Analysis of the Energetics of Gas-Phase Immunophilin–Ligand Complexes by Ion Spray Mass Spectrometry

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Abstract: Receptor–ligand and other macromolecular host–guest interactions comprise a wide range of noncovalent forces, ranging from hydrogen bonding and electrostatic effects to ion–dipole and dipole–dipole interactions, charge-transfer complexation, π-stacking, and hydrophobic effects. Since many such complexes have recently been observed by ion spray mass spectrometry, it became of interest to ascertain whether meaningful information about solution binding constants might be obtained from ion current abundances for the gas-phase noncovalent ions. To evaluate the prospects for success in a favorable case, we chose to investigate the binding of rapamycin and four synthetic analogs to the cytoplasmic receptor FKBP. Here we extend our previous studies by applying tandem mass spectrometry (MS/MS) at different collision energies to investigate the relative energetics of noncovalent binding in immunophilin–ligand complexes in the gas phase.

The drugs FK506, rapamycin (RM), and cyclosporin constitute a family of powerful immunosuppressive agents with important applications in organ transplant surgery.[1-3] When liganded to their appropriate receptor proteins, these drugs block gene transcription normally turned on by stimulation of the T-cell receptor, and also inhibit T-cell proliferation.[4,5] Such antiproliferative effects are thought to result from disruptions in two distinct cytoplasmic signaling processes at different points in the cell cycle. FK506 and cyclosporin disrupt the same step in a family of Ca^{2+}-dependent signaling pathways, while RM interferes with a family of Ca^{2+}-independent signaling processes.

Even though FK506 and RM (Figure 1A) act on different intracellular pathways, their effects are transmitted by binding to a common protein, the cytoplasmic receptor FKBP, which is a member of the immunophilin family of immunosuppressive binding proteins.[5,6] Human FKBP is a small, hydrophilic protein (MW 11 812 Da) which has been cloned and overexpressed in *Escherichia coli*.[7] The immunophilin binds both FK506 (K_d = 0.4 nM) and rapamycin (K_d = 0.2 nM) with good affinity[8] and possesses peptidyl prolyl cis–trans isomerase activity which can be inhibited by these tight-binding ligands (FK506, K_i = 1.7 nM;[9] RM, K_i = 0.2 nM). Elegant synthetic studies by Schreiber *et al.* have identified separate binding domain and effector regions of the FKBP–FK506 complex[4] and established its role in calcium-dependent binding to the protein phosphatase calcineurin. The FKBP–RM complex blocks a family of calcium-independent signaling pathways triggered by ribosomal kinases.

Recently, as part of a program to develop methods for studying biologically important noncovalent complexes, we reported the use of ion spray mass spectrometry (MS) to obtain the first mass spectra of the immunophilin–ligand complexes FKBP–FK506 and FKBP–RM under physiological conditions.[10] In electrospray or ion spray MS, ions formed by protonation in solution are transferred into the gas phase at atmospheric pressure by desorption from highly charged droplets. Although the ion evaporation mechanism is not fully understood, specific immunophilin–ligand noncovalent complexes survive the desolvation process and display a distribution of multiply-charged noncovalent ions reflecting the distribution of multiply-charged ions in solution. We[11-17] and subsequently others[18-25] have since discovered that ion spray (and electrospray) MS can also be employed to detect

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(1) Showstack, J.; *et al. N. Engl. J. Med.* **1989**, *321*, 1086–1090.
(2) Starzl, T. E.; Demetris, A. J.; Thiel, D. V. *N. Engl. J. Med.* **1989**, *321*, 1092.
(3) Oates, J. O.; Wood, A. J. J.; Kahan, B. B. *N. Engl. J. Med.* **1989**, *321*, 1725–1726.
(4) (a) Schreiber, S. L. *Science* **1991**, *251*, 283–287. (b) Schreiber, S. L.; Albers, M. W.; Brown, E. J. *Acc. Chem. Res.* **1993**, *26*, 412–416.
(5) Sehgal, S. N.; Molnar-Kimber, K.; Ocain, T. D.; Weichman, B. M. *Med. Res. Rev.* **1994**, *14*, 1–22.
(6) Rosen, M. K.; Schreiber, S. L. *Angew Chem., Int. Ed. Engl.* **1992**, *31*, 384–400.
(7) Standaert, R. F.; Galat, A.; Verdine, G. L.; Schreiber, S. L. *Nature* **1990**, *346*, 671–674.
(8) Bierer, B. E.; Mattila, P. S.; Standaert, R. F.; Herzenberg, L. A.; Burakoff, S. J.; Crabtree, G.; Schreiber, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9231–9235.
(9) Harrison, R. K.; Stein, R. L. *Biochemistry* **1990**, *29*, 3813–3816.
(10) Ganem, B.; Li, Y. T.; Henion, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 6294–6296.
(11) Ganem, B.; Li, Y.-T.; Henion, J. D. *J. Am. Chem. Soc.* **1991**, *113*, 7818–7819.
(12) Ganem, B.; Li, Y.-T.; Henion, J. D. *Tetrahedron Lett.* **1993**, *34*, 1445–1448.
(13) Li, Y.-T.; Hsieh, Y.-L.; Henion, J. D.; Ganem, B. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 631–637.
(14) Li, Y.-T.; Hsieh, Y.-L.; Henion, J. D.; Senko, M. W.; McLafferty, F. W.; Ganem, B. *J. Am. Chem. Soc.*, **1993**, *115*, 8409–8413.
(15) Ganem, B.; Li, Y.-T.; Hsieh, Y.-L.; Henion, J. D.; Kaboord, B. F.; Frey, M. W.; Benkovic, S. J. *J. Am. Chem. Soc.* **1994**, *116*, 1352–1358.
(16) Hsieh, Y.-L.; Li, Y.-T.; Henion, J. D.; Ganem, B. *Biol. Mass Spectrom.*, in press.
(17) Reviews: (a) Ganem, B.; Henion, J. D. *Chemtracts* **1993**, *6*, 1–22. (b) Ganem, B. *Am. Biotechnol. Lab. News* **1993**, *11* (10), 32–35.
(18) Baca, M.; Kent, S. H. *J. Am. Chem. Soc.* **1992**, *114*, 3992–3993.
(19) Ganguly, A. K.; Pramanik, B. N.; Tsarbopoulos, A.; Covey, T. R.; Huang, E.; Fuhrman, S. A. *J. Am. Chem. Soc.* **1992**, *114*, 6559–6560.
(20) Goodlett, D. R.; Camp, D. G., II; Hardin, C. C.; Corregan, M.; Smith, R. D. *Biol. Mass Spectrom.* **1993**, *22*, 181–183.
(21) Huang, E. C.; Pramanik, B. N.; Tsarbopopoulos, A.; Reichert, P.; Ganguly, A. K.; Trotta, P. P.; Nagabhushan, T. L.; Covey, T. R. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 624–630.
(22) Jaquinod, M.; Leize, E.; Potier, N.; Albrecht, A.-M.; Shanzer, A.; Dorsselaer, A. V. *Tetrahedron Lett.* **1993**, *34*, 2771–2774.

