C_2H_2 and PCH. In this regard, the NR spectra (Figure 2) are most informative for confirmation of the neutral losses from the CA or CR experiments: in the case of A^+, and not for B^+, the ions $C_2H_2^{\bullet+}$ and $PCH^{\bullet+}$ are present as reionized fragments. The marked intensity of the recovery signals provides complementary evidence of the existence of the corresponding phosphatropylium and phosphabenzyl radicals in $A^\bullet$ and $B^\bullet$. Furthermore, the different fragmentation patterns of Figure 2 vs Figure 1 are indicative of some extent of structural reorganization during the neutralization-reionization events. Consistently, $C_6H_6^{\bullet+}$ remains always absent, corroborating the nonoccurrence of any phosphorus-benzene π-adduct.

Further work is underway to address the evaluation of the relative stabilities of the phosphatropylium and phosphabenzylium structures as well as the accessibility to suitable precursors of "pure" isomers.[14,15]

(14) Following a reviewer's suggestion, atomic P^+ (generated by 70 eV electron impact ionization of PI_3) has been reacted with benzene in the low-pressure regime using our 7 T FTICR mass spectrometer (for a description and operation of the machine, see: Eller, K. Ph.D. Thesis, Technische Universität Berlin, D83, 1991). In addition to charge transfer (80%) to generate $C_6H_6^{\bullet+}$, ionic products were observed which are in keeping with the intermediate formation of a phosphatropylium ion **2**: $PC_4H_4^+$ (2%), $C_5H_5^+$ (3%), $PC_2H_2^+$ (5%), $C_3H_3^+$ (3%). The $C_6H_5^+$ ion (7%) is due to hydride atom abstraction by P^+.

(15) Financial support of our work by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is appreciated. We are grateful to Professor D. K. Bohme for helpful comments.

Analysis of Artificial Proteins by Matrix-Assisted Laser Desorption Mass Spectrometry

Ronald C. Beavis,[†,‡] Brian T. Chait,[*,‡] Howard S. Creel,[§] Maurille J. Fournier,[∥] Thomas L. Mason,[∥] and David A. Tirrell[*,§]

Laboratory for Mass Spectrometry and Gas Phase Ion Chemistry, Rockefeller University New York, New York 10021
Department of Polymer Science and Engineering and Department of Biochemistry and Molecular Biology University of Massachusetts Amherst, Massachusetts 01003
Received April 13, 1992

The growing use of recombinant DNA methods for the preparation of artificial proteins has created a pressing need for techniques for the rapid analysis of macromolecular structure. We report herein the use of matrix-assisted laser desorption mass spectrometry[1,2] to determine the structure and purity of an artificial copolypeptide prepared as part of our ongoing investigation of the crystallization behavior of periodic proteins.[3,4] The mass spectra demonstrate that SDS polyacrylamide gel electrophoresis (SDS-PAGE) is insensitive to the presence of degraded fragments of the artificial protein and that molecular masses estimated by SDS-PAGE may be in error by more than 100%. Furthermore, accurate mass determination by matrix-assisted laser desorption mass spectrometry allowed the discovery of two previously undetected mutations in the DNA code for the protein.

A synthetic DNA was constructed by multimerizing oligonucleotide fragments encoding two copies of the repeated undecapeptide of the target protein **1**.[5] The multimers were cloned and isolated, and a fragment encoding 14 undecapeptide repeats was inserted into the expression vector pET3-b.[3,6] The recombinant plasmid was used to transform *Escherichia coli* strain BL21(DE3)pLysS. After fermentation, the artificial protein was purified via acid precipitation of contaminants followed by ethanol precipitation of the target copolypeptide.[3] Amino acid analysis confirmed the composition of purified **1**, and Coomassie Blue staining of SDS-PAGE gels[7] revealed no contaminating proteins—the product was observed to migrate as a single tight band. On the other hand, the molecular weight reported by SDS-PAGE was found to be 43 000, which is more than twice the expected 17 207. Anomalously slow migration in SDS-PAGE is not uncommon, particularly for acidic proteins,[8] and has been attributed to reduced binding of SDS.

