Conformational Changes of Rapamycin and Analogs upon Complexing with FKBP Associated with Activity: An Application of Second Derivative CD Spectroscopy

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · MARCH 1994

Impact Factor: 12.11 · DOI: 10.1021/ja00085a084

Conformational Changes of Rapamycin and Analogs upon Complexing with FKBP Associated with Activity: An Application of Second Derivative CD Spectroscopy

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Received December 27, 1993

Although rapamycin 1[1] and FK-506[2] inhibit T and B cell proliferation through different pathways,[3] their immunosuppressive actions are thought to involve binding to FKBP.[4] The structure of the rapamycin–FKBP complex has been determined by X-ray[5a] and NMR.[5b] We report that CD studies show rapamycins **1–6** (Chart 1)[6] to interact differently with FKBP and that conformational changes of **1–4** upon binding to FKBP, better illustrated in second-derivative CD, can be associated with drug activity.

CD spectra were measured by a JASCO 720 instrument at ambient temperature. The protein[7] and drugs[8] were purified by HPLC, the purity of FKBP being confirmed by SDS-PAGE. The CD of FKBP (Figure 1) agrees with the published spectrum[9,10] which is consistent with the protein structure determined by X-ray[11] and NMR.[12] All six drugs (**1–6**) display positive and negative CEs at *ca.* 210 and *ca.* 300 nm, respectively (Figure 2),

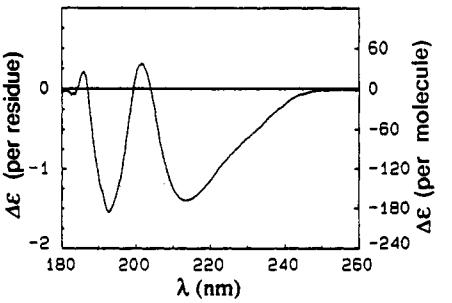


Figure 1. CD curve of FKBP (21.2 μM, 0.1-cm cell) in 20 mM Na phosphate buffer, pH 7.2, containing 0.002% reduced Triton X-100. The concentration of FKBP was calculated from the ε value of 9860 (278 nm).[7b]

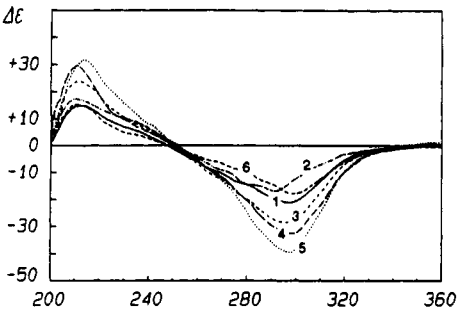
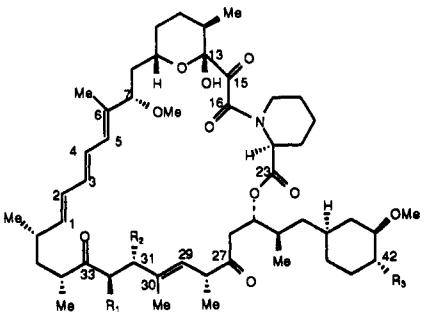


Figure 2. CD spectra of rapamycin **1** and analogs **2–6** (1-cm cell) in ethanol. The concentration of rapamycin **1** was calculated from the ε value of 50 200 (277 nm, measured in ethanol) and 48 500 (276 nm) for demethoxyrapamycin **2**;[8b] those for analogs **3–6** were assumed to be the same as for rapamycin **1**.

