrin) test.[19] Coupling

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reaction was repeated if more than 10% of unreacted amino groups were detected.[19]

c. Deprotection. Removal of the Fmoc protecting group was accomplished by washing the resin twice (for 15 min each) with 55% piperidine in DMF. The free amino group of the deprotected resin-bound peptides may attack the peptide−resin linkage and liberate a cyclic diketopiperazine compound. This intramolecular animolysis is residue specific and is known to occur readily in peptides containing a Gly residue. It has been found that washing with 55% piperidine/DMF for 5 min minimizes the dipeptide cleavage.[20,21] After Fmoc removal, the resin was washed 3 times with DMF followed by decarboxylation with 2:1 (v/v) dioxane/water. The resin was then washed with DMF and chloroform 5 times each to yield a free amino group.

The resin was suspended in chloroform and measured in a home-built cell. This cell was made of aluminum with microscope slides as windows (with a clear optical aperture of 1.9 cm × 3.8 cm) and has a path length of 6.0 mm.

RESULTS AND DISCUSSION

The six amino acids (Gly, Ala, Leu, Met, Phe, Val) used in the synthesis of peptides were selected because their structures are very similar to one another. Thirty-one resin-bound peptides were synthesized from these amino acids. As illustrated in Figure 1, in most cases, these peptides have more than one common amino acid residue. Because the structures of these peptides are very similar, they should serve as effective model compounds for the evaluation of a method to identify and determine the sequences of peptides.

Two different polystyrene-based solid supports (bead without a linker (AMS) and bead with a linker (Rink)) were used in this study so that the effect of the linker on the peptide synthesis and on the NIR analysis method can be evaluated. According to the manufacturer, the polystyrene core of both resin supports is lightly cross-linked (∼1%). Because of this rather low cross-linkage, it is expected that polar solvents can permeate into core of the beads. The reactive amino group in the resin without a linker (i.e., AMS resin) is attached to the polystyrene support through a methylene group. In the resin with a linker (i.e., Rink resin), the amino group is separated from the polystyrene core by the acid-labile group.

1. NIR Spectra of Peptides Attached to Resin without linker (AMS Resin). Figure 2 shows the NIR absorption spectrum of AMS resin suspended in chloroform as well as the absorption spectrum of pure chloroform. As illustrated, differences between these spectra include well-resolved bands at 1526, 2022, and 2160 nm and a distinct shoulder at 2300 nm. The bands at 1526 and 2022 nm may be due to the aliphatic amino groups attached to the resin backbone.[22−25] The complex band centered at 2160 nm may be assigned to the aromatic C−H stretch of the polystyrene backbone.[22−25] Aliphatic C−H combination transition may be responsible for the shoulder at 2300 nm.[22−25] Further, this band can be traced to methylenes in the resin as well as the amino group−polystyrene bridge.[22−25] Since the amino group is involved in all solid-phase syntheses, it may be possible to use the bands at 1526 and 2022 nm to monitor the formation of the amide bond during coupling reactions. Moreover, multivariate analysis of the aliphatic CH combination band at 2300 nm is expected to provide information on side groups of amino acid residues which, in turn, would facilitate identification of resin-bound amino acids.

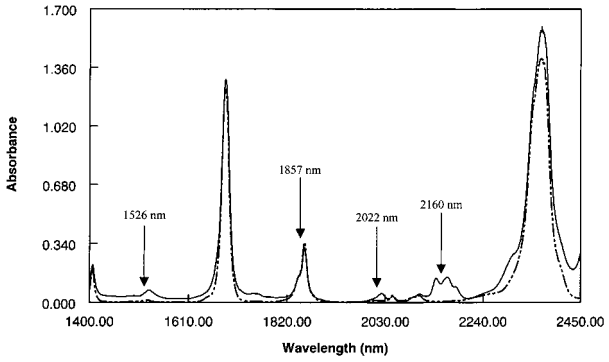

Figure 2. Near-infrared absorption spectra of the AMS beads in chloroform (solid line) and chloroform alone (dotted line).