ASMTGGQQMGRDPMFKYSRDPMG-
$[AGAGAGAGPEG]_{14}$ ARMHIRPGRYQLDPAAN-
KARKEAELAAATAEQ (**1**)

In order to resolve the apparent molecular weight discrepancy, the copolypeptide was analyzed by matrix-assisted laser desorption mass spectrometry.[1,2] A small amount (1-10 pmol) of the polypeptide was mixed with a 10^4-fold molar excess of 3,5-dimethoxy-4-hydroxy-*trans*-cinnamic acid in an aqueous 30% acetonitrile solution containing 0.1% trifluoroacetic acid. The mixture was placed inside a time-of-flight mass spectrometer and irradiated with a neodymium/YAG laser (355 nm, 10 ns pulse). The ions formed by each laser pulse were accelerated by a 30-kV potential into a 2-m evacuated tube and detected using a Lecroy TR8828D transient recorder. This technique allows the rapid analysis of protein samples as small as 1 pmol containing relatively high concentrations of non-proteinaceous material. More importantly, the matrix-induced ionization results in the production of only intact ions, so that the molecular weight is readily accessible with no requirement for elaborate interpretation of the mass spectrum.

The mass spectrum of **1** is shown in Figure 1a. Although the observed value of m/z, 17 264 ± 2, shows the electrophoretically determined mass to be grossly in error, the observed value remains significantly different from the predicted m/z of 17 208.[9] In order to determine the origin of the difference, the sequence of a 741 base pair DNA fragment containing the protein coding region of the expression plasmid was determined. This analysis revealed C → T transitions in codons 96 and 101, causing two alanine-to-valine substitutions in the expressed protein. The calculated m/z value of the protein with the altered repetitive sequence **2** is 17 264—the experimentally determined value. The source of the sequence errors is not clear; because the sequence of the oligonucleotide was verified prior to multimerization, mutations must have arisen in a subsequent culturing step. Other multimers of similar sequence have been isolated and shown to be free of defects.

$-[AGAGAGAGPEG]_6[AGAGAGVGPEG]-$
$[VGAGAGAGPEG][AGAGAGAGPEG]_6-$ (**2**)

Signals arising from low molecular weight polypeptides are also visible in the spectrum shown in Figure 1a. Analysis of these signals, expanded in Figure 1b, shows that the line at m/z 5730 corresponds to fragment **3**, which consists of the intact N-terminal sequence of **1** followed by four copies of the repeating undecapeptide. The calculated mass of the molecular ion derived from

* Authors to whom correspondence should be addressed.
† Present address: Department of Physics, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X7.
‡ Rockefeller University.
§ Department of Polymer Science and Engineering, University of Massachusetts.
∥ Department of Biochemistry and Molecular Biology, University of Massachusetts.

(1) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299. Karas, M.; Hillenkamp, F.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(2) Beavis, R. C.; Chait, B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6873. Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1990**, *62*, 1836.
(3) Creel, H. S.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *Macromolecules* **1991**, *24*, 1213.
(4) McGrath, K. P.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 727.
(5) Standard single-letter abbreviation for the amino acids; see, for example: Mathews, K.; van Holde, K. E. *Biochemistry*; Benjamin/Cummings: Redwood City, CA, 1990.
(6) Studier, F. W.; Rosenberg, A. H.; Dunn, J. J.; Dubendorff, J. W. *Methods Enzymol.* **1990**, *185*, 60. Dunn, J. J.; Studier, F. W. *J. Mol. Biol.* **1983**, *166*, 477.
(7) Laemmli, U. K. *Nature* **1970**, *227*, 680.
(8) Dunker, A. K.; Ruebert, R. R. *J. Biol. Chem.* **1969**, *244*, 5074.
(9) All m/z values refer to the protonated form of the polypeptide, $(M + H)^+$.