0002-7863/94/1516-7487$04.50/0

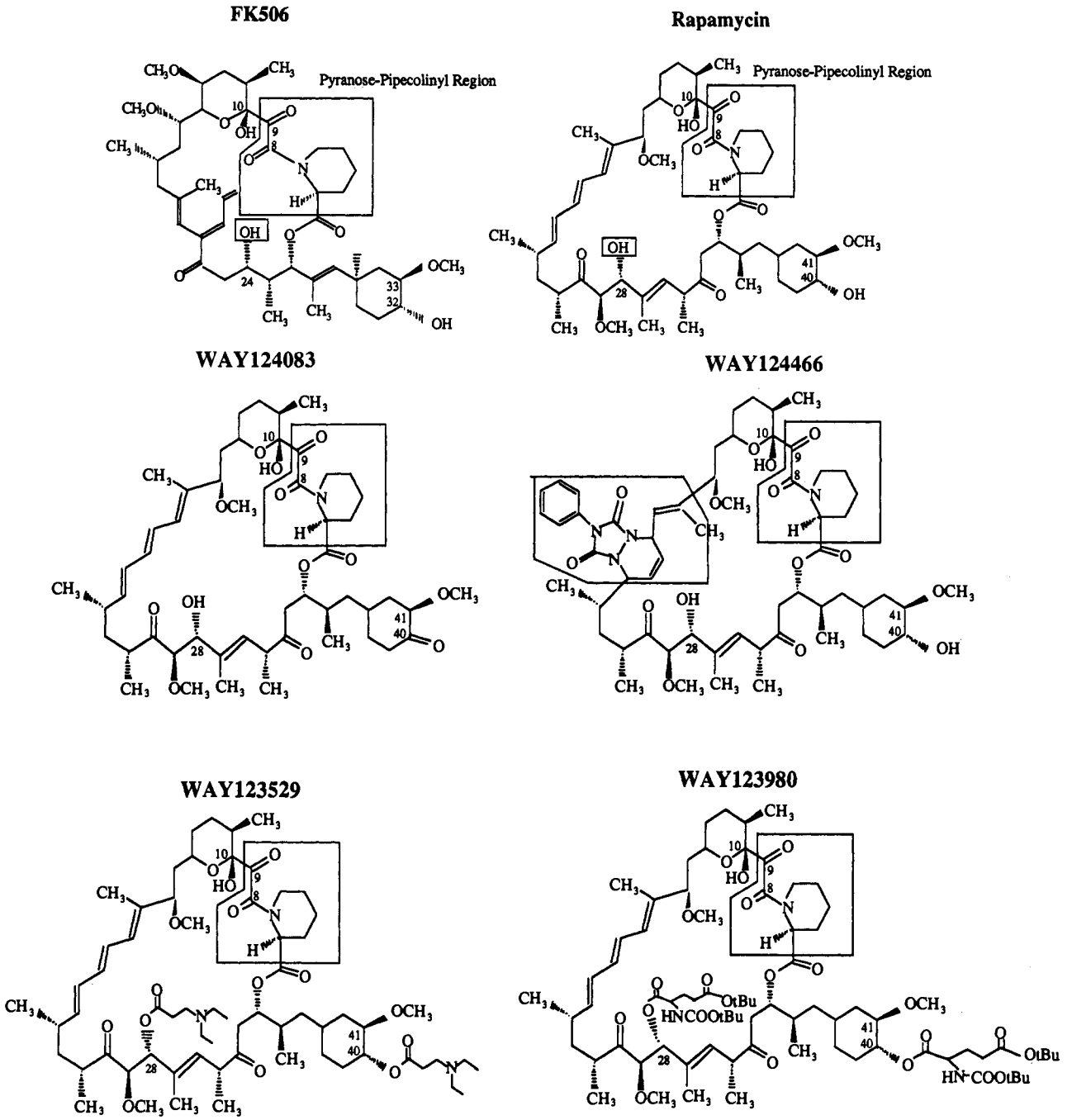

Figure 1. Structures of FK506, rapamycin, and four synthetic RM derivatives.

and analyze enzyme–substrate, receptor–ligand, antibody–antigen, protein–protein, protein–cofactor, and other noncovalent, host–guest complexes of biologically interesting molecules. In a preliminary report,[10] we showed that a mixture of FKBP with FK506 and RM gives rise to noncovalent ions for both receptor–ligand complexes, thus raising two intriguing issues for further study.