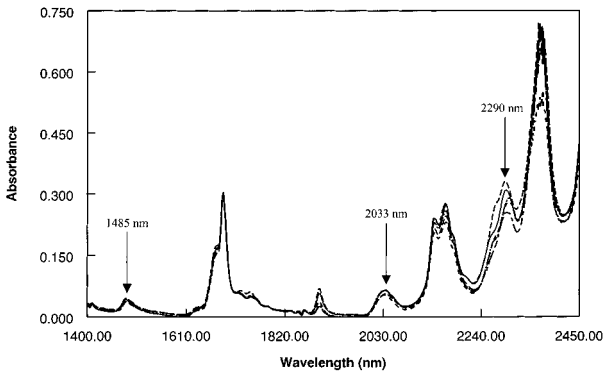

Figure 3. Near-infrared absorption spectra of Fmoc-protected dipeptides on AMS resin: AMS-Gly-Ala-Fmoc (—), AMS-Gly-Leu-Fmoc (- - -), AMS-Gly-Met-Fmoc, (— — —), AMS-Gly-Phe-Fmoc (— - -), and AMS-Gly-Val-Fmoc (—. —).

Shown in Figure 3 are the NIR absorption spectra for five Fmoc-protected dipeptides (i.e., AMS-Gly-Ala-Fmoc, AMS-Gly-Leu-Fmoc, AMS-Gly-Met-Fmoc, AMS-Gly-Phe-Fmoc, and AMS-Gly-Val-Fmoc) on AMS resin. The spectra in this figure were corrected for influence of chloroform. Since AMS resin absorbs a significant amount of chloroform (∼6.0 mL/g), the spectra were corrected by subtracting the chloroform spectrum using an empirical weighting factor of 0.85. The correction factor of 0.85 was selected because, as illustrated in Figure 3, the band at 1857 nm is due primarily to the absorption of chloroform. The absorbance of AMS resin in chloroform at this wavelength is ∼85% as intense as that of the chloroform spectrum. Therefore, it is assumed that a large proportion (∼85%) of the resin volum is due to chloroform, and bands common to both spectra may be accounted for using a chloroform weighting factor of 0.85.

As illustrated in Figure 3, following coupling, the 1526-nm band of the amino group on the AMS resin (in Figure 3) disappears with concomitant growth of a band at 1485 nm. The latter band is

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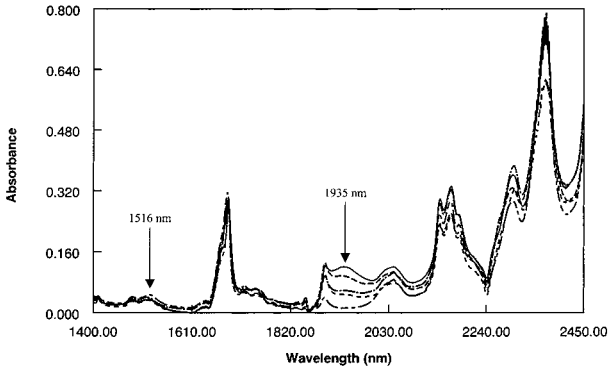

Figure 4. Near-infrared absorption spectra of deprotected dipeptides on AMS resin: AMS-Gly-Ala-NH_2 (—), AMS-Gly-Leu-NH_2 (- - -), AMS-Gly-Met-NH_2 (— — —), AMS-Gly-Phe-NH_2 (— - -), and AMS-Gly-Val-NH_2 (—. —).

