Formation of Protein−Protein Complexes in Vacuo

J. Mitchell Wells, Paul A. Chrisman, and Scott A. McLuckey*

Department of Chemistry, Purdue University
West Lafayette, Indiana 47907-1393

Received September 10, 2001

Protein−protein interactions are among the most important of all intermolecular events in living systems. The study of such interactions in the gaseous state has been enabled through the development of soft ionization techniques, principally electrospray ionization.[1] Many examples have been presented whereby gaseous ions of specific protein−protein complexes known to exist in solution have been liberated into the gas phase via electrospray.[2] The ease with which such complexes can be observed has been correlated with the nature of the protein−protein interactions. Complexes bound largely by electrostatic interactions tend to be relatively stable in the gaseous state, whereas those bound in solution largely due to hydrophobic effects are often difficult to form and preserve as gaseous ions.[3] Thus, the degree of correlation between solution and gas-phase binding strengths depends on the nature of the interactions. The initial formation of the complex in the condensed phase plays an important role in the ability to form stable protein complexes in the gas phase. The study of the intrinsic interactions of proteins (i.e., in the absence of solvent) has heretofore been limited to the study of complexes formed initially in solution. In this report, we describe phenomenology associated with the formation of protein complexes in the gaseous state via the interactions of multiply charged proteins of opposite polarity.

Protein ions of each polarity were formed via nanoelectrospray and were subjected to mutual storage in a quadrupole ion trap in the presence of helium (1 mTorr) for 150 ms.[4] General reaction phenomenologies are summarized in Figure 1, which shows spectra obtained via reactions of charge-state selected ions derived from bovine cytochrome *c* (denoted as C) and bovine ubiquitin (denoted as U).[5] The positive ion spectra resulting from four experiments are shown. They include the following reactant ion combinations: $(C)^{8+}/(C)^{5-}$, $(U)^{8+}/(U)^{5-}$, $(U)^{8+}/(C)^{5-}$, and $(C)^{8+}/(U)^{5-}$. The first three combinations show competition between complex formation and various extents of proton transfer. The $(C)^{8+}/(U)^{5-}$ experiment, on the other hand, shows essentially exclusive complex formation. The relative abundances of the products shown in Figure 1, along with the products in the negative ion mode (data not shown), were essentially invariant with ion/ion reaction time. Only the fractional conversions of precursor ions to product ions were reaction-time dependent.

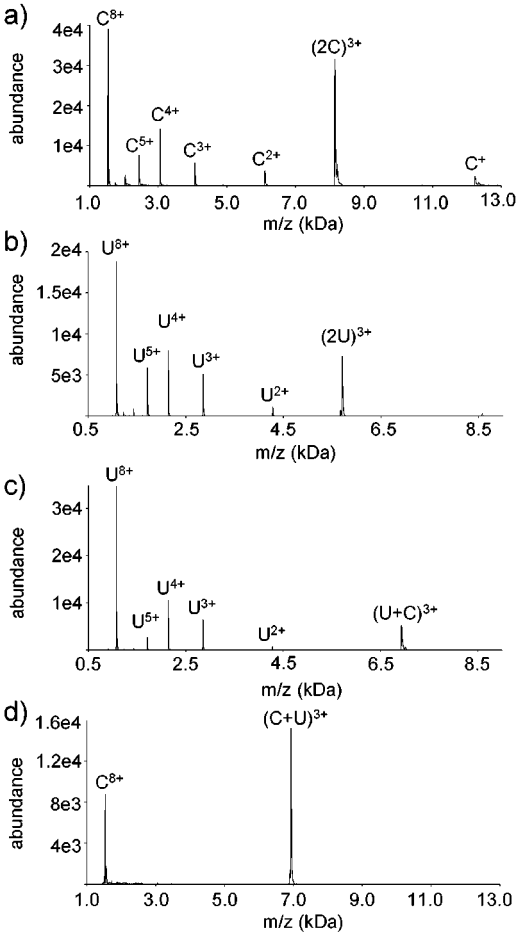


Figure 1. Positive product ion spectra for the reaction of opposite polarity protein ions. (a) $(C)^{8+} + (C)^{5-}$, (b) $(U)^{8+} + (U)^{5-}$, (c) $(U)^{8+} + (C)^{5-}$, and (d) $(C)^{8+} + (U)^{5-}$.

Furthermore, the relative abundances of the products in the negative ion mode were consistent with those of the complementary positive ion products. These observations indicate that in both ion polarity cases, the product distribution arises largely from single ion/ion collision events, and that, under the conditions used here, sequential ion/ion reactions play little role.

