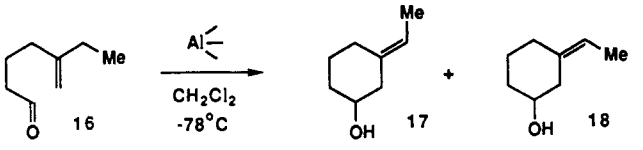
state of type **9** for trans selectivity, suggesting the intervention of an alternative transition state **10** with both R and the carbonyl group equatorial. This finding prompted us to further examine the intramolecular ene reaction of rigidly maintained cyclic substrate **11**, in which only the equatorial conformation (as in **12**) of both the α-alkyl substituent and the carbonyl group should afford the desired trans alcohol **13**. Indeed, treatment of **11** with MABR in CH_2Cl_2 at −78 °C for 2 h and at −40 °C for 1 h gave trans alcohol **13** predominantly.[5] Consequently, in the type II intramolecular ene reactions of δ,ϵ-unsaturated aldehydes **1**, the trans selectivity is best accounted for by the transition state **10** with both R and the carbonyl group equatorial rather than the alternative **9**.

Another interesting feature of MABR in the intramolecular ene reactions is the remote stereochemical control observed in the transformation of substrate **16** to *E*-olefinic alcohol **17** exclusively.[2]



Supplementary Material Available: Experimental details of the Lewis acid preparation, ene reactions with MABR, and preparation of compounds **11** and **13** (2 pages). Ordering information is given on any current masthead page.

(5) The structure of **13** was confirmed by conversion to the known *trans*-decalin-1,3-diol (Grutzmacher, H.-F.; Tolkien, G. *Tetrahedron* **1977**, *33*, 221).

Probing Conformational Changes in Proteins by Mass Spectrometry

Swapan K. Chowdhury, Viswanatham Katta, and Brian T. Chait*

The Rockefeller University, New York, New York 10021

Received August 3, 1990
Revised Manuscript Received October 11, 1990

Mass spectrometry has found wide application for the elucidation of the primary structures of proteins.[1] However, with the exception of topographical studies of membrane-bound proteins,[2] mass spectrometry has not previously been utilized to obtain information concerning the three-dimensional conformation of proteins. In the present communication, we describe the first use of mass spectrometry for probing conformational changes in proteins in a manner analogous to that employed in techniques like optical rotary dispersion, circular dichroism, and spectrophotometry.[3,4]

The new technique for probing the protein conformational changes makes use of electrospray ionization, which is a gentle method of ionization that produces intact multiply charged gas-phase ions from protein molecules in solution.[5,6] The multiply charged ions observed in the positive ion spectra are produced primarily as a result of proton attachment to available basic sites in the protein molecule. The availability of ionizable basic sites is determined by the conformation of the protein under the conditions of study, which include pH, temperature, and the presence of denaturing agents. In general, a protein in a tightly folded conformation will have fewer basic sites available for protonation compared to the same protein in an unfolded conformation. If the charge states of the gas-phase ions observed in the electrospray