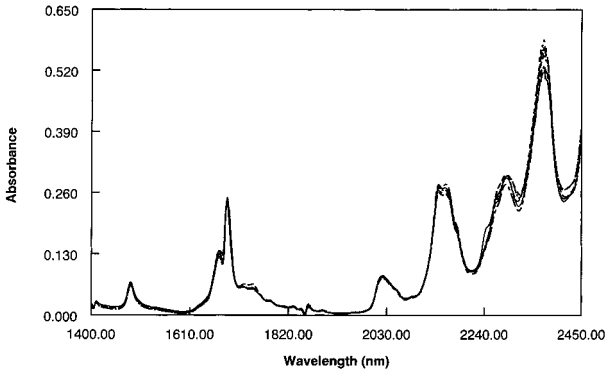

Figure 5. Near-infrared absorption spectra of Fmoc-protected dipeptides on Rink resin: Rink-Gly-Ala-Fmoc (—), Rink-Gly-Leu-Fmoc (- - -), Rink-Gly-Met-Fmoc, (— — —), Rink-Gly-Phe-Fmoc (— - -),and Rink-Gly-Val-Fmoc (—. —).

attributed to the amide bond formed during coupling.[22−25] Similarly, the 2022-nm band, which is due to the amino group on the AMS resin (in Figure 3), is shifted to 2033 nm, affirming formation of the amide bond during the coupling reaction.[22−25] Combination bands centered at 2290 nm are due to the absorption of amino acid residue side groups. They should, therefore, provide means to differentiate the dipetides. For instance, the absorption bands for AMS-Gly-Met-Fmoc and AMS-Gly-Phe-Fmoc are at 2291 and 2296 nm, respectively. Expectedly, these bands exhibit much greater variation compared to the other bands. Specifically, the combination bands of Gly-Ala, Gly-Leu, Gly-Met, Gly-Phe, and Gly-Val are different from one another in size, shape, and maximum position.

Illustrated in Figure 4 are the spectra for Fmoc-deprotected dipeptides on AMS resin. As depicted in this figure, following removal of the Fmoc protection group, several new spectral features are apparent when compared to the Fmoc-protected dipeptides. Most notably, bands at 1516 and 1935 nm as well as a subtle shoulder at 2179 nm are observed. The band at 1516 nm is due to the deprotected amino group. Expectedly, maximum absorption of this band is only slightly shifted from that of the amino group on the AMS resin (at 1526 nm in Figure 2). Additionally, the amide band at 1482 nm is similar (in position and intensity) to the amide band for the protected dipeptides at 1485 nm in Figure 3. Further, the broad band at 1935 nm and the shoulder at 2179 nm are indicative of the amino group formed following Fmoc deprotection. In the present figure, combination bands due to the amino acid residue side groups occur at 2298 nm. The differences in the peak position among different dipeptides are not as wide as those of the corresponding protected dipeptides. For example, the band at 2297 nm for AMS-Gly-Met is at the shortest wavelength, and the longest wavelength is at 2302 nm (for AMS-Gly-Leu). This 5-nm spread is relatively shorter than the spread of 9 nm which was found for protected dipeptides (i.e., the shortest band at 2291 nm for AMS-Gly-Met-Fmoc and the longest at 2300 nm for AMS-Gly-Val-Fmoc). The result seems to suggest that bands of the amino acid residue side groups are coalesced by the more polar amino group generated following deprotection.

2. NIR Spectra of Peptides Attached to Resin with Linker (Rink Resin). Recently, it has become desirable to use resin with a linker for the solid-phase peptide synthesis. The popularity stems from the fact that the peptides can be cleaved from the resin easier if they are attached to the resin through a labile linker. Such consideration prompted us to investigate the Rink resin as the solid support for the peptide synthesis. As described in the previous section, in this Rink resin, the amino group is attached to the resin through an acid-labile linker. Synthesized peptides can be readily released from the resin by use of a weak acid (e.g., by treating it with 50% trifluoroacetic acid in DCM for 15 min at room temperature[26])

