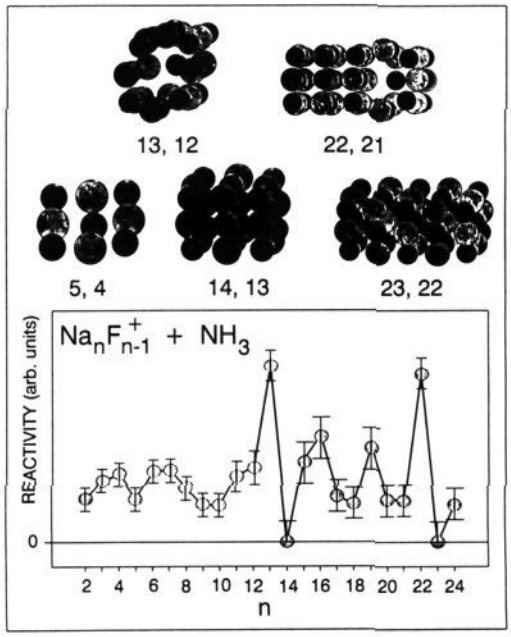

Figure 1. Relative rates for process 1 plotted vs cluster size, n = 2–24 (bottom) and calculated minimum energy structures for the most reactive (n = 13, 22) and the least reactive (n = 5, 14, 23) clusters (top). Small, dark spheres represent Na^+ ions and large, light spheres represent F^- ions. The upper structures are oriented to show the basketlike defect obtained by removing a single NaF unit from the n = 14 and 23 clusters. Point group symmetries are as follows: 5, C_{4v}; 13, C_{4v}; 14, O_h; 22, C_s; 23, D_{4h}.

near the cluster center where a halide ion X^- would have been.[15] The hydrogen atoms would lie in the interstices between the middle and outermost layers. For the case of $Na_{13}F_{12}^+$, the Na–F nearest-neighbor distance is near 2.2 Å. This is very close to the computed bond distance in the linear $Na^+ \cdot NH_3$ complex (2.25 Å);[16] which has a measured binding energy of 1.26 eV,[17] as compared to the computed energy gain of 3.3 eV by adding NaF to this site.[10] While this type of lock-and-key arrangement could neatly explain the high reactivity, and also the nonreactivity of the negatively charged analogs like $Na_{12}F_{13}^-$, the electronic character of the sites may also be important. The greatly lowered reactivity of the negatively charged clusters suggests that an important factor is the ability of NH_3 to act as a Lewis base, which could explain the results of $Si_n^{\pm}$ reactivity toward NH_3.[4]

In conclusion, the relative reactivity of charged alkali halide clusters $M_nX_{n-1}^+$ (M = Na, K; X = F, Cl) toward NH_3 has been measured under isothermal conditions using pulsed flow reactor and mass spectrometric techniques. By comparing relative reactivities to computed cluster structures, the reaction process is interpreted as molecular adsorption at favorable sites of cluster structures. Clusters with closed structural shells (n = 14, 23, 32, 38, and 53) are nonreactive at ambient temperature, whereas a specific structural defect (a "basket" or diatomic hole, present for n = 13, 22, 31, 37, and 52) greatly enhances initial adsorption. Negatively charged clusters ($Na_{n-1}F_n^-$) are much less reactive. In this approach, one can use clusters of known structures to obtain difficult-to-measure elementary reaction rates for other molecules (e.g., H_2O, CO_2, and NO_2), activation energies, and equilibrium quantities for real surfaces by extrapolating from larger clusters to the infinite.

Acknowledgment. This research has been supported by the Office of Naval Research and the National Science Foundation. R.L.W. acknowledges support from a David and Lucille Packard Foundation Fellowship.

(15) Previous experiments on AHCs have shown that it is possible to integrate different species (e.g., an electron (ref 7) or F_2^- (ref 9)) into a cluster lattice site while still maintaining the integrity of the lattice.

(16) Marchi, M.; Sprik, M.; Klein, M. L. *J. Phys.: Condens. Matter* **1990**, *2*, 5833.

(17) Keesee, R. G.; Castleman, A. W. *J. Phys. Chem. Ref. Data* **1986**, *15*, 1040.

