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361 Highly Accurate, High Sensitivity Amino Acid Analysis with Novel Activated Carbamates as Pre-column Derivatizing Reagents. STEVEN A. COHEN and DENNIS P. MICHAUD, Millipore Corp., Waters Chromatography Division, Milford MA, 01757,

Pre-column derivatization of amino acids has often become the method of choice for amino acid analysis due to several key advantages it offers, including higher sensitivity, faster analysis times, and the use of more flexible chromatographic equipment. Despite these potential improvements, most methods based on such reactions suffer from one or more deficiencies such as poor derivative stability, reaction sensitivity to buffer components, formation of multiple derivatives, low reactivity with selective components, or a complete lack of reactivity with secondary amino acids. We have synthesized a series of new, activated carbamates that exhibit none of these detrimental characteristics and provide a rapid, one-step, quantitative derivatization procedure for all amino acids studied. The synthesis route chosen allows the tagging group to be chosen from a wide variety of commercially available amines and yields high purity product with few components that interfere with analysis. The carbamate analog with aminoquinoline is the basis for an extremely powerful, versatile method for amino acid analysis that can provide quantitative analysis for peptides and proteins that rivals results form post-column derivatization procedures, yet has significant advantages such as detection limits in the 100-200 fmol range. The method requires no removal of the reagent as it is converted to a poorly detected component during the reaction, the derivatives are stable for weeks at room temperature, and low sensitivity to buffer interference has allowed the analysis of a wide variety of samples including foods and feeds, biological fluids and salt-containing protein solutions. This new reagent thus provides the first complete solution for amino acid analysis based on pre-column derivatization.

362 HPLC Analysis of Carbohydrate Weight Compositions in Glycoproteins. MICHAEL KUNITANI* and LILIA KRESIN (Analytical Chemistry Dept., Cetus Corporation, 1400 53rd St., Emeryville, CA 94608)

An HPLC method has been developed which determines the carbohydrate weight composition (weight carbohydrate per weight protein) in glycoproteins. The method is similar to that developed for the characterization of PEG-proteins (1) and involves the use of two detectors in series for size exclusion HPLC. The output of the UV (280 nm) detector, sensitive to only the protein moiety, and the refractive index detector, sensitive to both protein and carbohydrate, are mathematically combined to determine the weight composition. After initial system calibration, a single injection of a glycoprotein on SE-HPLC will yield the weight composition. Validation experiments show that nearly any carbohydrate that behaves well on SEC can be used for calibration. However, accurate protein calibration may be complicated by the lack of an appropriate deglycosylated protein. Data on accuracy and precision of this method will be presented.

1. M. Kunitani, G. Dollinger, D. Johnson and L. Kresin, On-Line characterization of PEG Modified Proteins, in press, J. Chromatogr.

363 Protein Sorting by Group-Selective Ion Exchange HPLC. JOHN FRENZ* JERRY CACIA AND CYNTHIA P. QUAN, Department of Medicinal and Analytical Chemistry, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080 USA.

N-linked oligosaccharides, that may contain sialic acid as well as sulfated or phosphorylated carbohydrates, can confer considerable charge heterogeneity on glycoproteins. This heterogeneity is reflected in multiple banding patterns of these proteins in isoelectric focusing (IEF) and in ion exchange chromatography. The availability of anion exchange columns developed for high performance liquid chromatography (HPLC) permits high resolution separations of classes of glycoforms that can facilitate characterization of the families of oligosaccharides present on the protein. The variety of positively charged groups on commercially available stationary phases permits broad variations in the selectivity attained for individual anionic groups on the protein. This report describes the behavior in anion exchange HPLC of recombinant DNase I expressed in mammalian cells. Selection of the appropriate column and mobile phase conditions permits separations of individual species that differ either in sialic acid or phosphate content of the N-linked carbohydrates on the protein. Hence, different anion exchange columns provide complementary separations. Furthermore, since HPLC separations are governed by interactions of the stationary phase with the surface of the protein, these separations are complementary to those provided by IEF. Careful selection of the conditions for HPLC and IEF therefore facilitates analysis and isolation of protein glycoforms required for characterization of therapeutic proteins.