Figure 5 shows the NIR absorption spectra of Fmoc-protected dipeptides (i.e., Rink-Gly-Ala-Fmoc, Rink-Gly-Leu-Fmoc, Rink-Gly-Met-Fmoc, Rink-Gly-Phe-Fmoc, and Rink-Gly-Val-Fmoc) synthesized on Rink resin. As illustrated in the figure, bands at 1484 and 2022 nm are due to amide bond formation during the coupling reaction and are similar to bands observed for Fmoc-protected dipeptides on AMS resin (Figure 3). Additionally, bands at 2150 and 2290 nm represent combination and overtone bands of aromatic CH and amino acid side groups, respectively. Further, these peak positions are in good agreement with those of Fmoc-protected peptides on AMS resin. It is noteworthy to add that the amino acid residue bands at 2290 nm is more sensitive to the chemical structure of the amino acid residues in the peptides than bands at 1484, 2022, and 2150 nm. Similarly to Fmoc-protected dipeptides on AMS resin, the variation in the position of this broad band can be attributed to different amino acid residues.

Shown in Figure 6 are absorption spectra for Fmoc-deprotected dipeptides on Rink resin. Bands at 1484, 2022, 2150, and 2290 nm occur in positions similar to those observed for Fmoc-protected dipeptides on Rink resin. A new band at 1518 nm and shoulders at 2035 and 2170 nm were observed following the removal of the Fmoc protecting group. They can be attributed to the free amino group.[22−25] The 1935-nm band due to the amino group that was seen for AMS resin (Figure 4) was not observed in this case. This may be due to the fact that, on Rink resin, the peptide and amino group are separated from the resin core by the Rink linker. Analogous to the effect observed on AMS resin (Figure 4), the amino acid residue combination bands at 2290 nm are coalesced compared to those for Fmoc-protected peptides on Rink resin.

Heavily overlapped bands throughout the NIR region make it very difficult, if not impossible, to use a single wavelength for the determination of any resin-bound peptides on AMS or Rink resin. However, it may be possible to use the partial least-squares

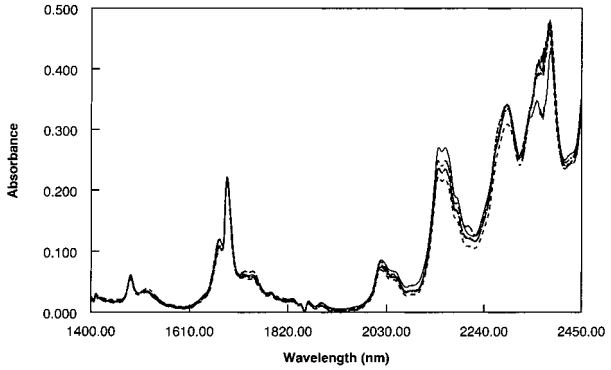

Figure 6. Near-infrared absorption spectra of deprotected dipeptides on Rink resin: Rink-Gly-Ala-NH_2 (—), Rink-Gly-Leu-NH_2 (- - -), Rink-Gly-Met-NH_2 (— — —), Rink-Gly-Phe-NH_2 (— - -), and Rink-Gly-Val-NH_2 (—. —).

regression (PLSR) method, a full spectrum chemometric technique, for the determination of resin-bound residues.

3. Analysis of Peptides. Inspection of Figure 3−6 reveals that the largest spectral variations useful for the identification of resin-bound peptides lie between 1972 and 2320 nm. Therefore, PLSR models were developed using this wavelength range. The calibrations were performed 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 ranges 1420−1455 and 1520−1655 nm. These regions were chosen because they contain little absorption information related to the peptide identity. PLS1 calibrations were developed using the solvent-corrected NIR absorption spectra of 1 monopeptide, 5 dipeptides, and 25 tripeptides on AMS resin and Rink resin as well as unreacted AMS resin and Rink resin. Moreover, calibrations were developed for Fmoc protected as well as deprotected peptides on both resins. Effects of chain length (i.e., monopeptide, dipeptide, or tripeptide) on the NIR spectra are expected since the numbers of C−H and N−H are increased with chain length. This facilitates the discrimination of dipeptides from tripeptides as well as monopeptides. Further, absorption due to the amino acid side groups is sensitive to peptide chain length. Accordingly, residues were classified into two groups depending on the 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 AMS-Gly-Ala-Leu-Fmoc, the Gly and Ala are internal residues while Leu is the terminal residue. Twelve PLS1 calibrations were developed to model the influence of internal residues and terminal residues on the absorption spectra of resin-bound (AMS and Rink resin) peptides. Additionally, a PLS1 calibration was developed to account for the absorption due solely to the unreacted resins.

