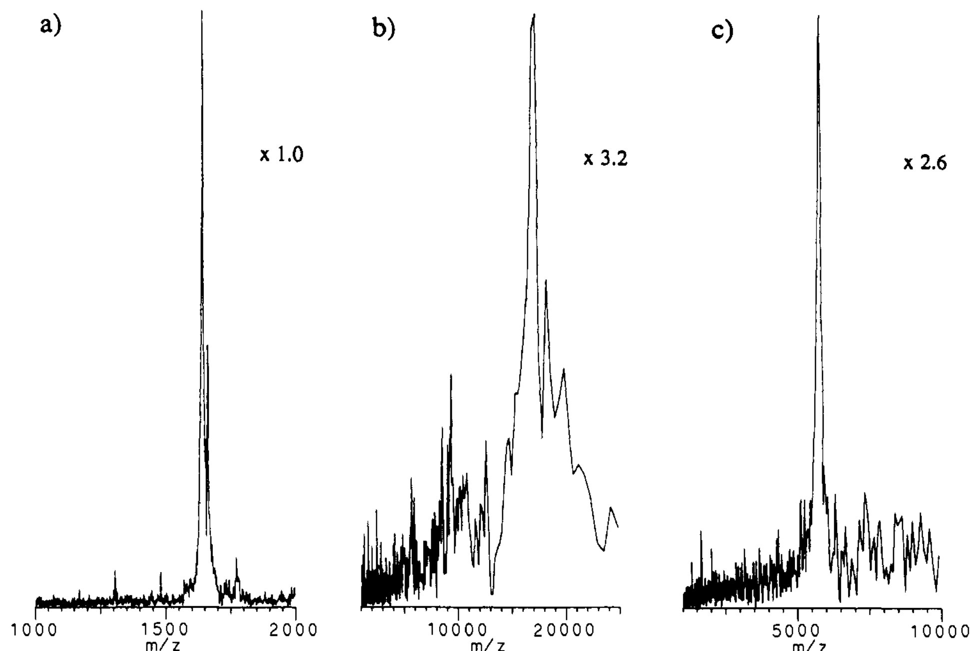

Figure 2. Matrix-assisted laser desorption Fourier transform mass spectra (355 nm) of (a) somatostatin; (b) equine myoglobin; and (c) bovine insulin.

A 200-nm UV detector senses the presence of component peaks and directs the entire peak volume of each component onto a separate probe tip position. Otherwise, effluent flow is diverted from the probe tip. For the first experiment, a 6 mM aqueous sodium bicarbonate solution containing a mixture of myoglobin and insulin was injected onto the CE column, introducing 4.9 pmol of myoglobin and 75.8 pmol of bovine insulin, subjected to electrophoresis,[20] and deposited, along with a 10^4 molar excess of 2,5-dihydroxybenzoic acid, upon a clean stainless steel probe tip. Following separation, the probe tip was transferred to the FTMS for analysis, where the single shot method, with the 308-nm laser beam impinging upon ca. 5% of the total sample surface area for each sample position, produced excellent spectra with signal to noise ratios in excess of 10:1. Up to five or six such spectra could be obtained for each spot, suggesting that a single spectrum corresponds to desorption of as little as 50 fmol of sample. As a second test of the method, a three-component mixture containing bovine insulin, somatostatin, and equine myoglobin in aqueous buffer solution was separated using similar CE conditions and the same column. Samples of 28, 16.2, and 6.2 pmol, respectively, were introduced onto the column. Figure 1 is the UV electropherogram for that separation, and Figure 2 shows the spectra obtained for these samples after transfer of a loaded Pyrex probe tip to the FTMS. Here, using the same considerations as described for the first sample, we estimate that between 60 and 300 fmol of sample were consumed per laser shot. Furthermore, on the basis of recent results with samples in a similar matrix,[16] it is expected that much higher mass resolution, with mass measurement accuracy better than the 0.01% commonly found[21] for MALDI-TOF, may be possible.

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(19) Spectra were recorded with a 7-T Millipore Extrel FTMS-2000 dual-cell mass spectrometer, equipped with an autoprobe. For laser desorption, a Lambda Physik EMG-201 MSC excimer laser (operating at 308 nm, 180 mJ/28 ns pulse) was used to pump a Lambda Physik FL-2001 dye laser. The 355-nm radiation was produced by pumping the dye laser cell containing a 0.60 g/L of dioxane solution of 2,2'''-dimethyl-*p*-quaterphenyl (BMQ, Lambda Physik), resulting in a maximum output energy of 5 mJ/pulse. For MALDI, the 355-nm laser light is attenuated by an iris and focused as a 0.06 mm^2 beam upon the probe tip. Alternatively, the 308-nm laser beam was used directly. Spectra were obtained using experimental parameters as described in ref 17.

