Direct Observation of a Ternary Complex between the Dimeric Enzyme HIV-1 Protease and a Substrate-Based Inhibitor

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Few techniques exist for the direct observation of noncovalent macromolecular complexes. Moreover, available techniques such as size exclusion chromatography, sedimentation equilibrium ultracentrifugation, and nondenaturing gel electrophoresis can provide only an approximate molecular weight of the complex. The new ion-spray and electrospray mass spectrometry (MS) technologies use an extremely mild means for the ionization of molecules.[1-4] Recently, these MS techniques have been used to observe noncovalent receptor-ligand,[5] enzyme-substrate,[6] and heme-protein[7a] complexes. In this communication, we report the direct observation by ion-spray MS of a noncovalent ternary complex consisting of the two subunits of the HIV-1 protease molecule and a substrate-based inhibitor.

HIV-1 protease (HIV PR) is an aspartyl protease essential for processing of the *gag-pol* polyprotein in the AIDS virus.[8] The active enzyme exists as a homodimer,[9] with one of the two active site aspartate residues contributed by each subunit. The correct three-dimensional structures of the free enzyme[10] and the enzyme complexed with peptide-based inhibitors[11-13] were initially solved using HIV PR prepared by total chemical synthesis,[14] in which cysteines were replaced by α-amino-*n*-butyric acid (Aba). Current interest in the enzyme has focused largely on the design of inhibitors, which may prove useful as therapeutic agents for the treatment of AIDS.[15]

In order to see whether it would be possible to observe dimeric HIV-1 protease complexed to a substrate-based inhibitor, the tight-binding (IC_{50} = 5.9 nM[16,17]) reversible inhibitor desmethyl-JG365 (Ac-Ser-Leu-Asn-Phe-ψ[CH(OH)CH_2N]-Pro-Ile-Val-OH, *S* isomer, MW = 847) was solubilized in a small amount of DMSO and diluted with buffer (5 mM NH_4OAc, pH 5.0). Purified [Aba^{67,95}]HIV PR was dissolved in a small amount of DMSO, and diluted with the desmethyl-JG365 solution to an approximate enzyme:inhibitor molar ratio of 1:7. Excess inhibitor was removed by concentration in a stirred Amicon ultrafiltration cell (10 kDa membrane), repeatedly diluting with buffer.

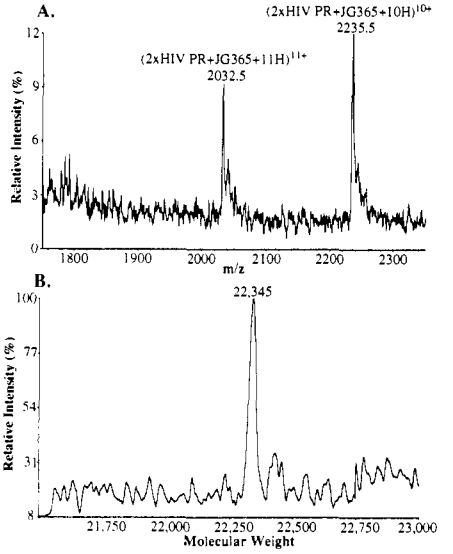

Figure 1. Mass spectra of the noncovalent ternary complex formed between dimeric [Aba^{67,95}]HIV PR and JG365. Under native conditions, the distribution of charge states is shifted to lower values of charge,[7b] so that ions corresponding to the folded enzyme-inhibitor complex were observed only at the high mass range of the spectrometer. A: Spectrum of the complex showing ions corresponding to the 11^+ and 10^+ protonation states. B: Deconvolution of the data in A to show the parent molecular species, MW 22 345.