First, it was of interest to ascertain whether meaningful information about solution binding constants might be obtained from ion current abundances for the gas-phase noncovalent complex ions. This question is complicated by the fact that noncovalent binding of macromolecules may involve a wide range of weak interactions, ranging from hydrogen bonding and electrostatic effects to ion–dipole and dipole–dipole interactions, charge-transfer complexation, π-stacking, and hydrophobic effects. Moreover, each type of interaction is influenced differently by solvation and displays qualitatively different structural and distance dependencies.

Second, the fact that noncovalently bound complexes of each drug could be detected without chromatographic separation suggested that ion spray MS might also be useful in evaluating more complex mixtures, and even combinatorial libraries, of small molecules targeted for a specific protein receptor. Therefore, we have extended our study of ion spray MS to FKBP complexes with four synthetic analogs of RM (Figure 1B). Here we present a complete account of our progress to date and extend our previous studies by applying tandem mass spectrometry (MS/MS) at different collision energies to investigate the relative energetics of noncovalent binding in a series of immunophilin–ligand complexes in the gas phase.

(23) Light-Wahl, K. J.; Springer, D. L.; Winger, B. E.; Edmonds, C. G.; Camp, D.G., II; Thrall, B. D.; Smith, R. D. *J. Am. Chem. Soc.* **1993**, *115*, 803–804.

(24) Ogorzalek Loo, R. R.; Goodlett, D. R.; Smith, R. D.; Loo, J. A. *J. Am. Chem. Soc.* **1993**, *115*, 4391–4392.

(25) Smith, R. D.; Light-Wahl, K. J. *Biol. Mass Spectrom.* **1993**, *22*, 493–501.

(26) WAY124466: Ocain, T. D.; Longhi, D.; Steffan, R. J.; Caccese, R. G.; Sehgal, S. N. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 1340–1346.

Table 1. K_i Values for Inhibition of the Peptidyl Prolyl Isomerase Activity of FKBP by Rapamycin (RM) and Several Synthetic RM Derivatives

	MW (Da)	K_i values
rapamycin	913	0.33 nM[a]
WAY124083	911	1.1 nM
WAY124466	1089	12.5 nM
WAY123529	1168	15.2 nM
WAY123980	1485	14.4 μM

[a] lit. K_i = 0.2 nM (refs 8 and 9).

Experimental Section

Cloned and overexpressed human FKBP[4] was prepared and purified in our laboratory. Samples of FK506 (MW 804 Da) were obtained from Professor Jon Clardy, Cornell University. Samples of RM and RM analogs (WAY124083, WAY124466,[26] WAY123529, and WAY123980; Figure 1B) were obtained from Wyeth Ayerst Research (Princeton, NJ). For molecular weights and solution binding constants of these analogs, see Table 1.[26] A solution of FKBP (33.3 μM) was prepared by dissolving the protein in 10 mM ammonium acetate at pH 7.5. Solutions of FK506, RM, WAY124083, WAY124466, and WAY123980 (100 μM in CH_3OH) as well as WAY123529 (100 μM in 10 mM ammonium acetate, pH 7.5) were prepared. Samples of FKBP and FK506, RM, or RM analogs were mixed (25–40 μM) at ambient temperature for binding studies.

A Sciex TAGA 6000E atmospheric pressure ionization (API) triple quadrupole mass spectrometer (Thornhill, Ontario) upgraded to an API-III with a scan range from *m/z* 10 to 2400 was used for all experiments. Samples were continuously introduced in solution at 2 μL/min at ambient temperature by an infusion pump (Harvard Apparatus, South Natick, MA) for MS and tandem mass spectrometry (MS/MS) experiments. The ion spray probe tip was positioned approximately 1 cm off-axis and 1 cm away from the ion sampling orifice and maintained at 4.5 kV with a flow of liquid N_2 blow off nebulizing gas maintained at 45 psi. Polypropylene glycol [PPG 1000 (0.1 mM) and PPG 2000 (0.2 mM)] dissolved in 4:1 CH_3CN/H_2O (3 mM NH_4OAc) was used for tuning and mass axis calibration for each mass-resolving quadrupole (Q_1 and Q_3). Single-MS and MS/MS experiments were performed at declustering potentials of 30 V in the positive-ion mode of detection. Argon collision gas was introduced into the collision cell (Q_2) for the MS/MS experiments with a collision gas thickness of 200 × 10^{12} atoms/cm^2. Mass spectra were acquired at a dwell time of 3 s per scan with 10 scans summed at a step size of 0.5 Da.

Results

The ion spray mass spectrum of FKBP (Figure 2) at pH 7.5 displayed a truncated series of multiply-protonated, multiply-charged ions ranging from the $(M + 5H)^{5+}$ (*m/z* 2364.0) to the $(M + 8H)^{8+}$ (*m/z* 1477.5) charge states of FKBP. The observed molecular weight of FKBP (11 815 ± 1.4 Da) was in good agreement with the calculated value (11 812 Da). However, the mass spectrum of FKBP unexpectedly also showed another protein (MW = 11 945 ± 1.1 Da) having MW 130 Da higher than FKBP (see *m/z* 1707.5, 7^+ charge state). This protein, which also possessed immunophilin-like properties (*vide infra*), appears to contain one additional amino acid residue (possibly methionine, lysine, glutamine, or glutamic acid) in the polypeptide backbone.