Given that these results constitute the first experiments in which charge-state selected multiply charged ions of opposite polarity undergo reactions in the dilute gas phase, it is important to consider possible models to rationalize the observed phenomena. The model must account for, inter alia, complex formation along with the simultaneous appearance of various proton-transfer products for some, but not all, experiments. One possibility is to invoke more than one neutralization mechanism. For example, it could be posited that complex formation arises from intimate collisions whereas the other products arise from proton transfers at relatively long range. However, it is difficult to rationalize why long-range proton transfers occur in the $(U)^{8+}/(C)^{5-}$ experiment while they do not occur in the $(C)^{8+}/(U)^{5-}$ experiment. An alternative picture is to consider the formation of all products as arising from competing processes from a single ion/ion collision complex. Such a picture appears to be consistent with all observations made to date and is outlined further below.

Figure 2 shows an energy diagram for the $(U)^{8+}/(C)^{5-}$ case. It shows only the reaction channels observed and does not reflect the possibility for fragmentation of amide bonds from either the collision complex or from excited proton-transfer products nor

* To whom correspondence should be addressed.

(1) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, 246, 64−71.

(2) (a) Loo, J. A. *Int. J. Mass Spectrom.* **2000**, 200, 175−186. (b) Light-Wahl, K. J.; Schwartz, B. L.; Smith, R. D. *J. Am. Chem. Soc.* **1994**, *116*, 5271−5278.

(3) Wu, Q. Y.; Gao, J. M.; Joseph-McCarthy, D.; Sigal, G. B.; Bruce, J. E.; Whitesides, G. M.; Smith, R. D. *J. Am. Chem. Soc.* **1997**, *119*, 1157−1158.

(4) Solutions for nano-electrospray were prepared at concentrations of 0.1 mg/mL protein in either aqueous 1% acetic acid (positive ions) or 5 mM NH_4HCO_3 (negative ions). A full description of the ion trap mass spectrometer equipped with two ESI sources and ion optics that allow sequential injection of opposite polarity ions will be the subject of a future publication.

(5) These proteins and charge states were chosen to illustrate the general phenomenology observed to date because this is the only system thus far examined for which all four protein/charge combinations were readily formed. All other multiply charged protein combinations thus far studied also show complex formation. Note that the ions are indicated by net charge only. Ubiquitin cations contain excess protons equal to the net charge. Ubiquitin and cytochrome *c* anions lack protons equal to the total charge. Cytochrome *c* cations contain a charged heme group in addition to excess protons.

10.1021/ja0170403 CCC: $20.00
Published on Web 11/13/2001

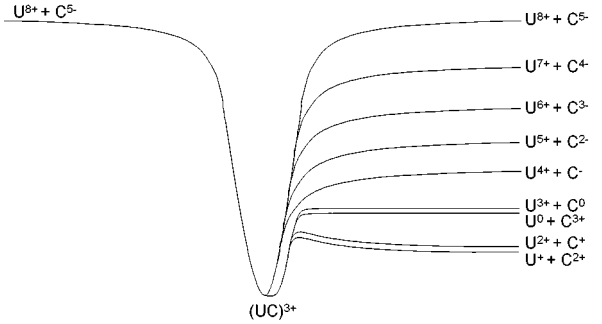
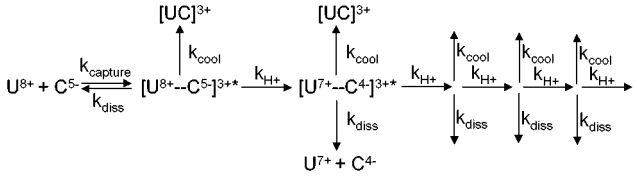


Figure 2. Hypothetical potential energy diagram for the reaction of $(U)^{8+}$ and $(C)^{5-}$.

Scheme 1. Kinetic Scheme for the Observed Protein/Protein Reactions



does it reflect proton-transfer reactions in which ubiquitin is converted from a positive ion to a negative ion, evidence for which was not present. Note that the relative energies of the product channels are indicated on a qualitative basis, as many of the relevant proton affinities and gas-phase acidities are not available to make a quantitative determination. However, the reaction enthalpies associated with these cation/anion reactions are remarkably high. For example, the ΔH_{rxn} for $(U)^{8+} + (C)^{5-} \rightarrow (U)^{7+} + (C)^{4-}$ is estimated to be at least -105 kcal/mol.[6] The energy liberated with each successive cation/anion proton transfer decreases slightly due to reduced Coulombic forces but adds to the overall energy released as the number of proton transfers increases. Hence, the total energy available within the collision complex for the $(U)^{8+}/(C)^{5-}$ case is expected to be on the order of 500 kcal/mol.

