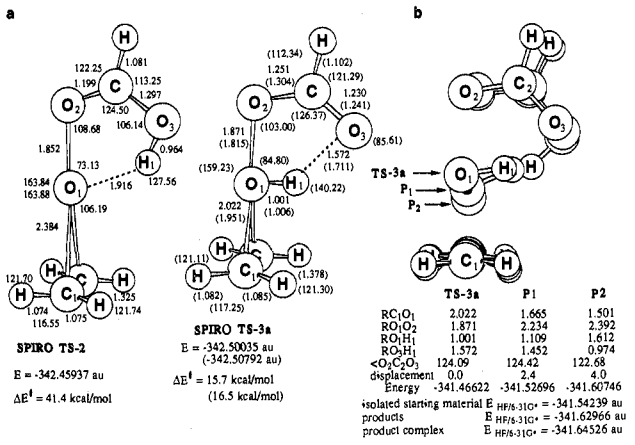

Figure 1. (a) Comparison between the transition states of peroxyformic acid zwitterion + ethylene (TS-**2**) and peroxyformic acid + ethylene (TS-**3a**) (optimized structures at the HF/6-31G* level, MP2/6-31G* geometry in parentheses; MP4SDTQ/6-31G*//HF/6-31G* energies in au, MP4SDTQ/6-31G*//MP2/6-31G* values in parentheses). (b) TS-**3a** and two intermediate points (P_1 and P_2) along the path toward products, ethylene oxide and formic acid (IRC at HF/6-31G*, displacement in amu$^{1/2}$·bohr).

kcal/mol more stable than a planar TS-**3b** and 8.4 kcal/mol lower in energy than an unsymmetrical structure[6] (both are second-order saddle points, MP4SDTQ/6-31G*//HF/6-31G*). As long as H_1 is strongly bound to its "electrophilic" oxygen, then the two lone pairs of electrons on that oxygen will differ in energy, potentially displaying a preference for orientation of attack on the alkene, which obviously arises from an electronic effect since steric interactions involved in this system are minimal. However, the oxenoid oxygen in TS-**2** has essentially spherical electron density, and no such stereoelectronic effects should be anticipated.

Figure 1b provides several "snapshots" along the intrinsic reaction path[2d] from TS-**3a** toward products. Rehybridization at ethylene is minimal at the TS while the O_2–C_2 bond shortens toward a carbonyl bond distance, the C_2–O_3 lengthens as the hydrogen is transferred, and the C_1–O_1 bonds of the epoxide develop. The relatively low activation barrier when compared to H_2O_2 is a reflection of the more idealized molecular architecture of the peracid functional group that has a developing carboxylate anion ideally poised to accept the migrating hydrogen *after the barrier is crossed* and to stabilize by resonance the charge on the leaving formate fragment as depicted in Figure 1b by the alternating C–O bond distances. This transition structure provides a theoretical corroboration of the generally accepted "butterfly" mechanism first disclosed by Bartlett[9] at this institution in 1950 and is consistent with a relatively low deuterium isotope effect, $k_H/k_D = 1.17$ (RO_1–H_1 = 1.001 Å).[10] This TS is also consistent with our earlier suggestion of an S_N2 attack by the alkene π-bond on the σ and σ^* orbitals of the O–O bond[11a,b] in consonance with the four-electron, three-MO frontier MO model.[11c] We attribute the electrophilicity of a peracid to its relatively weak O–O bond that can provide an empty (electrophilic) σ^* orbital early along the reaction coordinate that can mix with the nucleophilic π-bond.

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FK506 and Rapamycin Binding to FKBP: Common Elements in Immunophilin–Ligand Complexation

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Complexes of immunophilins and their ligands have been shown to inhibit signal transduction pathways that result in exocytosis and transcription.[1] For example, recent studies demonstrate that FK506[2,3] and rapamycin[4] bind to the same immunophilin, FKBP, and suggest that distinct signaling pathways are inhibited by complexes formed between an immunophilin (possibly FKBP) and either FK506 or rapamycin.[5,6]

Investigations of human recombinant FKBP,[7,8] isotopically labeled FK506,[9,10] and the nonnatural FKBP ligand 506BD led to the proposal that FK506 and rapamycin bind FKBP with similar structural elements, which include the pipecolinyl ring, and in a similar orientation.[6] The common immunophilin binding element of these agents, which is responsible for rotamase inhibition, is fused to distinct effector elements that apparently determine which signaling pathway will be inhibited. We have prepared several selectively deuterated variants of FKBP and examined the resultant FKBP/FK506 complexes by 1D and 2D NMR spectroscopy. The results show that the common pipecolinyl moiety of FK506 and rapamycin is involved in binding to FKBP and is likely to be oriented in a similar manner in the two ligand–receptor complexes.

