$^{13}C\{^1H\}$ NMR spectrum (Figure 1), which shows two singlets at 91.97 and 10.49 ppm for the Cp* ligand and a somewhat broader doublet ($^1J_{CF}$ = 295 Hz) at 103.50 ppm for the carbons of the C_5F_5 ligand. The single ^{13}C resonance strongly coupled to a single fluorine provides compelling evidence for the pentafluorocyclopentadienyl ligand. The value of $^1J_{CF}$ for the C_5F_5 ligand is similar to that found in $[Cr(\eta^6\text{-}C_6H_6)(\eta^6\text{-}C_6F_6)]$ (303 Hz).[14c] Finally, the mass spectrum of **3a** exhibits fragmentation behavior quite different from that of precursor **2a**, in which the base peak results from loss of the fluorinated portion of the molecule as C_6F_5OH. In contrast, the parent ion peak of **3a** is the most intense, and no peak due to $[Ru(C_5Me_5)]^+$ is observed. However, the peak corresponding to $[Ru(C_5F_5)]^+$ (28.6%) is prominent. Together with the microanalytical data,[15] the NMR and mass spectra provide an unambiguous characterization of **3a**.

Although crystals of **2a** and **3a** suitable for X-ray crystallography were obtained, attempts to refine both structures revealed a 2-fold disorder.[16] In an attempt to circumvent this problem, the η^5-C_5Me_4Et analogues **2b** and **3b** were also prepared[17] and found to have spectroscopic properties similar to **2a** and **3a**. Unfortunately, they were also found to suffer from the same disorder problem.[16]

Further work to extend this methodology to other complexes, to explore the chemistry of the perfluorocyclopentadienyl ligand, and to obtain suitable crystallographic samples is in progress.

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(17) Data for **2b**: ^1H NMR (C_6D_6) δ 1.90 (q, ^3J = 7.6 Hz, 2 H, CH_2CH_3), 1.44 (s, 6 H, CH_3), 1.42 (s, 6 H, CH_3), 0.64 (t, 3 H, CH_2CH_3); ^{19}F NMR (C_6D_6) δ -185.4 (m, 2 F, *m*-C_6F_5O), -190.1 (m, 2 F, *o*-C_6F_5O), -196.8 (tt, 1 F, *p*-C_6F_5O), J_{om} = 30.4 Hz, J_{op} = 14.1 Hz, J_{mp} = 42.7 Hz; IR (KBr) $\nu_{C=O}$ 1618 cm^{-1}. Anal. Calcd for $C_{17}H_{17}F_5ORu$: C, 47.11; H, 3.95. Found: C, 47.18; H, 3.82. For **3b**: ^1H NMR (C_6D_6) δ 2.13 (q, ^3J = 7.6 Hz, 2 H, CH_2CH_3), 1.71 (s, 6 H, CH_3), 1.69 (s, 6 H, CH_3), 0.85 (t, 3 H, CH_2CH_3); ^{19}F NMR (C_6D_6) δ -212.56 (s, C_5F_5). Anal. Calcd for $C_{16}H_{17}F_5Ru$: C, 47.41; H, 4.23. Found: C, 47.57; H, 4.39.

Probing Qualitative Conformation Differences of Multiply Protonated Gas-Phase Proteins via H/D Isotopic Exchange with D_2O

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We report a method for qualitatively probing the higher order structure of protein molecular ions in the gas phase and demonstrate the ability to distinguish the native and disulfide bond reduced forms in solution by gas-phase reactions of multiply protonated bovine proinsulin and α-lactalbumin. With the recent development of soft ionization methods based upon electrospray ionization (ESI)[1,2] allowing the formation of multiply charged ions from large polypeptides and proteins, new opportunities for probing structural characteristics and reactivity have arisen. Previous studies of multiply protonated cytochrome *c* reactions with dimethylamine[3] and 1,6-diaminohexane[4] in a quadrupole ion trap mass spectrometer have shown differences in the reactivity of various charge states. Chait and co-workers have also shown that H/D isotopic exchange with D_2O in solution can be useful for qualitatively probing protein structure.[5] Recent reports have indicated that various noncovalent associations, and perhaps elements of the higher order structure of macromolecular ions, may be preserved in the gas phase after ESI.[6] The method reported here invokes the use of thermal energy ion/molecule reactions of multiply protonated proteins with D_2O in order to probe *gas-phase* structural differences.