(20) Electrophoresis was carried out using a 99-cm fused silica capillary column (Polymicrotechnologies, 360 μm o.d., 50 μm i.d.) connected to the UV detector (Model 500, Scientific Systems Inc.) via an additional 11 cm of column at 25 kV (Glassman High Voltage, Inc.) A solution of 30 mM aqueous sodium biocarbonate (pH 9.0) was used as the running buffer.

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High-Resolution Matrix-Assisted Laser Desorption/Ionization of Biomolecules by Fourier Transform Mass Spectrometry

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Since the introduction of matrix-assisted laser desorption/ionization (MALDI) mass spectrometry,[1-3] its applicability to a large number of organic molecules, primarily proteins and polymers, has been investigated. Unfortunately, mass resolution employing time-of-flight (TOF) mass spectrometers typically used with MALDI rarely exceeds 1000.[4] However, by using a Fourier transform mass spectrometer (FTMS), and a new approach to MALDI, it is now possible to obtain high mass resolution of protein molecular ions. This communication presents the first demonstration of this new technique, which appears to have great promise.

The present work builds upon the earlier results of Castoro and co-workers[5] who successfully demonstrated the adaptation of MALDI to Fourier transform mass spectrometry for analysis of molecular ion species of polymers and biomolecules with masses

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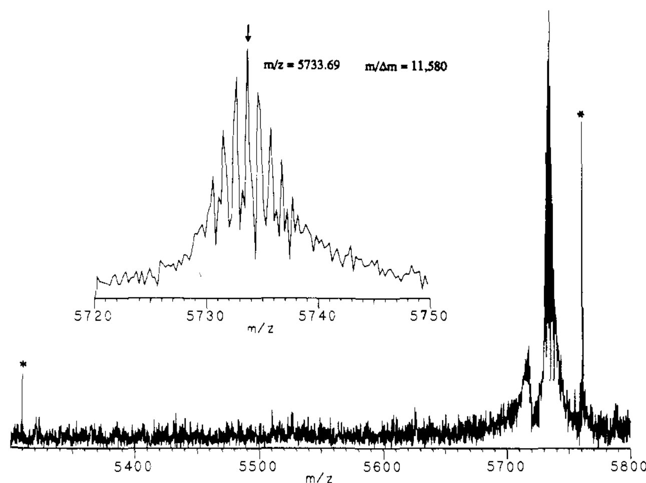

Figure 1. MALDI-FT mass spectrum of bovine insulin (MH^+) using *d*-ribose as co-matrix (mass resolution of 11 580 for m/z 5733.69 and mass accuracy of 0.001%). Poly(ethylene glycol) 1000 was used as calibrant in a separate measurement. * indicates an electronic noise artifact.

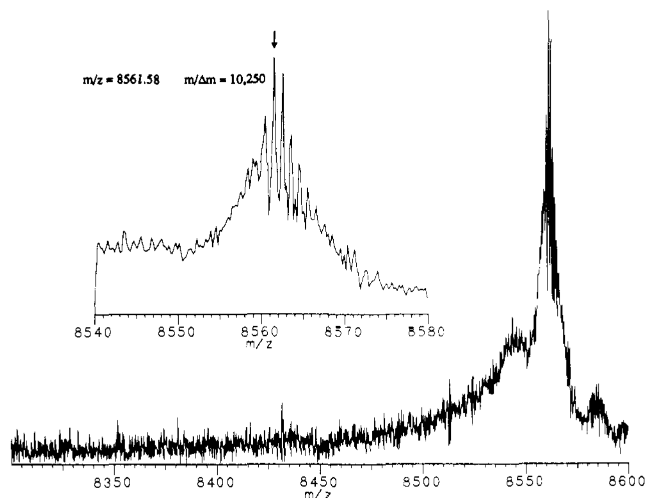

Figure 2. MALDI-FT mass spectrum of ubiquitin from bovine red blood cells using *d*-fructose as co-matrix (mass resolution of 10 250 for m/z 8561.95). Poly(ethylene glycol) 4000 was used as calibrant in a separate measurement.