Comparison of the ^{1}H NMR spectra of free FKBP, the FKBP/FK506 complex, and the FKBP/rapamycin complex reveals several strongly upfield shifted resonances that are similar in the spectra of the two complexes (Figure 1). To minimize signal overlap between drug and protein, several selectively deuterated FKBPs were overexpressed in *Escherichia coli*.[7] The variant discussed below contained only the four protonated amino acid types: proline, tryptophan, tyrosine, and valine.[11,12] 2D DQF-COSY spectra of FKBP/FK506 were used to identify a single spin system consisting of three methylene groups (δ 0.22 to −1.91) within these upfield resonances (Figure 2A). One of these resonances shows J-coupling as well as strong NOESY cross peaks to a downfield methylene group (δ 2.47, 3.17). A methylene group at the other end of the spin system shows strong NOEs from both hydrogens to a resonance at δ 4.52. There is no coupling in the DQF-COSY spectra between these resonances. Thus, six resonances in the upfield region belong to a spin system consisting

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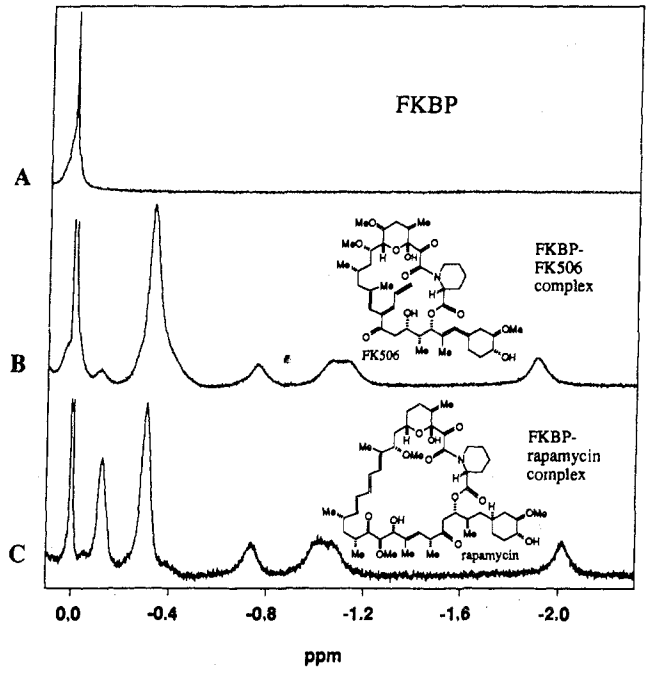

Figure 1. Comparison of the 1D 500-MHz 1H NMR spectra of (A) native FKBP, (B) the native FKBP/FK506 complex, and (C) the native FKBP/rapamycin complex. Spectra are referenced to 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt. All resonances in this region (due to protein and drug) are shown.

of X-$CH_2CH_2CH_2CH_2$CH-Y,Z. This pattern is uniquely satisifed by the pipecolinyl ring of the drug (all lysine residues of FKBP are deuterated).[13] Investigation of the fully protonated FKBP/rapamycin complex reveals an identical upfield spin system (Figure 1C) with the same patterns of cross peaks in the 2D NMR spectra (spectra not shown). The large high-field shifts of the pipecolinyl protons suggest shielding by at least two aromatic residues (the range of chemical shifts for the β, γ, and δ methylenes of *N*-BOC-protected pipecolinic acid is δ 1.80–1.25 in chloroform[9]). As aromatic shielding effects are highly sensitive to the relative positions and orientations of the interacting groups,[14,15] the similar chemical shifts of individual hydrogens of this fragment provide strong evidence that the orientation of the pipecolinyl groups is similar in the two complexes.

A NOESY spectrum of the FKBP/FK506 complex shows NOEs between several protons of the pipecolinyl ring of FK506 and the unique Trp 59 (W59) of FKBP (Figure 2B). (The Trp 59 ring protons are identified in the DQF-COSY spectra of the complex of FK506 and selectively deuterated FKBP by the distinctive cross-peak pattern (Figure 2C).) The Trp 59 ring protons H(5), H(6), and H(7) (indole numbering, see Figure 3) interact with the face of the pipecolinyl ring that is opposite the acyloxy group (the axial orientation of which[10] is confirmed by analyses of scalar couplings and NOE connectivities); the strongest NOEs are to the β- and δ-axial and the γ-equatorial protons of the ring. At least two other aromatic spin systems also show NOEs to the pipecolinyl ring of FK506 (Figure 2B). Figure 3 provides a schematic drawing of the aromatic-rich binding region of free FKBP, based on the structure determined by NOE-restrained molecular dynamics simulations.[16] Proximity of the pipecolinyl group to Trp 59 requires it to be near some of these aromatics;

(13) Preliminary isotope-edited NMR experiments with uniformly ^{13}C labeled FKBP and unlabeled FK506 confirm this assignment (spectra not shown).

