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Inverted Peptides - Single Bead Analysis by Methionine Scanning and Mass Spectrometry.

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Abstract: A method for sequencing peptides from single solid phase beads is presented. Unlike Edman and termination sequencing methods "methionine scanning" is applicable to peptides attached to the solid phase by either the amino or carboxyl terminus.

Combinatorial "Split and Mix" methodologies allow huge numbers of compounds to be generated in such a manner that a single bead supports only one compound.[1] For Split and Mix to realise its full potential as a method of generating chemical diversity it is necessary to be able to determine the structure of a compound on a single isolated bead. However, as a library may contain 10^5-10^6 structural possibilities this poses some major analytical challenges.

To date, this problem has been approached in two ways. Firstly by tagging beads with reporter groups[2,3] the tag defining the chemical history of any particular bead and hence the structure of the compound it supports. A range of tags have been used including halogenated aromatics, peptides, DNA, secondary amines and dyes. Secondly, by mass spectrometry analysis of library based material.[3,4] For example Youngquist *et al.*[4] have reported an elegant technique which permits the sequencing of resin bound library members using matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) by terminating 10% of the library component at each stage of the synthesis. On cleavage from the resin, MS analysis furnishes a molecular ion for the full-length compound and a "ladder" of truncated fragments. The sequence is deduced by mass differences between members of the termination synthesis family. Both MALDI-TOF and electrospray (ES) mass spectrometry can be used for direct bead interrogation and we have demonstrated that these techniques are capable of providing valuable information about a wide range of solid phase synthetic transformations from a single bead.[5]

We recently reported a method in which peptides are synthesised using traditional solid phase methods and subsequently manipulated such that the amino terminus becomes resin bound and the carboxyl terminus exposed.[6] This process has been termed "peptide inversion" and the products "inverted peptides". We demonstrated that C-terminally modified peptide based libraries can be prepared in this

manner, although a consequence of inversion, Edman sequencing of the peptide is not possible and thus for identifying materials from single beads following screening, methods of sequencing the inverted library had to be developed. Here we report a method we have termed "methionine scanning", which allows ladder sequencing of peptides regardless of their orientation on the solid support, from a single resin bead, using ES MS.

The method is based on the incorporation of a small amount of Fmoc-Methionine (5-10%) with each amino acid during the synthesis of the peptide library. After synthesis, each bead carries a number of related peptides, the predominant species being the unmodified peptide as well as a series of peptides with methionine "scanned" into the sequence. The possibility of two methionine residues occurring in the same peptide is 0.25-1% depending on the incorporation level and is not significant.

Thus the peptide H_2N-Val-Phe-Ala-Asp-Gly-Ser-Leu-Ala-Lys-Phe-OH was prepared with 10% methionine incorporated at positions from Val to Leu giving the parent peptide and a family of seven "methionine scanned" daughters (Scheme 1).[7] The electrospray mass spectrum of material prior to inversion (Figure 1) shows the MH_2^{++} mass envelope with the methionine scanned daughters surrounding the parent peptide (MH_2^{++} = 528.0). Following peptide inversion a number of single resin beads were treated with CNBr in TFA/H_2O for 24hrs and each bead was independantly analysed by ES MS.[8] A "ladder" of peptides, each differing in mass from its neighbours by one amino acid residue was released (Scheme 1 and Figure 2), allowing the peptide to be sequenced by mass differences.

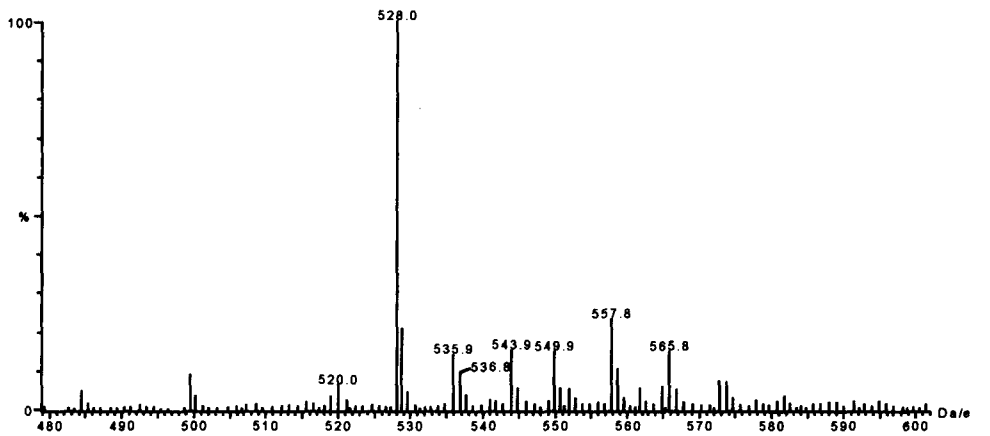

Figure 1

In conclusion we have demonstrated a method by which peptides can be sequenced from a single solid phase bead using a new process we have termed "methionine scanning". The method is similar to the termination method of Youngquist, however importantly for our purposes, it is applicable to inverted peptide based libraries which are under active investigation within our research group.