Results from the PLS cross-validation show that calibrations for 13 models developed using Fmoc-protected peptides on AMS and Rink resin require relatively small number of factors for optimal performance (from 7 to 21). 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. Both groups (peptides on AMS and peptides on Rink resin) show good linearity with correlation coefficients for all calibrations are greater than 0.97.

Similar results were obtained for deprotected peptides on AMS and Rink resins. For instance, the RMSEP and SEP are on the order of 10^{-5} mmol/g. All correlation coefficients are greater than 0.97.

For each resin, 18 resin-bound peptides (9 Fmoc protected and 9 deprotected) were randomly selected to evaluate the performance of these calibrations. The selected peptides were (AMS or Rink bound) Gly, Gly-Ala, Gly-Ala-Met, Gly-Leu-Val, Gly-Met, Gly-Met-Leu, Gly-Phe-Leu, Gly-Phe-Phe, and Gly-Val-Leu. Following remeasurement of the NIR spectra and MSC, the PLS calibrations were used to predict the amino acid residue concentration.

Results obtained are listed in Tables1 and 2 for peptides attached to AMS and Rink resin, respectively. As listed in the tables, the PLS models can distinguish terminal residues from internal residues (i.e., Terminal-Val (Term-Val) vs internal-Val (Int-Val)) as well as residues with different side groups (i.e., Term-Val vs Term-Leu) for the protected and deprotected AMS-peptides and Rink-peptides. As expected, concentrations of amino acid residues in the peptides on the AMS resin predicted by the PLS models are close to the value of 1.83 mmol/g, which is the loading of the AMS resin. Similarly, the predicted concentrations of amino acid residues in peptides attached to the Rink resin are close to the loading value of 0.91 mmol/g (Table 2). Interestingly, it is evident from the tables that, in addition to predicting concentrations of residues present in the peptides, the PLS models can also predict concentrations of residues that are not in the peptides. In all cases, for peptides on the AMS as well as on the Rink resin, predicted concentrations of residues that are not present are close to 0.00 mmol/g. More importantly, the PLS can effectively predict concentrations of resin-bound peptides with exactly the same number of amino acid residues but different sequences, i.e., Gly-Leu-Val and Gly-Val-Leu. As an example, for the Rink-Gly-Leu-Val-Fmoc, the actual concentrations of the Int-Gly, Int-Leu, and Term-Val residues are that of the resin loading, which is 0.91 mmol/g. The concentrations of the Term-Leu and Int-Val (which are not present in the peptide) are expected to be 0.0 mmol/g. As listed in the Table 2, the PLS models predict 0.96, 0.90, and 0.95 mmol/g for the Int-Gly, Int-Leu, and Term-Val residues, respectively. These correspond to 5.5, 1.1, and 4.4% error. For the Term-Leu and Int-Val, the models predict concentrations of only 0.02 and 0.00 mmol/g, respectively. In comparison, for the Rink-Gly-Val-Leu-Fmoc, the models predict 0.91, 0.87, and 0.84 mmol/g for Int-Gly, Int-Val, and Term-Leu, respectively. These are only 0.0, 4.4, and 7.7% different from the actual concentration of 0.91 mmol/g. For the residues that are not there, the models predict 0.07 mmol/g for Int-Leu as well as for Term-Val. These results are especially important considering that the Leu and Val side groups differ by only a methylene group.