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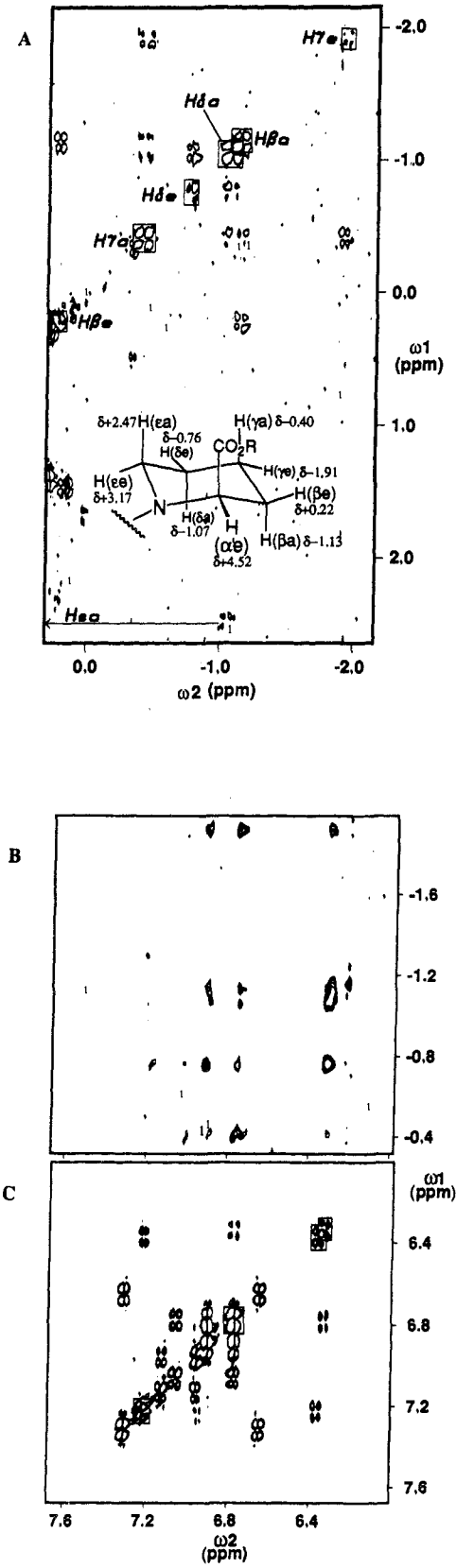

Figure 2. (A) Upfield region of DQF-COSY spectrum of selectively deuterated FKBP/FK506 complex. Diagonal peaks of six pipecolinyl ring protons are boxed and labeled. The protein sample was prepared as in ref 12a. (B) Aromatic/upfield region of NOESY spectrum (τ = 100 ms) of selectively deuterated FKBP/FK506 complex (same sample as in Figure 2A). Cross peaks correlate NOEs between aromatic protons of FKBP and the pipecolinyl ring of FK506. (C) Aromatic region of DQF-COSY spectrum of selectively deuterated FKBP/FK506 complex. The protein sample was prepared as in ref 12b. Tyr and Trp protons are present; Phe residues are deuterated. The Trp diagonal peaks are boxed.

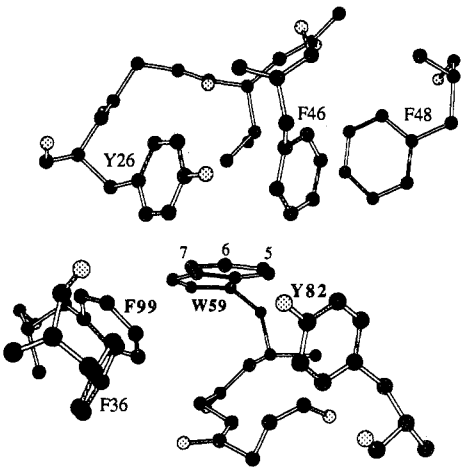

Figure 3. External view of the drug binding site of the unbound protein. The pipecolinyl ring of FK506 is in close proximity to W59 [H(5), H(6), and H(7)] and two additional aromatic residues, possibly F99 and Y82.

preliminary analyses suggest that the additional pipecolinyl–aromatic NOEs may be due to Tyr 82 and Phe 99. The FK506- and rapamycin-binding immunophilins of M_r 13000 and 30000[4] have sequences related to that of FKBP and, like FKBP sequences from other organisms, have high conservation of the aromatic residues that comprise the binding site, including Trp 59, Tyr 82, and Phe 99 (Figure 3).[17] Thus, we expect the structural insights concerning the ligand–receptor complexes reported herein to be relevant to other members of this family of immunophilins.