0002-7863/92/1514-7584$03.00/0

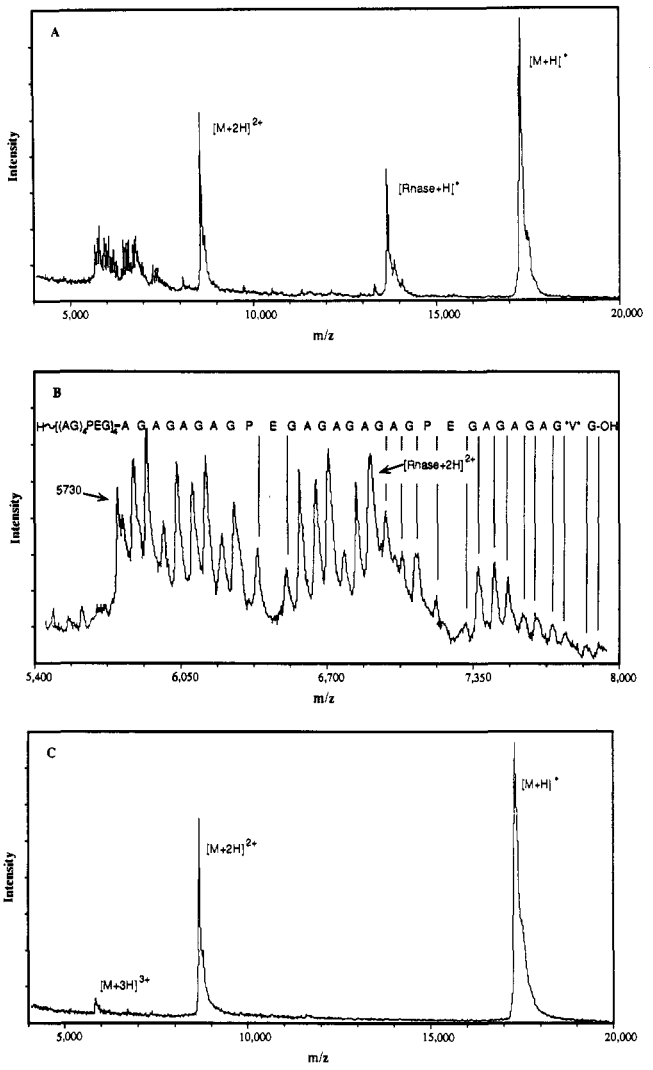

Figure 1. Analysis of artificial proteins by matrix-assisted laser desorption mass spectrometry. (A) Spectrum of target copolypeptide **1** with RNase A as an internal calibrant. The m/z value of the molecular ion $[M + H]^+$ is 17 264 ± 2. (B) Spectrum expanded in region of low molecular weight contaminants. The peaks progress starting with N-terminal fragment **3** (calcd m/z = 5733, found 5730) with the sequential addition of amino acid residues through three repeats of the target repetitive sequence. The substituted valine is apparent in the seventh repeat. (C) Mass spectrum of the protein sample after removal of the contaminating fragments by dialysis.

3 is 5733, in good agreement with the observed value. More striking, however, is the fact that each succeeding signal can be rationalized by addition of a single amino acid residue, proceeding in the N- to C-terminal direction along sequence **1**. Thus, one can read portions of the periodic sequence directly from the mass spectrum, including one of the substituted valines. These fragments appear in all preparations of the copolypeptide and probably arise from the action of exo- and endopeptidases, either in vivo and in vitro or both. The fragments are easily removed by dialysis as shown in Figure 1c, which shows only the singly, doubly, and triply ionized molecular ions of the intact protein. Fragmentation is not an artifact of the ionization technique.

H-ASMTGGQQMGRDPMFKYSRDPMG-[AGAGAGAGPEG]$_4$-OH (**3**)

Subsequent to the discovery of the contaminating fragments, the sample was rerun on a 25-cm 15% SDS polyacrylamide gel, and the proteins were visualized by Coomassie Blue staining. The fragments were not detected by this method, even when the sample was overloaded (up to 50 μg of protein per lane). In contrast, matrix-assisted laser desorption mass spectrometry provides rapid, accurate determination of molecular weight, reveals minor protein contaminants, and provides direct amino acid sequence information.

Acknowledgment. This work was supported by grants from the National Science Foundation (DMR 8914359) and the National Institutes of Health (RR00862, GM38724, and BRSG S07 RR07065).