Mass Spectrometric Detection of the Noncovalent GDP-Bound Conformational State of the Human H-*ras* Protein

Ashit K. Ganguly,*,† Birendra N. Pramanik,† Anthony Tsarbopoulos,† Thomas R. Covey,‡ Eric Huang,† and Shella A. Fuhrman§

Schering-Plough Research Institute
60 Orange Street, Bloomfield, New Jersey 07003
Sciex, Division of MDS Health Group Limited
55 Glen Cameron Road, Thornhill
Ontario L3T 1P2, Canada
Agouron Pharmaceuticals, 3565 General
Atomics Court, San Diego, California 92121

Received February 18, 1992

In recent years, the development of several new ionization methods[1-4] has allowed the direct analysis and sequencing of biomolecules previously not amenable to mass spectrometry (MS).[5-9] However, it is only recently that noncovalent receptor–ligand,[10] enzyme–substrate,[11] and enzyme–inhibitor[11,12] complexes have been detected via direct infusion ion-spray MS.[13]

Sample solutions analyzed by ion-spray MS generate a series of multiply protonated ions which, under normal operating conditions, do not undergo molecular fragmentation. Experiments can be performed in aqueous solutions without the addition of organic solvents or acidic modifiers, thus making the detection of noncovalent association complexes feasible. In this report, we describe the use of ion-spray MS for the study of the noncovalent complex of human *ras* protein with GDP, as well as the effect of pH and cosolvent on the stability of the *ras*:GDP complex system.

The *ras* proteins are regulatory guanine nucleotide binding proteins which, only in the GTP-bound active form, serve as signal transducers controlling cell proliferation or differentiation.[14] Malignacies in many different tissue types are in part brought on by mutations in the *ras* oncogene. For our studies, C-terminally truncated human H-*ras* (1–166) (Figure 1) was expressed in *Escherichia coli* from a synthetic *ras* gene under the control of both the *lpp* and *taq* promoters,[15] while *ras*:GDP (1–166) was purified from fermentor-grown, IPTG-induced cells.[16] The ac-

* Author to whom correspondence should be addressed.
† Schering-Plough Research Institute.
‡ Sciex.
§ Agouron Pharmaceuticals.

(1) Macfarlane, R. D.; Torgerson, D. F. *Science* **1976**, *191*, 920.
(2) Barber, M.; Bordoli, R. S.; Sedgwick, R. D.; Tyler, A. N. *J. Chem. Soc., Chem. Commun.* **1981**, 325.
(3) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. *Anal. Chem.* **1985**, *57*, 675.
(4) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
(5) Biemann, K.; Martin, S. A. *Mass Spectrom. Rev.* **1987**, *6*, 1.
(6) (a) Roepstorff, P. *Acc. Chem. Res.* **1989**, *22*, 421. (b) Jardine, I.; Scanlan, G. F.; Tsarbopoulos, A.; Liberato, D. J. *Anal. Chem.* **1988**, *60*, 1086.
(7) (a) Barber, M.; Green, B. N. *Rapid Commun. Mass Spectrom.* **1987**, *1*, 80. (b) Tsarbopoulos, A.; Pramanik, B. N.; Reichert, P.; Siegel, M. M.; Nagabhushan, T. L.; Trotta, P. P. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 81.
(8) (a) Biemann, K. *Biomed. Environ. Mass Spectrom.* **1988**, *16*, 99. (b) Carr, S. A. *Adv. Drug Delivery Rev.* **1990**, *4*, 113. (c) *Methods in Enzymology, Vol. 193: Mass Spectrometry*; McCloskey, J. A., Ed.; Academic Press: San Diego, CA, 1990.
(9) (a) Tsarbopoulos, A.; Becker, G. W.; Occolowitz, J. L.; Jardine, I. *Anal. Biochem.* **1988**, *171*, 113. (b) Tsarbopoulos, A. *Peptide Res.* **1989**, *2*, 258.
(10) Ganem, B.; Li, Y.-T.; Henion, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 6294.
(11) Ganem, B.; Li, Y.-T.; Henion, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 7818.
(12) Baca, M.; Kent, S. B. H. *J. Am. Chem. Soc.* **1992**, *114*, 3992.
(13) Bruins, A. P.; Covey, T. R.; Henion, J. D. *Anal. Chem.* **1987**, *59*, 2642.
(14) (a) Hall, A. *Science* **1990**, *249*, 635. (b) McCormick, F. *Cell* **1989**, *56*, 5.
(15) Ghrayeb, J.; Kimura, H.; Takahara, M.; Hsiung, H.; Masui, Y.; Inouye, M. *EMBO J.* **1984**, *3*, 2437.
(16) Jacob, J.; Schlichting, I.; Schiltz, E.; Rösch, P.; Wittinghofer, A. *J. Biol. Chem.* **1989**, *264*, 13086.

