criminating diradical intermediates [9]. The uncovering of the biosynthetic pathway to the enediynes opens up another route to improve upon these natural products by engineering new enediynes with the potential for increased selectivity. The finding that both nine- and ten-membered enediynes are biosynthesized through a common polyketide pathway indicates that it may be possible to bioengineer this pathway to produce completely novel enediynes [10]. However, the bioengineering of novel enediyne cores will require much more work to determine the way in which the enediyne PKS, together with other biosynthetic enzymes, work in concert to effect the construction of the enediyne chromophore.

In contrast, bioengineering of novel enediynes based upon manipulating the genes involved in the synthesis of groups attached to the enediyne core, such as the unusual sugars and aromatic groups, should proceed rapidly. In fact, Shen and coworkers report the preparation of a novel C-1027 analog by disrupting a specific hydroxylase gene involved in the biosyntheses of the β-amino acid portion of the natural product [6]. Interestingly, the resulting deshydroxy C-1027 (**2**, see Figure) analog is more stable than C-1027 itself. The availability of bioengineered enediynes will provide additional insights into these fascinating natural products and may lead to improved drugs to fight cancer.

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Converting Solution Macromolecular Thermodynamic Properties into Gas-Phase Mass Spectrometry Observations

Solution dissociation constants and changes in free energies associated with ligand binding to proteins have been measured in the gas phase using mass spectrometry.

Can a protein, nucleic acid, or other macromolecular complex retain a memory of its solution state as solvent and counterions are removed in vacuo? At first glance, the notion seems improbable [1]. However, since the early 1990s, noncovalent complexes of proteins, nucleic acids, and their ligands have been moved intact from solution into the gas phase using electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS). The solution characteristics of these complexes, such as their dissociation constant (K_D), have been measured in the gas phase using ion abundances from the various species, and these ESI-MS results are in good agreement with solution values over a wide range (nM to mM) [2–4]. Additional information on ligand stoichiometry, ligand binding sites, and activation energy for gas-phase dissociation is available directly or through gas-phase dissociation of ions from the complex or free macromolecule [5, 6].

The solution exchange rates of hydrogen atoms for deuterium (HDX) in peptides, proteins, and nucleic acids can be monitored using mass spectrometry [7–10]. Solvent-exposed amide hydrogen atoms will exchange with deuterons, and each incorporated deuteron increases the mass of the molecule by 1 Da, easily measured by modern mass spectrometers. The measured difference between the theoretical maximum incorporation and the experimental result quantitatively reflects the protection from exchange. Various experimental strategies (quenching, digestion, etc.) can be used with MS to measure HDX rates for surface amide residues that exchange rapidly or for slowly exchanging amides sequestered in the core of a protein [11]. MS HDX studies have been used to establish the folding of proteins, to observe changes in folding induced by various perturbations, and to map the locations of amides protected from exchange by ligand binding. MALDI-TOF mass spectrometry is well suited to measurement of HDX, since exchange is quenched by addition of the acidic matrix substrate.

Powell, Ghaemmaghami, and coworkers recently have described a general mass-spectrometry-based assay (called SUPREX) for quantitation of noncovalent complexes of proteins in solution [12]. Rather than vary the concentration of ligand and directly observe the ratio of free and bound protein, they have estimated the free energy of ligand binding and dissociation constant by

measuring the change in protein HDX as a function of denaturant concentration and ligand concentration. The technique has been demonstrated for complexes with K_Ds ranging from 0.16 nM to 520 nM, including TrpR with W and DNA, S-protein with S-peptide, B1 Domain with Fc Frag, and FIV Pr with TL3. Most MS K_Ds were within 2- to 3-fold of values measured using other solution techniques. This approach overcomes a significant limitation of MS methods: proper Scatchard determinations of K_D and ΔG tax the sensitivity of most MS systems for $K_D < 200$ nM. SUPREX offers a number of experimental advantages over non-MS methods, including the ability to work with purified or unpurified protein-ligand complexes, good sensitivity from MALDI ionization, and the ability to make measurements in a high-throughput format.

SUPREX complements existing MS high-throughput screening methods and should have direct application in biochemistry, biophysics, and drug discovery of protein targets. Combination of the method with tryptic digestion will provide information on ligand binding sites and changes in protein folding resulting from ligand binding. In addition, comparative studies of wild-type and mutant proteins should allow a detailed evaluation of the structural features essential to ligand binding affinity and specificity. Mass spectrometry can now provide protein identity and changes in protein levels using proteomics approaches. The current work adds another MS tool for high-throughput structural biology and drug discovery.

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