A Substoichiometric Pyridine–Lithium Enolate Complex: Solution and X-ray Data and Implications for Catalysis in the Aldol Reaction

Paul J. Pospisil, Scott R. Wilson, and Eric N. Jacobsen*

Roger Adams Laboratory, Department of Chemistry
University of Illinois, Urbana, Illinois 61801

Received May 4, 1992

The coordination chemistry of lithium enolates has been the subject of extensive investigation,[1-4] and the relative reactivity of different aggregates has emerged as an area of central concern.[5] In the context of a broad approach to the design of asymmetric catalytic aldol reactions, we are investigating the effect of substoichiometric amounts of Lewis bases on the aggregation state and reactivity of enolates. Enantioselective catalysis is achievable in principle with chiral ligands that enhance enolate reactivity, effectively induce asymmetry in the aldol condensation, and turn over due to preferential binding to free enolate relative to the metal aldolate product.[6] We report herein our initial studies directed toward this goal, including the unexpected reaggregation of lithium pinacolate (**1**) with substoichiometric amounts of pyridine and the first definitive observation of a coordinatively unsaturated lithium enolate complex.[7]

(1) For a review on enolates: Mekelburger, H. B.; Wilcox, C. S. In *Comprehensive Organic Synthesis*; Pergamon Press: Oxford, 1991; Chapter 1.5.

(2) For reviews on lithium enolates: (a) Jackman, L. M.; Lange, B. C. *Tetrahedron* **1977**, *33*, 2737. (b) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1988** *27*, 1624. (c) Jackman, L. M. In *Comprehensive Carbanion Chemistry*, in press.

(3) For X-ray structures of organolithium compounds: Setzer, W. N.; Schleyer, P. v. R. *Adv Organomet. Chem.* **1985**, *24*, 353.

(4) (a) Arnett, E. M.; Fisher, F. J.; Nichols, M. A.; Ribeiro, A. A. *J. Am. Chem. Soc.* **1990**, *112*, 801 and references cited therein. (b) Arnett, E. M.; Palmer, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 7354. (c) Williard, P. G.; Carpenter, G. B. *J. Am. Chem. Soc.* **1986**, *108*, 462. (d) Hahn, E.; Maetzke, T.; Plattner, D. A.; Seebach, D. *Chem. Ber.* **1990**, *123*, 2059. (e) Seebach, D. In *Proceedings of the Robert A. Welch Foundation Conferences on Chemical Research*, Houston, TX, 1984; Welch Foundation: Houston, TX, 1984. (f) Laube, T.; Dunitz, J. D.; Seebach, D. *Helv. Chim. Acta* **1985**, *68*, 1373. (g) Hayes, R. N.; Grese, R. P.; Gross, M. L. *J. Am. Chem. Soc.* **1989**, *111*, 8336. (h) Jackman, L. M.; Szeverenyi, N. M. *J. Am. Chem. Soc.* **1977**, *99*, 4954. (i) Jackman, L. M.; Lange, B. C. *J. Am. Chem. Soc.* **1981**, *103*, 4494. For related work on solution structures of phenolates, see: Jackman, L. M.; Rakiewicz, E. F. *J. Am. Chem. Soc.* **1991**, *113*, 1202 and references cited therein.

(5) (a) Jackman, L. M.; Dunne, T. S. *J. Am. Chem. Soc.* **1985**, *107*, 2805. (b) Williard, P. G.; Hintze, M. J. *J. Am. Chem. Soc.* **1990**, *112*, 8602. (c) Williard, P. G.; MacEwan, G. J. *J. Am. Chem. Soc.* **1989**, *111*, 7671. (d) Hall, P. L.; Gilchrist, J. H.; Collum, D. B. *J. Am. Chem. Soc.* **1991**, *113*, 9571 and references cited therein. (e) Hall, P. L.; Gilchrist, J. H.; Harrison, A. T.; Collum, D. B. *J. Am. Chem. Soc.* **1991**, *113*, 9575. (f) Jackman, L. M.; Rakiewicz, E. F. *J. Am. Chem. Soc.* **1991**, *113*, 4101 and references cited therein.

(6) Arnett has previously demonstrated that Lewis bases such as THF and TMEDA bind to lithium enolates yet do not interact measurably with the corresponding aldolates. Arnett, E. M.; Fisher, F. J.; Nichols, M. A.; Ribeiro, A. A. *J. Am. Chem. Soc.* **1989**, *111*, 748.

(7) Although the ligand-free hexamer **1** is formally coordinatively unsaturated, Williard noted that the unusually short contacts between the lithium centers and the enolate double bonds may reflect a weak dative interaction. The resulting stabilization was proposed to account for the hexameric structure, since such chelation is geometrically less accessible in a tetramer. Williard, P. G.; Carpenter, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 3345.

0002-7863/92/1514-7585$03.00/0