In summary, it has been demonstrated that synergistic use of the NIR spectrometry and partial least-squares analysis makes it possible to develop a new method capable of determining the

Table 1. PLS-Predicted Concentrations of Peptides on AMS Resin (mmol/g)

peptide	Term-Gly	Int-Gly	Term-Ala	Int-Ala	Term-Leu	Int-Leu	Term-Met	Int-Met	Term-Phe	Int-Phe	Term-Val	Int-Val
AMS-Gly-Fmoc	1.79	0.12	0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.12
AMS-Gly-NH_2	1.76	0.03	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.05	0.00	0.00
AMS-Gly-Met-Fmoc	0.00	1.83	0.09	0.00	0.17	0.00	1.73	0.07	0.00	0.01	0.00	0.00
AMS-Gly-Met-NH_2	0.05	1.82	0.00	0.13	0.00	0.10	1.78	0.21	0.34	0.00	0.05	0.00
AMS-Gly-Ala-Fmoc	0.02	1.84	1.77	0.07	0.00	0.13	0.00	0.00	0.12	0.00	0.09	0.00
AMS-Gly-Ala-NH_2	0.10	1.64	1.70	0.10	0.15	0.00	0.09	0.00	0.00	0.20	0.00	0.08
AMS-Gly- Ala-Met-Fmoc	0.00	1.92	0.03	1.80	0.14	0.00	1.57	0.28	0.10	0.00	0.02	0.14
AMS-Gly- Ala-Met-NH_2	0.00	1.87	0.14	1.66	0.00	0.06	1.98	0.00	0.00	0.00	0.14	0.03
AMS-Gly-Leu-Val-Fmoc	0.02	1.82	0.00	0.00	0.04	1.67	0.07	0.12	0.02	0.00	1.90	0.00
AMS-Gly- Leu-Val-NH_2	0.00	1.86	0.00	0.12	0.04	1.73	0.00	0.06	0.00	0.09	1.91	0.00
AMS-Gly-Met-Leu-Fmoc	0.04	1.74	0.00	0.00	2.10	0.00	0.03	1.86	0.08	0.08	0.00	0.00
AMS-Gly- Met-Leu-NH_2	0.12	1.70	0.00	0.25	2.10	0.00	0.03	1.86	0.08	0.08	0.00	0.00
AMS-Gly-Phe-Leu-Fmoc	0.03	1.81	0.01	0.00	1.60	0.03	0.17	0.00	0.17	1.68	0.00	0.16
AMS-Gly-Phe-Leu-NH_2	0.08	1.71	0.00	0.25	1.89	0.00	0.16	0.00	0.00	1.98	0.00	0.09
AMS-Gly- Phe-Phe-Fmoc	0.00	1.88	0.00	0.04	0.00	0.22	0.00	0.17	2.03	1.45	0.00	0.06
AMS-Gly- Phe-Phe-NH_2	0.00	1.82	0.11	0.00	0.03	0.00	0.00	0.02	1.72	1.63	0.06	0.07
AMS-Gly- Val-Leu-Fmoc	0.00	1.94	0.00	0.14	1.76	0.01	0.05	0.00	0.00	0.10	0.20	1.69
AMS-Gly-Val-Leu-NH_2	0.00	1.91	0.01	0.00	1.72	0.02	0.00	0.04	0.00	0.00	0.13	1.80