0002-7863/92/1514-6559$03.00/0

```
  1  MTEYK LVVVG AGGVG KSALT IQLIQ NHFVD
 31  EYDPT IEDSY RKQVV IDGET CLLDI LDTAG
 61  QEEYS AMRDQ YMRTG EGFLC VFAIN NTKSF
 91  EDIHQ YREQI KRVKD SDDVP MVLVG NKCDL
121  AARTV ESRQA QDLAR SYGIP YIETS AKTRQ
151  GVEDA FYTLV REIRQ H
```

Figure 1. Primary structure of human H-*ras* (1-166) protein (calculated isotopically averaged molecular weight, 18 853.3).

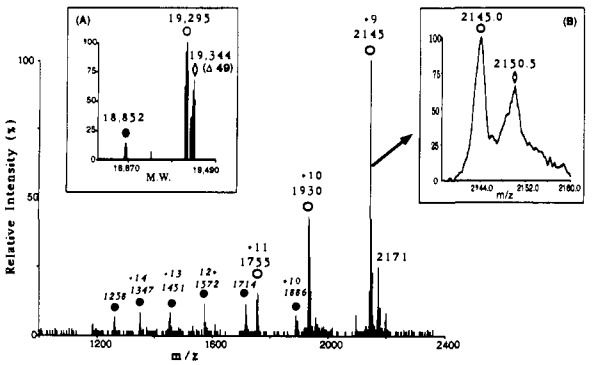

Figure 2. Ion-spray mass spectrum of *ras*:GDP complex at pH 5.8 (2 μg/μL in 2 mM NH_4OAc buffer), with the deconvoluted spectrum (inset A) and part of the narrow scan from m/z 1700 to 2400 (inset B). Open (○) and full (●) circles denote ion signals corresponding to the *ras*:GDP complex and the free *ras* protein, respectively.

tivity of this protein in GDP binding assays and GTPase assays was comparable to published data.[16-18]

The *ras*:GDP sample solution was analyzed by ion-spray MS using a Sciex API III triple quadrupole mass spectrometer. An aqueous solution of *ras*:GDP (2 μg/μL in 2 mM NH_4OAc buffer, pH 5.8) was infused at 5 μL/min through the ion-spray interface.[19] Several scans (10-20) from m/z 300 to 2400 at a scan rate of 2 s/scan were summed to yield the final profile spectrum.

The ion-spray mass spectrum of *ras*:GDP showed two envelopes of peaks corresponding to multiply protonated ions of the *ras*:GDP complex and the *ras* protein (Figure 2). The first envelope comprising intense signals at m/z 1755, 1930, and 2145 corresponding to the +11, +10, and +9 charge states of the *ras*:GDP protein-ligand complex, respectively, yields an average mass of 19 295 Da (Figure 2, inset A), thus confirming the 1:1 stoichiometry of the *ras*:GDP complex (calculated MW 19 293.5 Da). The much less abundant ions at m/z 1258, 1347, 1451, 1572, 1714, and 1886 representing the +15 to +10 charge states of the *ras* protein, respectively, provide an average mass of 18 852 Da (Figure 2, inset A); this spectrum also reveals the presence of a third component with a molecular mass 49 Da higher than that of the *ras*:GDP signal, probably due to the attachment of two magnesium atoms. This additional component was clearly resolved in the narrow scan mass spectrum (Figure 2, inset B).

The stability of the *ras*:GDP complex is highly dependent on the solvent system employed in the ion-spray analysis. Our studies indicate that dissociation of the *ras*:GDP noncovalent complex is brought about either by adding methanol or by lowering the pH of the *ras*:GDP aqueous solution or both. For example, denaturation of the *ras*:GDP complex is not brought on until the pH of the aqueous solution is lowered to ca. 3.5, whereas addition of 10% methanol at that pH induces extensive dissociation of the complex.