In our studies, multiply protonated protein molecules formed via ESI are transported through a dual "inlet/reaction" capillary interface, described elsewhere.[7] The first (inlet) capillary is temperature-regulated in order to assist droplet evaporation and ion desolvation. At the junction with the second capillary, D_2O (or H_2O) gas is added, and subsequent thermal energy reactions between the partially or completely desolvated ions occur in a second temperature-regulated capillary prior to expansion into vacuum. The extent of deuterium isotopic exchange is determined from the change in molecular weight measured with a triple quadrupole mass spectrometer.[2,7]

The extent of H/D exchange for the individual proteins is dependent upon the reaction temperature. Most of the presented data was obtained with the reaction capillary at an externally measured temperature of 145 °C. Above 145 °C thermally induced dissociation[8] increases for ion formed from the reduced species. When multiply protonated molecules generated via ESI from native bovine proinsulin react with gas-phase D_2O, the measured average molecular mass (M_r) calculated from the ESI m/z spectrum is 8715.0 ± 1.4 Da, 33.2 Da higher than the nondeuterated molecule (M_r = 8681.8 ± 1.4 Da).[9] This corresponds to approximately 25.2% deuterium incorporation (based upon 132 potentially labile hydrogens in the native molecule).[10] Upon reduction of the three disulfide bonds in proinsulin with 1,4-dithiothreitol, the number of potentially labile hydrogens increases to 138 and the measured M_r to 8687.8 ± 1.4 Da. However, when this species is electrosprayed and allowed to react with gas-phase D_2O, the measured M_r is 8705.2 ± 1.4 Da. This corresponds to an increase in M_r of only 17.4 Da or approximately 12.6% deuterium incorporation for ions formed from reduced proinsulin. Therefore, the ratio of the differences in molecular weight after reaction with D_2O for ions formed from the native (N) and reduced (R) proteins, $\Delta N/\Delta R$, is 1.91 ± 0.25,[11] after

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(9) To ensure that the increase in M_r was not due to the addition of solvent molecules, H_2O was added to the reaction capillary and the M_r of the protein was measured. The M_r value obtained in these experiments matched exactly the M_r measured without H_2O added.

(10) Determined by totaling the number of hydrogens attached to the heteroatoms N, O, and S. The total number of potentially labile hydrogens for α-lactalbumin is 227 for the native form and 235 for the reduced form.

(11) The experimental error associated with the ratio is based on the precision in the m/z measurement, obtained from the summation of five scans.

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Table I. Experimentally Determined Average Molecular Weight (M_r) for the Native and Reduced Forms of the Proteins Bovine Proinsulin and α-Lactalbumin after Reaction with D_2O, % H/D Exchange, and $\Delta N/\Delta R$ (the Ratio of the Difference in Molecular Weight after Reaction with D_2O for the Native and Reduced Forms of the Protein)

	M_r after D_2O reaction	% H/D exchange	$\Delta N/\Delta R$
native proinsulin (120 °C)[a]	8703.8 ± 1.4	16.7	1.87 ± 0.25
reduced proinsulin (120 °C)[b]	8699.6 ± 1.4	8.9	
native proinsulin (145 °C)[a]	8715.0 ± 1.4	25.2	1.91 ± 0.25
reduced proinsulin (145 °C)[b]	8705.2 ± 1.4	12.6	
native α-lactalbumin (120 °C)[c]	14277.0 ± 2.2	44.8	1.18 ± 0.06
reduced α-lactalbumin (120 °C)[d]	14269.6 ± 2.2	36.9	

[a] M_r = 8681.8. [b] M_r = 8687.8. [c] M_r = 14 175.2. [d] M_r = 14 183.0.

accounting for the M_r change due to the reduction of three disulfide bonds.