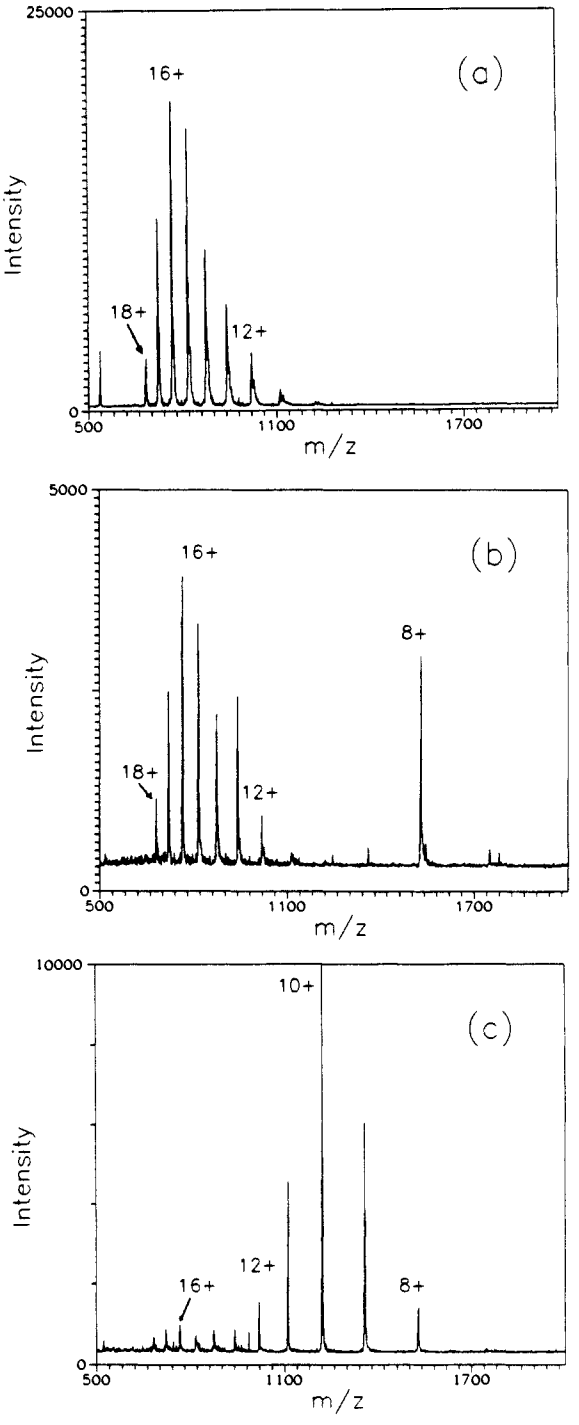


Figure 1. Electrospray ionization mass spectra of bovine cytochrome *c* obtained with different acetic acid concentrations in aqueous protein solutions. Protein concentration is 1×10^{-5} M: (a) 4% acetic acid, pH = 2.6, (b) 0.2% acetic acid, pH = 3.0, and (c) no acid, pH = 5.2. The labels on the peaks, *n*+, indicate the number of protons, *n*, attached to the protein molecule.

(1) Biemann, K.; Martin, S. *Mass Spectrom. Rev.* **1987**, *6*, 1-75. Hunt, D. F.; Yates, J. R., III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 6233-7.
(2) Falick, A. M.; Mel, S. F.; Stroud, R. M.; Burlingame, A. L. In *Techniques in Protein Chemistry*; Hugli, T. E., Ed.; Academic: San Diego, 1989; pp 152-9.
(3) Ghelis, C.; Yon, J. *Protein Folding*; Academic Press: New York, 1982.
(4) Lapanje, S. *Physicochemical Aspects of Protein Denaturation*; Wiley-Interscience: New York, 1978.
(5) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64-71.
(6) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882-99.

0002-7863/90/1512-9012$02.50/0

mass spectrum reflect the charge states of the protein in solution, then the spectrum will yield information regarding the conformational state of the protein.

The mass spectra of bovine cytochrome *c*, shown in Figure 1, were obtained[7] by electrospraying aqueous solutions over a range of pH values (2.6–5.2) where the cytochromes *c* are known to undergo conformational changes.[9-15] The mass spectrum obtained from a solution at pH 2.6 (Figure 1a) exhibits eight peaks, each one corresponding to a different protonation state of cytochrome *c*. These protonation states range from 11+ to 18+ with 16+ being the most intense. Mass spectra of this type, exhibiting ions with a wide distribution of charge states and a single maximum, are typical of the electrospray ionization mass spectra of proteins that have been reported.[5,6,8] Surprisingly, as the pH of the sprayed solution was increased to 3.0, a second maximum with a protonation state of 8+ appears in the mass spectrum (Figure 1b). The width of this second distribution is narrow and is largely composed of the 8+ ion peak. Upon further increase of the pH to 5.2, the intensity of the distribution centered around 16+ decreases substantially and a third distribution centering around 10+ (with protonation states ranging from 8+ to 12+) is observed to dominate the spectrum (Figure 1c). Determination of molecular mass from the observed mass-to-charge ratios confirmed that all the peaks designated as 8+ to 18+ arise from intact bovine cytochrome *c*. We interpret the dramatic changes observed in the cytochrome *c* mass spectra (Figure 1) to result from differences in the conformational states of the protein in solution.