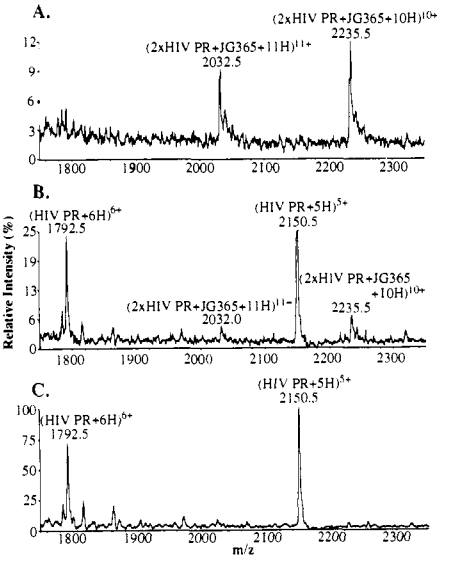

Figure 2. Ion-spray mass spectra of the complex as a function of the orifice potential. A: By setting the declustering voltage at the ion sampling orifice sufficiently low (40 V), it was possible to observe the ternary complex between dimeric HIV PR and JG365. B: At an intermediate orifice potential (70 V), both the ternary complex and monomeric HIV PR were observed. C: At high orifice potential (120 V), only the HIV PR monomer was detected.

This enzyme-inhibitor solution (approximately 90 μM) was analyzed on a Sciex API-III triple quadrupole mass spectrometer by infusion at 10 μL/min through the ion-spray interface. Mass spectra were obtained by averaging a sufficient number of scans to give an adequate signal-to-noise ratio. Ions were observed at m/z 1792.5 and 2150.5, corresponding to the $[M + 6H]^{6+}$ and $[M + 5H]^{5+}$ protonation states of monomeric [Aba^{67,95}]HIV PR.[18] In addition, ions at m/z 2032.5 and 2235.5 were observed, which

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(18) Under denaturing conditions, [Aba^{67,95}]HIV PR gave ions at 827.5, 897.0, 978.5, 1076.0, 1195.5, 1345.0, 1536.5, 1792.5, and 2150.5, corresponding to a measured MW of 10 748.5 (calcd monoisotopic 10 747.0, average 10 753.7).

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corresponded to the $[M + 11H]^{11+}$ and $[M + 10H]^{10+}$ protonation states of the native (i.e., homodimeric) enzyme–inhibitor complex. The data are shown in Figures 1 and 2. The ternary complex had a mass of 22 345 Da, as expected for 2 HIV PR subunits and JG365 ($C_{1021}H_{1672}N_{270}O_{281}S_4$ calculated monoisotopic mass 22340.4, average mass 22354.4). By raising the declustering voltage at the ion sampling orifice, it was possible to dissociate the complex either partially (Figure 2b), thus observing both the ternary complex and monomeric HIV PR, or entirely, so that only the enzyme monomer was observed (Figure 2c).

Observation of the ternary complex is direct proof of 1:1 stoichiometry between the dimeric enzyme and inhibitor and is consistent with crystallographic[12] and kinetic[17] data. Interestingly, the unique internal water molecule observed in the crystal structure of this[12] and other HIV PR–inhibitor complexes[11,13,19,20] was not observed. This is surprising given that this water molecule is internal to the folded structure and appears to be tightly coordinated, with two hydrogen bonds to the amide backbone of the enzyme and two to the inhibitor.

The demonstration that noncovalent macromolecular complexes can be directly observed will extend the applications of mass spectrometry to the study of protein–protein, protein–DNA, receptor–ligand, enzyme–substrate, and enzyme–inhibitor macromolecular complexes. Dissociation of such complexes by raising the declustering voltage at the ion sampling orifice will make possible the facile distinction between covalent and noncovalent complexes.

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New Trialkylsilyl Enol Ether Chemistry. Conjugate Additions without the Enone