Detection of Noncovalent Immunophilin–Drug Complexes. When FKBP was mixed with 1.6 equiv of FK506 at pH 7.5 (10 mM NH_4OAc buffer, receptor/ligand 1:1.6), a new abundant ion current signal appeared at *m/z* 1804.5, corresponding to the complex $(FKBP–FK506 + 7H)^{7+}$ (Figure 3A). A signal for the same complex in the 6^+ charge state was also evident at *m/z* 2104.5, as were weaker signals for complexes of the minor FKBP structural variant. No adduct was detected in a control experiment when FKBP was mixed with cyclosporin A, which binds to the immunophilin several orders of magnitude more weakly than FK506.[4a]

If the observed noncovalent ions represent specific drug–receptor interactions, then unfolding the protein's binding pocket should cause the complex ion signal at *m/z* 1803 to disappear. FKBP is known to undergo reversible denaturation at low pH.[27] In fact, when the 1:1.6 mixture of FKBP/FK506 was acidified to pH 3.3, the envelope of multiply-charged protein ions shifts to higher charge states, as might be expected in stronger acid. More importantly, signals for the receptor–ligand complex ion disappear (Figure 3B). However when the pH of the sample is restored to 7.5, a mass spectrum virtually identical to that of Figure 3A is obtained, indicating that FKBP spontaneously refolds and binds FK506 as before, thus providing further evidence of binding specificity. The FKBP–RM complex, with signals at *m/z* 1592, 1820, and 2123 corresponding to the 8^+, 7^+, and 6^+ charge states (Figure 4), also undergoes reversible denaturation with disappearance and reappearance of the receptor–ligand noncovalent complex.

To measure binding affinities with FKBP, K_i values were obtained for several synthetic Wyeth Ayerst RM analogs (Figure 1B) along with an independent K_i determination for RM (Table 1). With these data in hand, formation of gas-phase noncovalent FKBP complex ions was also examined by ion spray MS. The mass spectrum of FKBP (25 μM, pH 7.5) with WAY124083 (40 μM; Figure 5) displayed ion current signals for the noncovalent FKBP–WAY124083 complex in the 6^+ (*m/z* 2122.0), 7^+ (*m/z*

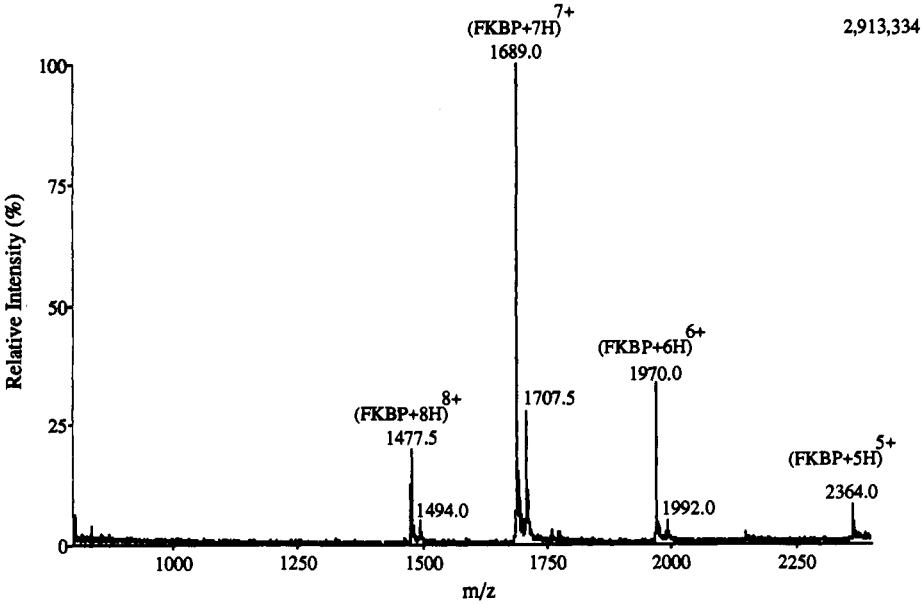

Figure 2. Ion spray mass spectrum of human FKBP in 10 mM NH_4OAc, pH 7.5.