At low pH, the protein unfolds (state A) so that it can accept a large number of protons (Figure 1a). As the pH is raised, some of the cytochrome *c* molecules fold into a relatively tight conformation (state B) that can accept far fewer protons and produces a second distribution centered at the 8+ charge state (Figure 1b). The simultaneous observation of two discrete distributions of ions with no ions having intermediate charge states provides evidence for a highly cooperative transition between the two conformations. The tight conformation, B, can readily be converted into the highly charged unfolded state, A, by the addition of a denaturing agent such as methanol. Upon a further increase in the pH, virtually all the protein molecules are converted into a second folded conformation (state C) that can accept a larger number of protons than B but a smaller number than A.

The acid unfolding of cytochrome *c* has been extensively studied by various other techniques including acid–base titrations,[9] optical rotation,[10] spectrophotometry,[9,11-15] circular dichroism,[15] fluorescence,[15] NMR,[16] temperature jump,[12,17] and viscometry.[14,15] The existence of at least three conformational states in acidic conditions has been reported.[9,11,12,15,17] The results of the present investigation also indicate the presence of three distinct conformational states of cytochrome *c* for electrosprayed solutions in the pH range 2.6–5.2. In the electrospray ionization process, small highly charged droplets are initially formed that rapidly evaporate before gas-phase ions are finally produced. Because the evolution of the effective pH of the rapidly evaporating charged droplets is not known, a direct correlation between the presently observed and previously reported conformational states cannot be made.

The technique has also been applied to the investigation of conformational changes in horse cytochrome *c*, bovine ubiquitin, and yeast ubiquitin induced by changes in pH and by addition of organic solvents.[18] Dramatic changes in the charge distributions were observed in each case that could be correlated with changes in protein conformation. It is noteworthy that the charge distributions of proteins containing disulfide bonds have also been observed to be increased by reduction of the disulfide bonds.[19]

Our findings demonstrate the viability of a new physical method for probing conformational changes in proteins. In addition, these studies provide the basis for a better understanding of the roles of solvent composition and protein conformation in the degree to which proteins are ionized in the electrospray process.

Acknowledgment. This work was supported in part by Grants RR00862 and RR07065, Division of Research Resources, NIH, and GM38274, National Institute of General Medical Sciences, NIH. Insightful discussions with Dr. Ronald Beavis are gratefully acknowledged.

(7) The aqueous protein solutions (without the addition of any buffers) were electrosprayed at room temperature through a 150-μm-i.d. stainless steel syringe needle, whose tip was etched to provide a sharp conical shape. The electrospray ionization mass spectrometer used in the present investigations has been described earlier.[8] All the experiments were performed under identical conditions, except for the amounts of acetic acid added to the spray solutions and the flow rates, which ranged between 0.15 μL/min at pH = 2.6 and 1.0 μL/min at pH = 5.2.