The excess energy liberated upon mutual neutralization can be dissipated in several ways under these experimental conditions. First, the complex can fragment either via cleavage of amide linkages, which is not observed, or via dissociation into product channels reflecting various numbers of proton transfers, which is observed. Second, energy can be dissipated via collisions with the bath gas.[7] The relative product ion abundances are also determined, in part, by the proton-transfer rates within the collision complex. A kinetic scheme for the overall process is shown in Scheme 1. In this picture, an initially formed $[U^{8+}\text{- -}C^{5-}]^{3+*}$ complex undergoes successive intracomplex proton-transfer reactions while competitive cleavage and cooling processes take place in parallel. The proton-transfer products arise from complexes with lifetimes sufficiently short to avoid being trapped as $[UC]^{3+}$. The relative abundances of the proton-transfer products are determined by the interplay between the consecutive proton-transfer steps within the complex and the dissociation rates associated with the various complex intermediates, $[U^{x+}C^{y-}]^{3+}$. When collisional cooling is competitive with dissociation, fragmentation of the complex is inhibited. Note that the various rate constants, k_{H+} and k_{diss}, will almost certainly be different for each step.

On the basis of the picture described herein, the distinct behavior noted in the $(C)^{8+}/(U)^{5-}$ experiment (Figure 1d) can arise from higher cooling rates, lower dissociation rates, or both. It is difficult to rationalize significantly different cooling rates associated with the $(U)^{8+}/(C)^{5-}$ and $(C)^{8+}/(U)^{5-}$ experiments, for example. It is more likely that the observed contrasting behaviors arise from differences in dissociation rates of the complexes. Dissociation-rate differences can arise from differences in reaction exothermicities, which affect the total energy available to drive dissociation, and differences in the stabilities of the complexes, which affects the competition between cooling and dissociation. While for a comparison such as $(U)^{8+}/(C)^{5-}$ versus $(C)^{8+}/(U)^{5-}$, differences in reaction exothermicities are expected to be only a small fraction of the overall values, the sensitivity of the competition between cooling and dissociation to small differences in reaction exothermicities is unclear. With respect to the complex stabilities, the initial interaction is expected to be between a protonated basic site of the cation and a deprotonated carboxylic acid site in the anion. Previous gas-phase studies have shown that acidic molecules tend to attach to neutral basic sites in protein ions due to the relatively strong dipole−dipole interaction.[8] Any differences in the strengths of these interactions are expected to be largely dependent upon the identities of the basic sites, assuming carboxylate moieties are the only negative charge-bearing sites. However, the overall binding strengths of the complexes cannot be predicted on this basis alone, as many other interactions can come into play once the reactants are brought into intimate contact. For example, the positively charged heme group may influence complex stability in the $(C)^{8+}/(U)^{5-}$ combination. Future work will explore the influence of protein structure on complex stability, and the role of stability in determining the extent of proton transfer versus complex formation in ion/ion reactions of multiply charged proteins of opposite polarity.

The results described herein demonstrate that protein−protein complexes can be readily formed in the dilute gas phase via reactions of oppositely charged protein ions. The so-formed complexes are not restricted to those formed initially in solution. Furthermore, proton-transfer reactions can compete with complex formation. This competition may reflect the binding strengths associated with the complexes. The capability for forming complexes from mass-to-charge selected reactants provides a new tool for studying intrinsic aspects of protein−protein interactions. For example, this now allows for a comparison of the stabilities and reactivities of complexes formed in solution with those of complexes formed in the gas phase.

Acknowledgment. We thank Dr. Gavin E. Reid for helpful discussions. This research was sponsored by the National Institutes of Health under Grant GM45372 and the U.S. Department of Energy under Award No. DE-FG02-00ER15105.

JA0170403

(6) This value is based on the apparent gas-phase acidity of $(U)^{8+}$, measured to be 212 kcal/mol by Cassady (Zhang, X.; Cassady, C. J. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 1211−1218), and the ΔG_{acid} of 317 kcal/mol for several amino acids (http://webbook.nist.gov).

(7) Cooling rates for large polypeptide systems in the ion trap are expected to be in the range of 10^2-10^3 s^{-1} (Goeringer, D. E.; McLuckey, S. A. *Int. J. Mass Spectrom.* **1998**, *177*, 163−174).

(8) Stephenson, J. L., Jr.; McLuckey, S. A. *J. Am. Chem. Soc.* **1997**, *119*, 1688−1696.