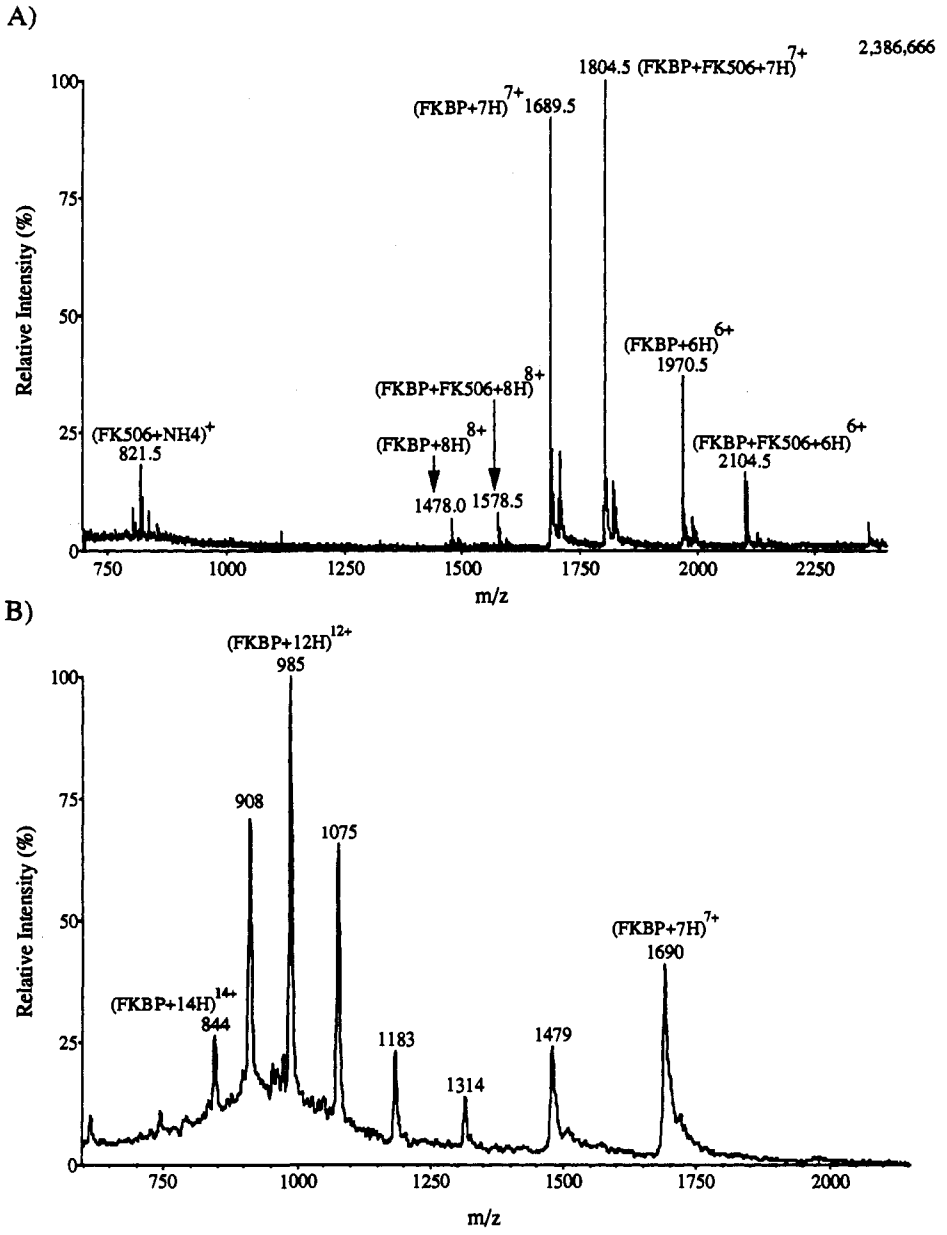

Figure 3. Ion spray mass spectrum of a 1.6:1 mixture of FK506 and FKBP in 10 mM NH_4OAc: (A) at pH 7.5; (B) at pH 3.3.

1820.0), and 8^+ (m/z 1592.0) charge states. Similarly for WAY124466 (40 μM; Figure 6), ions were detected at m/z 2152, 1845, and 1614 corresponding to the 1:1 receptor–ligand complex in the 6^+, 7^+, and 8^+ charge states, respectively. Although the binding affinity of FKBP for WAY123529 was comparable to that of the two analogs just discussed, a noncovalent ion at m/z 1855.5 for the FKBP–WAY123589 complex in the 7^+ charge state was only observed at low (10 V) declustering potential (data not presented). With WAY123980, having a thousandfold weaker affinity for FKBP, little or no complexation was detected under identical experimental conditions.

Competition of Immunophilin Ligands for FKBP. A competition experiment between FKBP (25 μM) and equimolar amounts of the natural products FK506 and RM (40 μM each) clearly showed both receptor–ligand complexes at m/z 1804.5 and 1820.5 (Figure 7). Inspection of relative ion currents in each signal revealed that the RM complex was approximately 9 times as abundant as the FK506 complex,[28] in good agreement with relative solution K_i values of 0.2 and 1.7 nM, respectively.[8,9]

An additional experiment was carried out in which the three most potent Wyeth Ayerst RM analogs (WAY124083, -124466, and -123529; Figure 8) competed for noncovalent binding to FKBP. Complex ions at m/z 1820, 1845, and 1856 were detected for each of the respective synthetic analogs. Surprisingly, the most abundant complex ion corresponded to the WAY123529 complex, which had previously been observed only at low declustering potential.

Estimation of Immunophilin–Ligand Relative Gas-Phase Binding Affinities by MS/MS. To probe the relative magnitude of noncovalent interactions in gas-phase complex ions of FKBP with RM analogs, a novel application of tandem mass spectometry (MS/MS) was developed. The collision-induced dissociation of a mixture of the FKBP–RM, FKBP–WAY124083, and FKBP–WAY124466 complexes was monitored as a function of increasing collision energy under conditions of constant declustering potential and collision gas thickness (Figure 9). Breakdown of the FKBP–RM complex into free FKBP and RM was plotted by measuring the abundance of the $[FKBP–RM + 7H]^{7+}$ ion current in proportion to the corresponding signal for $[FKBP + 7H]^{7+}$. Similar data recorded for the FKBP–WAY124083 and FKBP–WAY124466 complexes were also plotted in Figure 9. Inde-

(27) Personal communication from Professor S. L. Schreiber.

(28) The 2:1 relative abundance observed for the RM and FK506 complexes of FKBP as reported in our original communication was incorrect. The error resulted from unintentional use of a tenfold more concentrated solution of FK506 in the competitive binding experiment. Also, the lower declustering energies in the present work give more reproducible results.