(8) Chowdhury, S. K.; Katta, V.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1990**, *3*, 81-7.
(9) Theorell, H.; Akesson, A. *J. Am. Chem. Soc.* **1941**, *63*, 1804-20.
(10) Knapp, J. A.; Pace, C. N. *Biochemistry* **1974**, *13*, 1289-94.
(11) Dickerson, R. E.; Timkovich, R. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1975; Vol. 11, pp 397-547. Drew, H. R.; Dickerson, R. E. *J. Biol. Chem.* **1978**, *253*, 8420-7.
(12) Dyson, H. J.; Beattie, J. K. *J. Biol. Chem.* **1982**, *257*, 2267-73.
(13) Kaminsky, L. S.; Miller, V. J.; Davison, A. J. *Biochemistry* **1973**, *12*, 2215.
(14) Babul, J.; Stellwagen, E. *Biochemistry* **1972**, *11*, 1195-1200.
(15) Goto, Y.; Calciano, L. J.; Fink, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 573-7. Goto, Y.; Takahashi, N.; Fink, A. L. *Biochemistry* **1990**, *29*, 3480-8.
(16) McDonald, C. C.; Phillips, W. D. *Biochemistry* **1973**, *12*, 3170-86.
(17) Tsong, T. Y. *Biochemistry* **1973**, *12*, 2209-14.
(18) Katta, V.; Chowdhury, S. K.; Chait, B. T., manuscript in preparation.
(19) Loo, J. A.; Edmonds, C. G.; Udseth, H. R.; Smith, R. D. *Anal. Chem.* **1990**, *62*, 693-8.

Surreptitious Involvement of a Metallacycle Substituent in Metal-Mediated Alkyne Cleavage Chemistry

Joseph M. O'Connor* and Lin Pu

Department of Chemistry (0506)
University of California at San Diego
La Jolla, California 92093-0506

Received August 7, 1990

Potential applications of metallacycles in organic synthesis[1] and, more recently, in the area of electronic materials[2] have stimulated extensive research into the properties and reactivity of this compound class. A virtually untapped source of metallacycle reactivity lies in the chemistry of the ring substituents, particularly the α-substituents due to their close proximity to adjacent coordination sites.[3,4] Herein we describe the iridiacycle-mediated alkyne cleavage reaction represented by eq 1. Although such a transformation is rare,[5] and remarkable in its own right, labeling studies have revealed a novel mechanism that includes surreptitious involvement of an α-metallacycle substituent.

$$M + H_2O + HC{\equiv}CR' \rightarrow MCO + H_3CR' \qquad (1)$$

When a wet chloroform-d_1 solution of $Ir(\overline{CR{=}CR\text{-}CR{=}CR})(PPh_3)_2(NCCH_3)_2^+BF_4^-$ (**1**)[6] (R = CO_2CH_3, 9.5 mM) and methyl propiolate (95 mM) is maintained at 23 °C for

(1) (a) Schore, N. E. *Chem. Rev.* **1988**, *88*, 1081. (b) Lindner, E. *Adv. Heterocycl. Chem.* **1986**, *39*, 237. (c) Vollhardt, K. P. C. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 539. (d) Chappell, S. D.; Cole-Hamilton, D. J. *Polyhedron* **1982**, *1*, 739.
(2) (a) Fagan, P. J.; Nugent, W. A. *J. Am. Chem. Soc.* **1988**, *110*, 2310. (b) Parshall, G. W. *Organometallics* **1987**, *6*, 687. (c) Bradley, D. C.; Faktor, M. M.; Scott, M.; White, E. A. D. *J. Cryst. Growth* **1986**, *75*, 101.
(3) Metallacycle α-substituents are known to react further with the carbon framework of metallacycles as in the cobalt-mediated enediyne chemistry of Vollhardt (ref 1c).
(4) O'Connor, J. M.; Pu, L.; Chadha, R. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 543.
(5) Sullivan, B. P.; Smythe, R. S.; Kober, E. M.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4701. Mountassir, C.; Hadda, T. B.; LeBozec, H. *J. Organomet. Chem.* **1990**, *388*, C13.
(6) O'Connor, J. M.; Pu, L.; Rheingold, A. L. *J. Am. Chem. Soc.* **1989**, *111*, 4129.

0002-7863/90/1512-9013$02.50/0