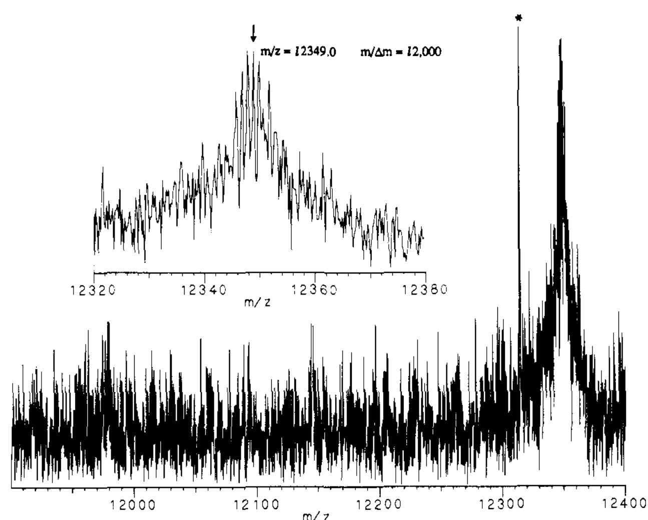

Figure 3. MALDI-FT mass spectrum of cytochrome *c* from horse heart using *d*-fructose as co-matrix (mass resolution of 12 000 for m/z 12349.0). Poly(ethylene glycol) 4000 was used as calibrant in a separate measurement. * indicates an electronic noise artifact.

as high as 34 000 Da. Because FTMS typically requires the trapping of analyte ions under low trapping potentials for reliable results, it had previously been suggested that MALDI might be difficult to implement with such instruments.[6] This suggestion derives from the observation that, in MALDI-TOF, desorbed ions have common velocities[6,7] resulting in translational energies that scale with mass, although not necessarily in a strictly linear fashion.[7] These observations prompted an experimental approach which employs the use of carefully timed retarding potentials to decelerate desorbed ions, permitting their low-voltage trapping in the FTMS cell. Even so, initial experiments showed that MALDI-FTMS under such conditions is accompanied by extensive metastable decay of molecular ions of biomolecules such as melittin and insulin, limiting observation time and, accordingly, attainable mass resolution.

An alternative to exclusive use of retarding potentials to slow ions is to cool them through some suitable collision process. Thus, we sought a means of suitably modifying the matrix both to minimize metastable fragmentation and to enhance ionization. Initially, this was accomplished by saturating with sucrose a mixture of analyte and 2,5-dihydroxybenzoic acid (DHB, 50 mmol/L) dissolved in a methanol/water/trifluoroacetic acid (TFA) solution (90:10:0.1). This solution was aerosprayed onto the probe tip,[5] and the probe was inserted into a 7-T FTMS[8] for laser desorption, producing spectra with mass resolutions in the range 6000–10 000 for melittin, bovine insulin B chain (oxidized), and bovine insulin.[9] Further studies revealed that even better results are obtained using *d*-glucose, *d*-ribose, or *d*-fructose as a co-matrix in a methanol/TFA solution. Thus far, the optimum ratios of analyte/DHB/sugar appear to be ca. 1:5000:5000. Figures 1–3 show the best results obtained to date. Figure 1 is the MALDI-FTMS mass spectrum of bovine insulin[9] with a resolution of greater than 11 000 and a mass measurement accuracy for the most abundant resolved isotopic ion of 0.001%. Figures 2 and 3 contain the MALDI-FTMS spectra of bovine ubiquitin[9] and equine cytochrome *c*,[9] each with similar mass resolution, obtained using fructose as the co-matrix. Each spectrum resulted from a single laser shot.[10] Experimental details are described elsewhere.[5]

In accord with a suggestion by Beavis and co-workers,[11] we speculate that sugars are efficacious as the co-matrix because they are relatively volatile and can also decompose to yield products such as water or carbon dioxide during the desorption process, possibly creating a momentarily dense gas environment which can serve to collisionally cool desorbing analyte molecules. Enhanced ionization, which is observed, might result from chemical ionization processes taking place during desorption. In conclusion, it appears that this new approach to matrix-assisted laser desorption is a promising technique which could allow high-resolution mass spectrometry of even higher mass biomolecules than those reported

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(8) Millipore Extrel (Madison, WI) FTMS 2000 dual-cell system equipped with a 7-T superconducting magnet.
(9) Sigma Chemical Co., St. Louis, MO.