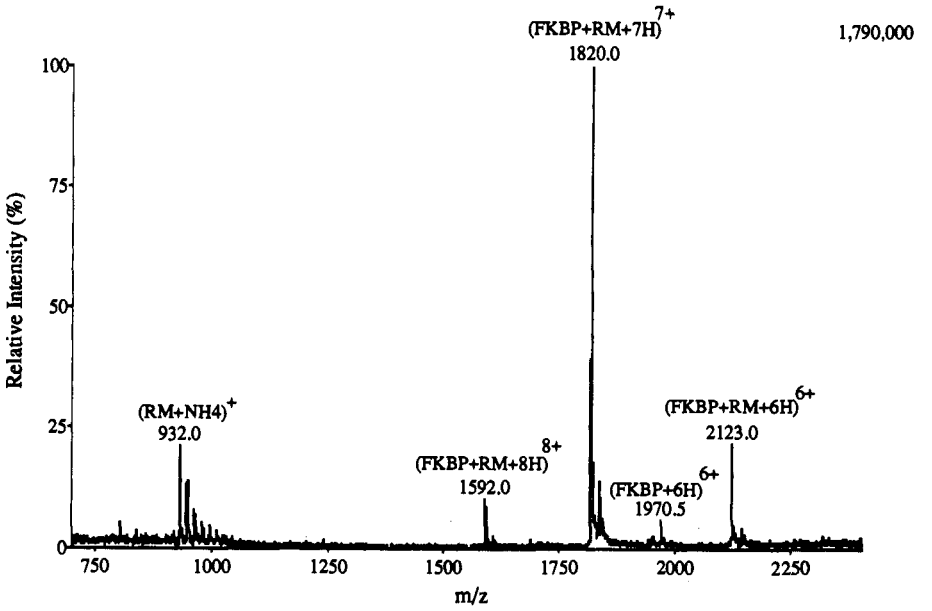

Figure 4. Ion spray mass spectrum of a 1.6:1 mixture of RM and FKBP in 10 mM NH_4OAc, pH 7.5.

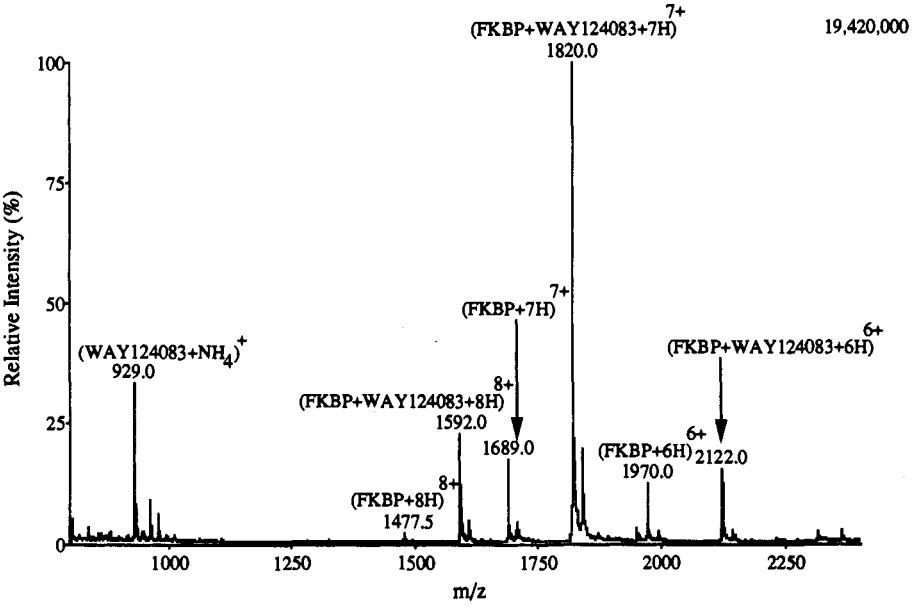

Figure 5. Ion spray mass spectrum of WAY124083 (40 μM) with FKBP in 10 mM NH_4OAc, pH 7.5.

pendent measurements on individual complexes gave the same results, indicating that, as expected, the gas-phase noncovalent ions in the mixture behaved independently of one another.

Although the relative abundance of each noncovalent complex at the lowest collision energy (200 eV) showed no correlation with K_i values for the ligands (Table 1), the ease of dissociation of each complex during MS/MS, judging from the apparent slope of each plot, revealed the expected trend in which the most tightly bound ligand was least readily dissociated. When plotted in the same fashion, data for the FKBP–FK506 complex (not shown) closely resembled that of the RM complex, even though their solution dissociation constants differ ninefold.

Discussion

Receptor–ligand and other macromolecular host–guest interactions comprise a wide range of noncovalent forces, ranging from hydrogen bonding and electrostatic effects to ion–dipole and dipole–dipole interactions, charge-transfer complexation, π-stacking, and hydrophobic effects. Besides involving different distance dependencies, these forces may further differ considerably in magnitude from case to case, depending on geometry and other structural parameters, and may also be strongly influenced by the presence or absence of solvent. Indeed, small changes in solvent composition are well-known to exert dramatic effects on total ion current in the electrospray mass spectrum.[29] Given the poorly understood physics of electrospray, the size and escaping tendency of multiply-charged ions may also be significant factors in the experiment.

We[14] and others[25] have called attention to the paucity of information about which types of noncovalent interactions may be detected by ion spray MS and about the relevance of gas-phase binding energetics to solution behavior. To date, very little experimental data addressing these questions have been reported for the noncovalent association of biological macromolecules. For example, the relative intensities of gas-phase ions corresponding to duplex forms of complimentary oligonucleotides, while proportional to the number of hydrogen bonds between base pairs, are unexpectedly low in overall abundance.[12] This finding may reflect (i) repulsive electrostatic terms between each multiply negatively charged oligonucleotide and/or (ii) the importance of hydrophobic effects, which would be significantly diminished in the gas phase. In the case of leucine zipper peptide

(29) Chowdhury, S. K.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1660–1664.