Comparison of H/D exchange observed for the native and reduced protein ions of the same charge state shows that ions formed from the native protein are more reactive than those from the reduced form. The charge-state distribution observed in the ESI m/z spectrum for native proinsulin consists primarily of the 7^+ and 8^+ charge states, while the distribution observed for the reduced proinsulin ranges from 7^+ to 11^+. Upon reaction with D_2O (or H_2O), the only charge state observed for the native protein is 7^+, while the charge states 7^+ to 9^+ are observed for the reduced protein.[12] While it is not possible to determine whether the extent of H/D exchange differs for the two initial charge states of the native protein, no significant difference is observed for the limited number of product charge states after reaction of the reduced protein.

To investigate whether $\Delta N/\Delta R$ is also dependent upon the reaction temperature, the same reactions were performed at 120 °C, and the value of $\Delta N/\Delta R$ was determined to be 1.87 ± 0.25. This suggests that, unlike the total extent of H/D exchange, the relative extent of H/D exchange rate is not strongly temperature-dependent over the range subject to investigation (typically 90–150 °C).[13] Table I summarizes these results as well as those obtained for ions formed from the native and reduced forms of α-lactalbumin at 120 °C where a smaller but still significant $\Delta N/\Delta R$ ratio of 1.18 ± 0.06 was observed.[10] These data show that gas-phase ions formed from these proteins exhibit opposite behavior toward H/D exchange than that observed in solution[5] where the denatured or reduced form is more reactive, presumably due to a more accessible solution structure.

The present results show that qualitative differences in the gas-phase structure of multiply charged macromolecules can be probed using high-pressure ion/molecule reactions. We speculate that the greater H/D exchange for the ions formed from native disulfide-bonded protein is due to Coulombic contributions[5,14,15] arising from the more compact gas-phase structure. Ogorzalek Loo et al. have recently reported that gas-phase proton-transfer reactions of multiply protonated proteins with diethylamine show higher reactivity with ions formed from the native protein as compared to ions from the reduced form having the same charge state,[16] a trend similar to that observed here. Possible Coulombic contributions to enhanced proton-transfer efficiency for highly charged ions have been noted.[3,4,7,15] In addition, Cooks and coworkers[17] have reported a dependency of H/D exchange rates on proton affinity differences in gas-phase ion/molecule reactions. It is reasonable that the gas-phase structure of the constrained disulfide-bonded protein will result in enhanced Coulombic effects compared to the disulfide reduced form. As a consequence, this increase in Coulombic energy may be sufficiently large to assist in overcoming reaction barriers, resulting in increased reactivity (e.g., H/D exchange, proton transfer, etc.) depending upon gas-phase structure and charge location.

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(12) The observed shift in the charge-state distribution is a result of proton-transfer reactions between multiply protonated protein ions and D_2O (or H_2O).[7]

(13) The minimum temperature is defined by the point below which substantial condensation of D_2O (or H_2O) occurs, resulting in loss of signal intensity due to extensive solvation and, at sufficiently low temperature, large clusters of droplets entering into the mass spectrometer. The upper limit is defined by the point above which thermally induced dissociation is prevalent. The actual temperature at which this occurs is dependent upon the specific protein,[9] but it is typically >150 °C for the experimental arrangement used in this work.

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Application of the Allylic Diazene Rearrangement: Synthesis of the Enediyne-Bridged Tricyclic Core of Dynemicin A

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Since its isolation and structure determination, the enediyne-containing antibiotic dynemicin A (**1**) has stimulated much research.[1,2] Certainly one of the most demanding synthetic challenges posed by dynemicin A is the dense array of sensitive functionality present in the molecule's enediyne core. Reported herein are studies that have culminated in a concise synthesis of the fully functionalized A–C rings of **1**. The synthesis features a previously described transannular Diels–Alder polycyclization[3] coupled with a highly efficient allylic diazene rearrangement[4] to rapidly assemble the enediyne-bridged A–C ring system, which is then elaborated to the fully functionalized dynemicin A core structure **2**.

As previously reported,[3] macrolactonization of seco acid **3** leads to efficient formation of polycyclization product **4**.[5] Attempts

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