(10) To measure MALDI spectra, the source side of a dual cell was used[5] for all experiments. Prior to firing the desorption/ionization laser pulse, the front trap plate of the cell is set to ground potential and the rear trap plate to 9 V. The laser is then triggered, and following a variable delay of between 50 and 200 μs (optimized for each sample), trapping potentials are adjusted to 1 V for melittin, 0.4 V for bovine insulin B chain, and 0.2 V for bovine insulin, ubiquitin, and cytochrome *c* measurements. Spectra are obtained using 200-V peak-to-peak chirp excitation of 50 Hz to 100 (melittin, bovine insulin B chain), 50 (bovine insulin), or 25 kHz (ubiquitin and cytochrome *c*) at −200 Hz/μs sweep rate, followed by detection. Each spectrum results from one time domain scan of 65 536 (or 131 072 for ubiquitin and cytochrome *c*) data points augmented by an equal number of zeros, and the spectra are base line corrected prior to magnitude-mode Fourier transform to produce mass spectra. No apodization was used. The resolution is estimated from the ratio of peak position to peak width at half-height.

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here. Furthermore, it could also be applicable to MALDI-TOF MS as a possible means of moderating metastable decomposition and enhancing mass resolution.

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Chiral Molecular Recognition in Intercalated Zirconium Phosphate

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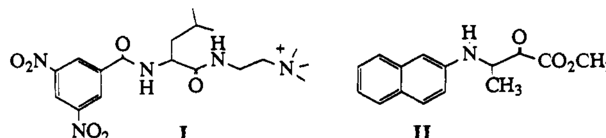
One of the challenges of modern inorganic and analytical chemistry is the synthesis of microporous solids that bind molecular analytes selectively and reversibly. The practical implications of such materials are very significant for separations and catalysis. For example, size- and shape-selective sorption and reactivity in the case of zeolites form the basis of a catalytic hydrocarbon chemistry that is carried out on an enormous scale. In recent years, layered metal phosphates and phosphonates have received considerable attention as metal-organic analogues of zeolitic solids,[1-8] because their structures can in principle be tailored, and there are now a few examples of interlayer molecular recognition in these materials. Small "templating" molecules can be introduced in some cases at interlayer coordination sites during the synthesis, and subsequently removed in order to create shape-selective binding pockets for aliphatic alcohols or amines.[9-11] These examples of shape-selective intercalation in the solid state are nevertheless rather primitive in comparison with the elegant host/guest chemistry that has been developed for purely molecular systems. The latter use multipoint noncovalent interactions in concert not only to bind analytes with a high degree of specificity but also to effect complex and often biomimetic reactivity.[12]

In terms of shape selectivity, enantioselective binding of molecules from a racemic mixture is significantly more demanding than binding of an achiral molecule. Both enantiomers possess chemically identical functional groups and are resolved by multipoint binding to a chiral host possessing complementary functionality. It is generally accepted that at least three points of contact between host and guest are required for enantioselectivity.[13] Because of this requirement, enantiomeric excesses achieved in the intercalation reactions of chiral microporous solids, most notably smectite clays,[14] have been modest. To our knowledge similar attempts to resolve enantiomers using zeolite beta one polymorph of which crystallizes[15] in the chiral space group $P4_122$, have as yet been unsuccessful.

While chiral molecules, particularly amino acids[16] and cyclodextrins,[17] have been studied as intercalants in metal phosphates, there have been no previous reports of enantioselective binding in these materials. We report here an approach that combines the ultrahigh internal surface area and structural versatility of these solids with the enantioselectivity of a well-studied chiral selector group. The chiral selector (I) and racemic analyte (II) molecule used in this study are structurally nearly identical to those



studied by Pirkle and co-workers,[18] the difference being that the (*S*)-(+)-*N*-(3,5-dinitrobenzoyl)-L-leucine derivative I contains a cationic quaternary ammonium group at the end of the molecule remote from the π-stacking and hydrogen-bonding sites. I was intercalated into microcrystalline α-$Zr(HPO_4)_2 \cdot H_2O$ by reaction with tetrabutylammonium hydroxide, followed by the iodide salt of I. The bulky tetrabutylammonium ion swells the layers, causing the solid to disperse in water; because of its poor electrostatic interaction with the anionic layers, this cation acts as a good "leaving group", being quantitatively displaced by I up to a loading of approximately 0.5 mmol/g. Introduction of an aqueous solution of I causes immediate flocculation of the solid, and X-ray powder diffraction patterns show a single phase in which the layer spacing is 19 Å, compared to 7.6 Å for the parent solid. Exposure of this solid to acetonitrile solutions of the racemic analyte II causes, at high concentrations, the immediate formation of an orange I·II charge-transfer complex within the solid.

The intercalation reaction of II is very selective for its (*S*)-(−)-isomer, as shown in Figure 1. The reaction shows an interesting dependence on the concentration of racemic II in solution,

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