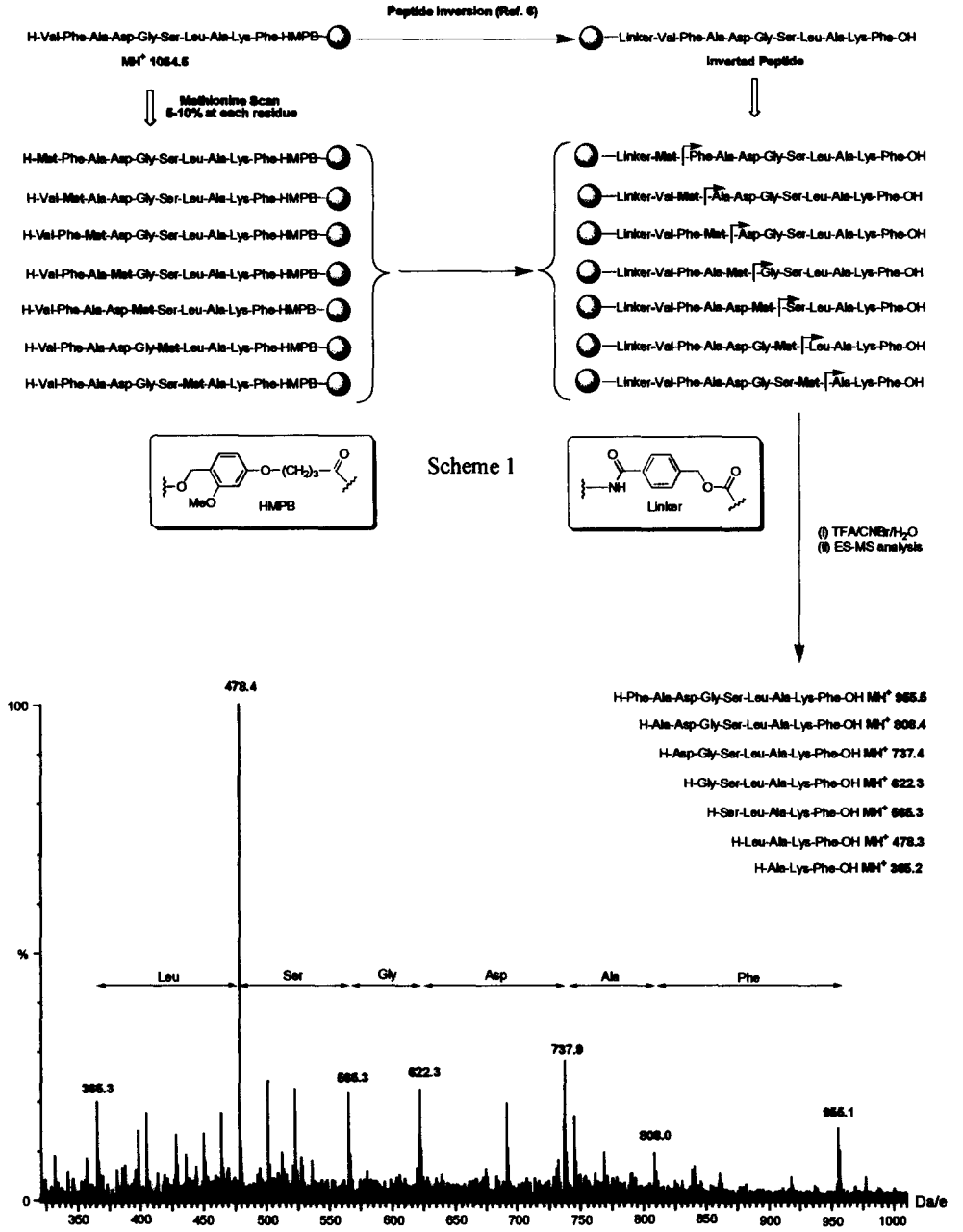

Figure 2: ES-MS Spectrum from a Single Bead

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7. Peptide synthesis was carried out on Polymer Laboratories polystyrene resin (250-300μm beads with an initial amine loading of 0.72 $mmolg^{-1}$, approximately 10nmoles/bead) using standard Fmoc chemistry with 3h HOBt/DIC couplings using monomers doped with 10% methionine.
8. Single beads were placed in 200μL capacity mass spectrometry vial inserts and treated with 100μL of CNBr $10mgml^{-1}$ in 1:1 TFA:H_2O for 24 hours. In each case, volatile materials were removed *in vacuo* and the residue and bead suspended in 200μL CH_3CN for 48 hours, 10μL injections were made using a Hewlett Packard (Palo Alto, CA, USA) HP 1050 autosampler. Electrospray mass spectra were recorded on a Micromass Platform quadrupole mass analyser (Micromass, Tudor Road, Altrincham, UK) with an electrospray ion source. The operating conditions were 3.50kV, HV lens 0.5kV, cone voltage 20V, source temperature 110°C, electrospray eluent acetonitrile at $100\mu Lmin^{-1}$ nitrogen drying gas $300Lh^{-1}$ and nebulising gas $20Lh^{-1}$.

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