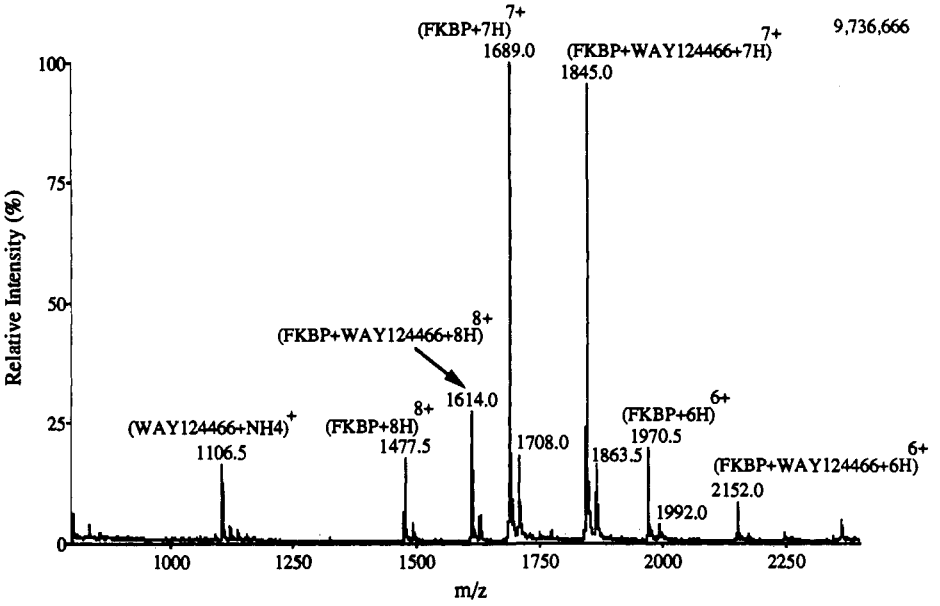

Figure 6. Ion spray mass spectrum of WAY124466 (40 μM) with FKBP in 10 mM NH_4OAc, pH 7.5.

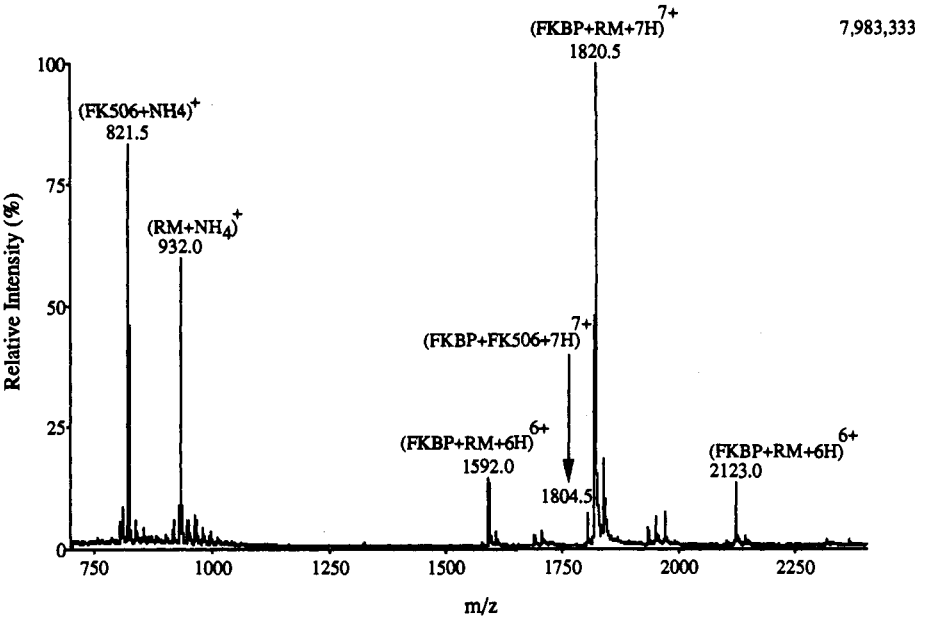

Figure 7. Ion spray mass spectrum of a mixture of FK506 and RM (40 μM each) with FKBP in 10 mM NH_4OAc, pH 7.5.

dimers, where the coiled-coil dimer structure is largely stabilized by hydrophobic interactions, gas-phase dimer ions can only be detected as weak ion current signals.[14] Furthermore, naturally occurring noncovalent dimers and trimers of gp45 (an accessory protein in the T4 DNA replication complex which displays many of the characteristics of a hydrophobic protein) are likewise detected only in very low abundance by ion spray MS, despite being readily observable by size-exclusion chromatography.[15]

Macromolecular noncovalent complexes may involve a variety of different weak interactions with different distance dependencies (e.g. charge–charge, varying as $1/r$; charge–dipole, varying as $1/r^2$; dipole–dipole, varying as $1/r^3$). Therefore, predicting solution binding constants from the relative abundance of the gas-phase noncovalent ions may be difficult. To evaluate the prospects for success in a favorable case, we chose to investigate a closely related series of receptor–ligand complexes in which noncovalent forces in solution are reasonably well-characterized. Crystallographic[30] and NMR[31] studies have shown that the pipecolinyl, pyranose, and cyclohexyl moieties of FK506 bind tightly in the hydrophobic pocket of FKBP consisting of Tyr-26, Phe-36, and Phe-99 through weak, van der Waals interactions. Additionally, the complex is stabilized by five hydrogen bonds, e.g. (i) Ile-56/C1-lactone, (ii) Glu-54/C24-OH, (iii) Gln-53/C24-OH (H_2O), (iv) Asp-37/C10-hemiketal, and (v) Tyr-82/C8-amide. Structurally and conformationally similar loci of noncovalent interactions in FK506 and RM are highlighted in Figure 1A. The FKBP-RM complex displays two additional hydrogen bonds (Glu-54/C28-OH and Gln-53/C40-OH) which cannot form in the FK506 complex because of conformational restraints on the cyclohexyl ring. The corresponding pyranose–pipecolinyl groups and other important regions of noncovalent interactions in the four synthetic RM analogs are highlighted in Figure 1B.