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Computer Software Reviews

Regression. Windows Version. Blackwell Scientific Software, BSP Inc.: 3 Cambridge Center, Cambridge, MA 02142. List price (single installation) $225.00, additional installations $85.00 each, department license (extra) $225.00, academic discount 20%.

Regression is a math-utility program that fits a function to data. Regression can be run on IBM compatibles (with Microsoft Windows version 2.0 or above, at least 512 Kbytes of memory and a mouse) or any Apple Macintosh (with at least 128K of ROM). The use of a hard disk greatly facilitates the use of this software.

The data consist of up to 100 pairs (x,y) and can be input by hand within the windows environment or imported as an ASCII file. The model function used to fit the data is constructed in the Windows environment just as one would write it as a line of computer code. The function ($y = f(x)$) can be of any form (linear or nonlinear) and can include up to 10 parameters to be determined by the program and 10 predefined constants. The model functions can be saved and retrieved. A number of model functions, such as exponential decay, Gaussian distribution, and reaction kinetic equations, are provided. It is very easy to edit both the function and the data in the Windows environment.

The parameters in the model function are determined by minimizing the sum of the squares of the residual errors between the model ($f(x)$) and the data (y). The minimization is accomplished by the Marquardt or simplex algorithm (user's choice). The iterations continue until a specified number of iterations is reached or the errors in the parameters fall below a specified maximum value. The documentation on the algorithms is minimal, but references are given for both algorithms and for the actual programming code.

The program outputs a plot of both the original data with the fitted function and the residuals. The program used in this laboratory gave some problems with the graphics output (the axes and the fitted function were missing). The program also outputs the sum of squares of the residuals and the standard deviation $(SS/df)^{1/2}$ to aid in the interpretation of the goodness-of-fit of the model function.

Regression achieves what it claims to do and is easy to learn to use. The ease of creating and fitting any type of function, especially nonlinear functions, makes Regression a valuable program. However, there are serious limitations to its use that are not readily apparent. The first and most important shortcoming is the limit on the number of data points allowed. While 100 data points may be sufficient in some types of work, much greater amounts of data are generated in most modern computer-oriented laboratories and a maximum input of 100 data points is a serious limitation. Furthermore, the user is only allowed a single independent variable which rules out the use of Regression in any multivariate experiment. These two limitations make Regression unsuitable for many applications.

One further limitation in the software and documentation is that the concept of goodness-of-fit and the significance of a model is given a very cursory treatment. The user needs to have some background in statistics in order to properly use this software. It is all too easy to produce untrustworthy results with any fitting algorithm and it is important for a program to include an indication of the uncertainty in the parameters. Regression does so in a very limited fashion.

Regression was tested against some software written in this laboratory for doing curve fitting. A spectrum was generated as the sum of three Lorentzian peaks (with no noise), and Regression and the in-house program were used to recover the parameters of the peaks. Both programs used the Marquardt algorithm, but the in-house program was written specifically for Lorentzian curve-fitting, i.e. it included the partial derivatives determined symbolically and not numerically as in Regression. Both programs were successful at fitting quite simple problems (resolved peaks) and both programs failed at fitting extremely ill-conditioned problems (heavily overlapped peaks). There were some cases where the in-house program was able to fit spectra that Regression could not (even when Regression was given a starting guess very close to the true answer). The conclusion is that a program written specifically for the work at hand can perform better than Regression.

One more point that should be noted is that the actual algorithms in Regression are quite simple. Most of the program is probably devoted to the input, editing, and interpretation of the model function. The price of Regression is quite high for the simplicity of the algorithmic work done on the data.

The researcher thinking of purchasing a function fitting program should consider several questions very carefully: how many data points will normally be used; will any multivariate data be used; how ill-conditioned is the problem; and can the necessary programming be done in-house? If the work being envisioned is within the capabilities of Regression then it is a useful program. If not, then one is better served by writing the software in-house specifically suited to the problem.

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Theorist. Version 1.01. Prescience, Inc.: 814 Castro St., San Francisco, CA 94114. (415) 282-5864.

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