Chart 1



COMPD	R1	R2	R3	LAF[15b] (IC_{50}, nM)	PPIase[15a] (K_i, nM)
1	-OMe	-OH	-OH	6.4	0.14
2	-H	-OH	-OH	58.0	4.4
3	-OMe	-OC(O)H	-OH	4.3	19
4	-OMe	-OAc	-OAc	198.0	26
5	-OMe	-OTBS	-OTBS	>1000	>1000
6	-OMe	BOC-Lys(BOC)	BOC-Lys(BOC)	>1000	>1000

suggesting similar conformations.[13] The positive 210-nm band is an overlap of the 23-lactone n–π* transition with the β,γ-enone CT band, while the negative 300-nm band is due to n–π* transitions of the 15-, 27-, and 33-ones. The CD of the triene at *ca.* 270 nm is weak, with some vibrational fine structures, and overlaps with the 300-nm carbonyl bands.

The CD show that the analogs interact with FKBP to varying degrees.[14] For **1–3** (Figures 3a-c), the CD of coincubated solutions of drugs and protein differ from the summation spectra of the

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(1) (a) Sehgal, S. N.; Baker, H.; Vezina, C. *J. Antibiotics* **1975**, *28*, 727. (b) Swindells, D. C. N.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491. (c) Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579.

(2) (a) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031. (b) Karuso, P.; Kessler, H.; Mierke, D. F. *J. Am. Chem. Soc.* **1990**, *112*, 9434.

(3) (a) Dumont, F. J.; Melino, M. R.; Staruch, M. J.; Koprak, S. L.; Fischer, P. A.; Sigal, N. H. *J. Immunol.* **1990**, *144*, 1418. (b) 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. (c) Liu, J.; Farmer, J. D., Jr.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807. (d) Calvo, V.; Crews, C. M.; Vik, T. A.; Bierer, B. E. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7571. (e) Chung, J.; Kuo, C. J.; Crabtree, G. R.; Blenis, J. *Cell* **1992**, *69*, 1227. (f) Price, D. J.; Grove, J. R.; Calvo, V.; Avruch, J.; Bierer, B. E. *Science* **1992**, *257*, 973.

(4) (a) Schreiber, S. L. *Science* **1991**, *251*, 283. (b) Sehgal, S. N.; Molnar-Kimber, K.; Ocain, T. D.; Weichman, B. M. *Med. Res. Rev.* **1994**, *14*, 1. (c) Schreiber, S. L.; Crabtree, G. R. *Immunology Today* **1992**, *13*, 136. (d) Rosen, M. K.; Schreiber, S. L. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 384.

(5) (a) Van Duyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433. (b) Wandless, T. J.; Michnick, S. W.; Rosen, M. K.; Karplus, M.; Schreiber, S. L. *J. Am. Chem. Soc.* **1991**, *113*, 2339.

(6) These six compounds are representative of a number of rapamycin-related compounds studied.

(7) (a) All experiments were carried out using recombinant human FKBP12 with an extended N-terminal sequence GSPGISGGGGGINST-FKBP. (b) The FKBP concentration was calculated from the known ε: *cf.* Standaert, R. F.; Galat, A.; Verdine, G. L.; Schreiber, S. L. *Nature* **1990**, *346*, 671.

(8) (a) Synthesis of rapamycin analogs **3–6**: Rakhit, S. U. S. Patent 4 316 885, 1982. Failli, A.; Steffan, R. J. U. S. Patent 5 120 842, 1992. Failli, A.; Caufield, C.; Steffan, R. J. U. S. Patent 5 130 307, 1992. (b) Findlay, J. A.; Liu, J. S.; Burnell, D. J.; Nakashima, T. T. *Can. J. Chem.* **1982**, *60*, 2046.

(9) Marquis-Omer, D.; Sanyal, G.; Volkin, D. B.; Marcy, A. I.; Chan, H. K.; Ryan, J. A.; Middaugh, C. R. *Biochem. Biophy. Res. Commun.* **1991**, *179*, 741.

(10) It was not possible to quantitatively estimate the protein secondary structure employing the commercial JSSE program (JASCO 720). The same difficulties were encountered by earlier workers using Manavalen and Johnson's program.[9]

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

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

(13) Amplitudes of published CD spectra of rapamycin and demethoxyrapamycin (in methanol) are about twice those of curves 1 and 2 (in ethanol, Figure 2),[8b] which were measured twice because of this discrepancy.