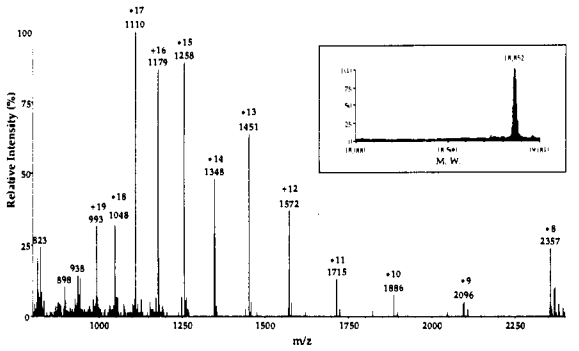

Figure 3. Ion-spray mass spectrum of the apo-*ras* protein at pH 2.7 (4 μg/μL in 1:1 methanol/water containing 2 mM NH_4OAc and 5% AcOH), with the deconvoluted spectrum shown in the inset.

In the analysis of the *ras*:GDP sample by infusion of a 1:1 methanol/H_2O solution containing 2 mM NH_4OAc and 5% acetic acid (pH 2.7), the complete denaturation of the *ras*:GDP complex was evidenced by the appearance of the multiply charged peak envelope of the free *ras* protein comprising the +12 up to +19 charge states (average mass 18 855 Da). This is in good agreement with the multiply charged peak envelope observed in the ion-spray mass spectrum of the apo-*ras* protein (not bound to GDP) shown in Figure 3. In addition, a second distribution centered around the +11 charge state was also present in the above mass spectrum of the *ras*:GDP complex (pH 2.7), yielding an average mass of 18 853 Da. This probably arises from another "tighter" conformational state of the *ras* protein in solution, identical to the one assumed by the free *ras* protein in Figure 2, wherein a smaller number of basic amino acids is exposed for protonation. Similar observations have been made in the electrospray analysis of bovine cytochrome *c*.[20]

In conclusion, we have successfully applied ion-spray mass spectrometry toward the detection of the GDP-bound noncovalent complex of the oncogenic human H-*ras* protein, and we believe this method could be extended for the study of the GTP-bound *ras* protein, as well as complexes of the *ras* protein with other pharmaceutically interesting compounds that could occupy the place of the nucleotide.

Position-Dependent Stabilizing Effects in α-Helices: N-Terminal Capping in Synthetic Model Peptides

Pingchiang C. Lyu,[‡] Hongxing X. Zhou,[†] Nazanin Jelveh,[†] David E. Wemmer,[‡] and Neville R. Kallenbach*[,†]

Department of Chemistry, New York University 4 Washington Place, New York, New York 10003 Lawrence Berkeley Laboratory, Department of Chemistry, University of California at Berkeley 1 Cyclotron Road, Berkeley, California 94720

Received March 16, 1992

The role of individual side chains in stabilizing or destabilizing α-helices has been analyzed by statistical approaches[1] and by host-guest experiments on model peptides or polypeptides.[2] While each NH group of a peptide in the "middle" of a helix is H-bonded

(17) Neal, S. E.; Eccleston, J. F.; Hall, A.; Webb, M. R. *J. Biol. Chem.* **1988**, *263*, 13086.
(18) Tucker, J.; Sczakiel, G.; Feuerstein, J.; John, J.; Goody, R. S.; Wittinghofer, A. *EMBO J.* **1986**, *5*, 1351.
(19) Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249.
(20) Chowdhury, S. K.; Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 9012.

* Author to whom correspondence should be addressed.
† New York University.
‡ University of California at Berkeley.

(1) Fasman, G. D. In *Prediction of Protein Structure and the Principles of Protein Conformation*; Fasman, G. D., Ed.; Plenum Press: New York, 1989; p 193.
(2) (a) Wojcik, J.; Altmann, K.-H.; Vasquez, M.; Scheraga, H. A. *Biopolymers* **1990**, *30*, 107. (b) O'Neil, K. T.; DeGrado, W. F. *Science* **1990**, *250*, 646. (c) Lyu, P. C.; Liff, M. I.; Marky, L. A.; Kallenbach, N. R. *Science* **1990**, *250*, 669. (d) Merutka, G.; Lipton, W.; Shalongo, W.; Park, S.-H.; Stellwagen, E. *Biochemistry* **1990**, *29*, 7511. (e) Padmanabhan, S.; Marqusee, S.; Ridgeway, T.; Laue, T. M.; Baldwin, R. L. *Nature* **1990**, *344*, 268. (f) Lyu, P. C.; Sherman, J. C.; Chen, A.; Kallenbach, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5317. (g) Chakrabartty, A.; Schellman, J. A.; Baldwin, R. L. *Nature* **1991**, *351*, 586.

0002-7863/92/1514-6560$03.00/0