Table 2. PLS-Predicted Concentrations of Peptides on Rink Resin (mmol/g)

peptide	Term-Gly	Into-Gly	Term-Ala	Int-Ala	Term-Leu	Int-Leu	Term-Met	Int-Met	Term-Phe	Int-Phe	Term-Val	Into-Val
Rink-Gly-Fmoc	0.88	0.02	0.01	0.00	0.00	0.01	0.00	0.02	0.01	0.00	0.00	0.00
Rink-Gly-NH_2	0.88	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.03	0.02	0.00	0.00
Rink-Gly-Ala-Fmoc	0.02	0.87	0.87	0.09	0.06	0.00	0.02	0.00	0.00	0.01	0.00	0.03
Rink-Gly-Ala-NH_2	0.00	0.88	0.91	0.00	0.00	0.12	0.00	0.11	0.00	0.00	0.06	0.00
Rink-Gly-Met-Fmoc	0.01	0.90	0.00	0.00	0.01	0.00	0.91	0.00	0.06	0.00	0.01	0.02
Rink-Gly-Met-NH_2	0.00	0.92	0.00	0.00	0.00	0.01	0.84	0.10	0.04	0.00	0.01	0.00
Rink-Gly- Ala-Met-Fmoc	0.00	0.95	0.07	0.84	0.09	0.00	0.77	0.13	0.04	0.00	0.00	0.00
Rink-Gly- Ala-Met-NH_2	0.03	0.91	0.05	0.88	0.02	0.03	0.88	0.03	0.12	0.00	0.00	0.00
Rink-Gly-Leu-Val-Fmoc	0.00	0.96	0.00	0.05	0.02	0.90	0.00	0.00	0.05	0.00	0.95	0.00
Rink-Gly- Leu-Val-NH_2	0.00	0.91	0.00	0.02	0.03	0.82	0.01	0.07	0.00	0.00	0.92	0.00
Rink-Gly-Met-Leu-Fmoc	0.00	0.92	0.00	0.06	0.95	0.00	0.11	0.80	0.00	0.04	0.01	0.06
Rink-Gly- Met-Leu-NH_2	0.00	0.89	0.00	0.00	0.86	0.00	0.02	0.88	0.00	0.01	0.02	0.03
Rink-Gly-Phe-Leu-Fmoc	0.00	0.92	0.03	0.00	0.84	0.06	0.00	0.04	0.08	0.83	0.03	0.00
Rink-Gly-Phe-Leu-NH_2	0.03	0.89	0.10	0.00	0.87	0.09	0.02	0.00	0.00	0.92	0.00	0.00
Rink-Gly- Phe-Phe-Fmoc	0.01	0.92	0.00	0.00	0.02	0.00	0.04	0.00	0.83	0.96	0.00	0.00
Rink-Gly- Phe-Phe-NH_2	0.00	0.89	0.00	0.01	0.00	0.07	0.00	0.08	0.93	0.81	0.10	0.00
Rink-Gly- Val-Leu-Fmoc	0.00	0.92	0.08	0.00	0.87	0.07	0.00	0.05	0.00	0.00	0.07	0.84
Rink-Gly-Val-Leu-NH_2	0.00	0.94	0.03	0.00	0.96	0.06	0.00	0.03	0.03	0.00	0.00	0.88

identity and sequences of amino acid residues in di- and tripeptides. The di- and tripeptides were synthesized from six amino acids (Gly, Ala, Leu, Met, Phe, Val) on two different polymer beads (bead with and without a linker) using the solid-phase peptide synthetic method. The developed method is capable of determining the identity and sequences of these peptides (with and without the protecting groups) directly on the polymer beads. It can distinguish not only dipeptides and tripeptides but also peptides with very similar structures ((e.g., bead-Gly-Ala-Ala, bead-Gly-Ala-Phe, bead-Gly-Ala-Leu, bead-Gly-Ala-Val, and bead-Gly-Ala-Met). More importantly, the method is capable of distinguishing peptides with the same amino acid residues but different sequences (e.g., bead-Gly-Leu-Val from bead-Gly-Val-Leu). Experiments are currently in progress to expand the applications of the method to larger peptides and to increase the throughput of the method by replacing the NIR spectrometer with the NIR multispectral imaging spectrometer (i.e., replacing the single-element detector with an NIR camera).[25,27,28]

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