Remarkably good agreement with solution K_i values was noted in the competitive binding study of FKBP with FK506 and RM. Noncovalent binding of FKBP with the three most potent Wyeth Ayerst RM analogs (WAY124083, -124466, and -123529; Figure 8) also generated signals for the individual noncovalent complexes, although their relative abundances did not accurately reflect the

(30) Van Duyne, G. D.; Standaert, R. E.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *252*, 839–842.

(31) (a) Moore, J. M.; Peattie, D. A.; Fitzgibbon, M. J.; Thomson, J. A. *Nature* **1991**, *351*, 248–250. (b) Michnick, S. W.; Rosen, M. K.; Wandless, T. J.; Karplus, M.; Schreiber, S. L. *Nature* **1991**, *252*, 836–839.

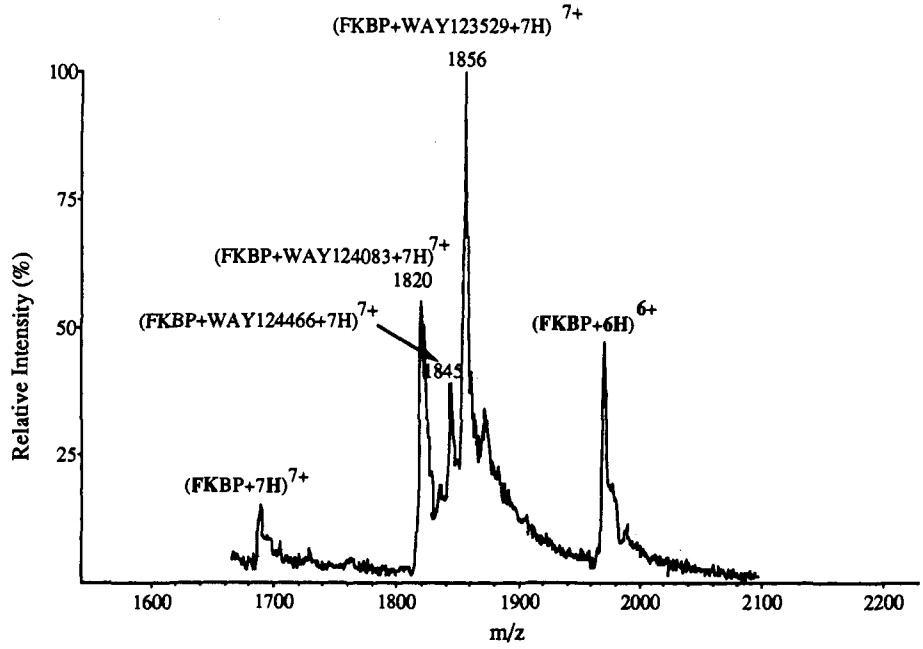

Figure 8. Ion spray mass spectrum of a mixture of WAY124083, WAY124466, and WAY123529 (40 μM each) with FKBP in 10 mM NH_4OAc, pH 7.5.

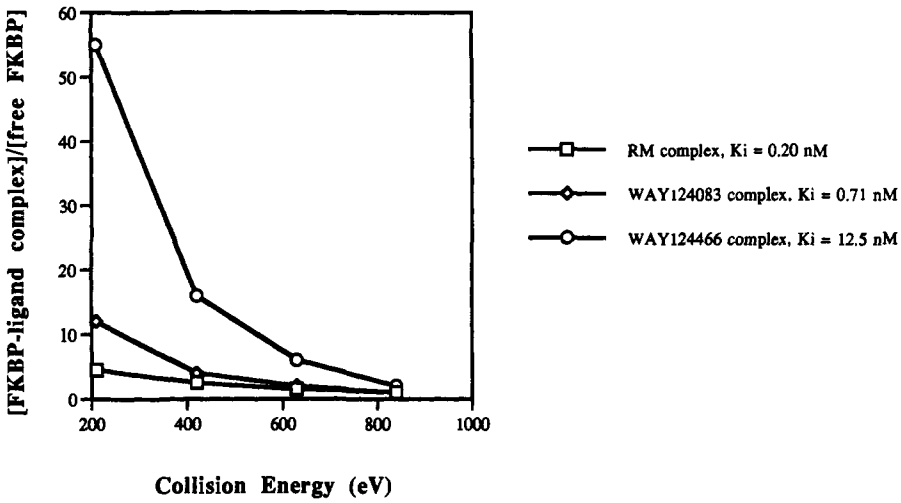

Figure 9. Plots of the extent of dissociation of three gas-phase FKBP–ligand complexes as a function of collision energy in a series of ion spray MS/MS experiments.

15-fold differences in solution K_i values. The modest agreement between gas-phase and solution data may be due to MW differences in the individual ligands (Table 1) or to the electrospray process itself or to collision-induced dissociation originating at the orifice region of the spectrometer. Nevertheless, judging from the apparent slope of each plot in Figure 9, the dissociation of each gas-phase complex during MS/MS (which mainly involves hydrogen bond dissociation) does appear to reflect the same general affinities evident in solution.

In conclusion, it is now apparent from the work of several groups that ion spray MS can be used to detect and characterize a wide variety of biologically important association complexes, although the extent to which the technique is useful for studying noncovalent interactions depends critically on the nature of the noncovalent interactions in the complex. Several independent experimental systems have established that in cases where hydrophobic interactions play a prominent role in complex formation (leucine zippers, DNA duplexes, gp45 oligomerization), the abundance of gas-phase complex ions does not accurately reflect solution binding affinities. In the current study of immunophilin–ligand complexes, where hydrogen bonding has been shown to play a predominant role in noncovalent complexation, the energetics of gas-phase binding more closely reflect aqueous solution behavior. Although obtaining accurate binding data on noncovalent FKBP–ligand complexes by ion spray MS proved more difficult than expected, the method may still find application in assaying host–guest complexation and in screening modest combinatorial libraries for significant noncovalent interactions.

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