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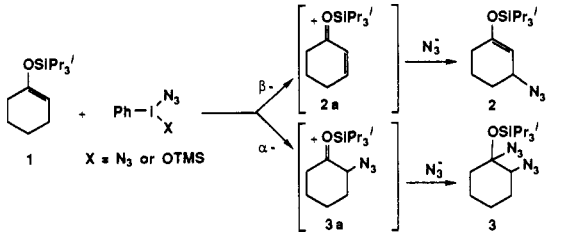
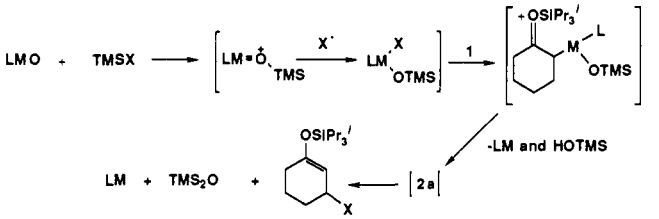
Triisopropylsilyl (TIPS) enol ethers undergo electrophilic substitution chemistry and retain the triisopropylsilyl group.[1] The normal desilylation that is observed in the case of trimethylsilyl enol ethers is prevented by the bulky isopropyl ligands. While attempting to introduce α-amino functionality into TIPS enol ethers, we examined a number of potential electrophilic nitrogen reagents.[2] Much to our surprise, it was found that treatment of a variety of TIPS enol ethers with Me_3SiN_3 (2.4 equiv)/PhIO (1.2 equiv)/CH_2Cl_2 at −15 to −18 °C gave excellent yields of the *β-azido adduct* **2** rather than the expected (normal) α-azido adduct **3** (Scheme I).[3] The β-azido adduct most probably arises from the enonium ion **2a** and the α-diazide from the onium ion **3a**. This new transformation appears to be a member of a general

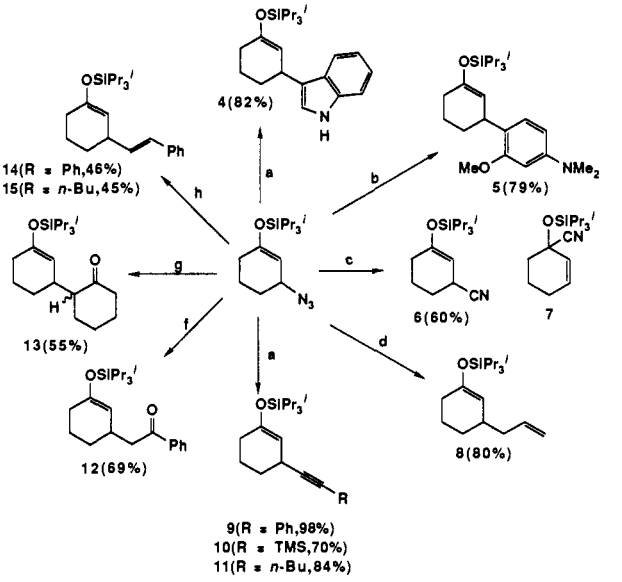
Scheme I



Scheme II



Scheme III[a]



[a] (a) Indole/Me_3Al/CH_2Cl_2, −70 °C. (b) *m*-$MeOC_6H_4NMe_2$/Me_2AlCl/hexane, 25 °C. (c) Et_2AlCN/THF, reflux. (d) Allyltri-*n*-butylstannane/Me_2AlCl/hexane, −70 °C. (e) RCCH/*n*-BuLi/Me_2AlCl/hexane, 25 °C. (f) Acetophenone TMS enol ether/Me_2AlCl/hexanes, 25 °C. (g) Cyclohexanone TMS enol ether/Me_2AlCl/hexanes, 25 °C. (h) RCCH/Cp_2ZrHCl/Me_2AlCl/hexane, 25 °C.

unexplored class of reactions that can be represented by Scheme II.[4]

To examine the trapping of the enonium ion **2a** with nucleophiles other than azide ion, we examined the response of the β-azido adduct **2** to carbon nucleophiles in the presence of Lewis acids. The preliminary results are shown in Scheme III.

Treatment of **2** with indole (2 equiv)/Me_3Al (2 equiv)/CH_2Cl_2 at −70 °C gave the 3-substituted derivative **4** (82%). Similarly, the presumed enonium ion **2a** could be trapped with *m*-$MeOC_6H_4NMe_2$ to give **5** (79%). The Nagata reagent[5] in dichloromethane gave a mixture of **6** and **7** (2:1), whereas the same reaction in THF at reflux gave only **6** (60%). While the addition

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(4) Treatment of **1** with n-$Bu_4N^+IO_4^-$/$TMSN_3$/CH_2Cl_2 gave **2** and **3** (1:1). B. Kersten, University of Texas at Austin.
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