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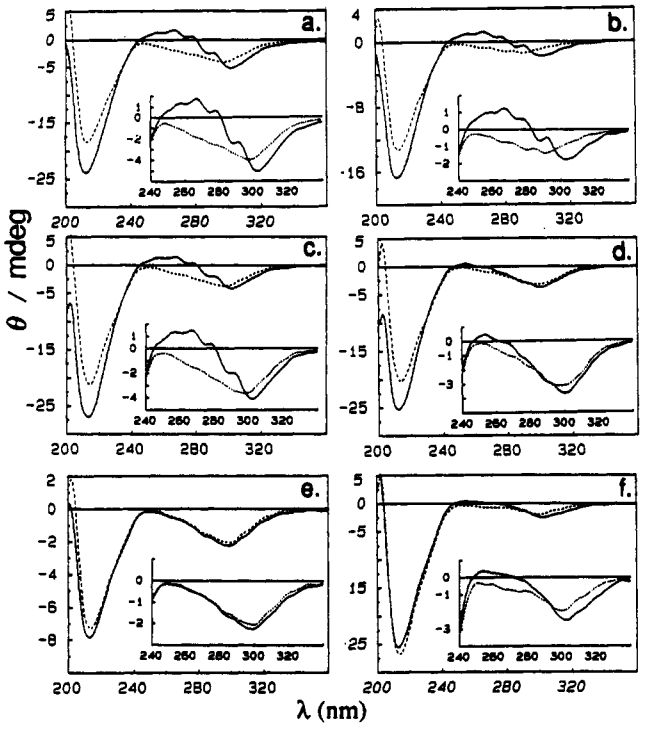

Figure 3. Solid curves: CD spectra of FKBP in 20 mM Na phosphate buffer, pH 7.2, containing 0.002% reduced Triton X-100 in the presence of drugs **1–6** (1-cm cell): (a) 5.1 μM FKBP + 5.4 μM **1** (1.1 equiv of **1**), (b) 2.9 μM FKBP + 2.5 μM **2** (0.9 equiv of **2**), (c) 3.8 μM FKBP + 3.8 μM **3** (1.0 equiv of **3**), (d) 3.7 μM FKBP + 3.2 μM **4** (0.9 equiv of **4**), (e) 1.5 μM FKBP + 1.6 μM **5** (1.1 equiv of **5**), and (f) 4.8 μM FKBP + 3.3 μM **6** (0.7 equiv of **6**). Dashed curves: summation CD curves of FKBP (in Na phosphate buffer) and drugs (in ethanol); same concentrations for FKBP and drugs as mentioned above. The insets are enlarged CD curves in the 240–340-nm region. The drug–protein mixtures were prepared by adding the drug in ethanol to the FKBP buffer solution (ethanol volume was less than 1% of the final volume), followed by 20-min incubation at room temperature.

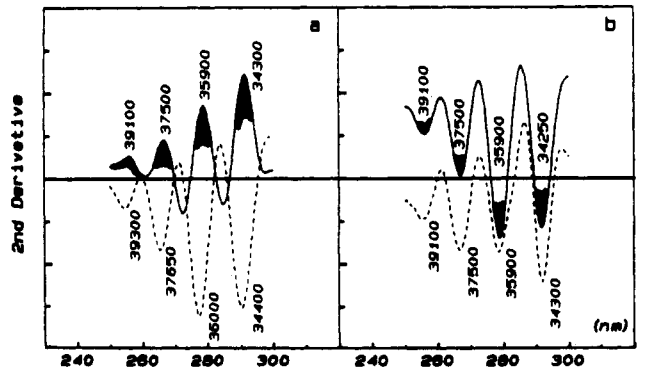

Figure 4. Second-derivative CD (solid) and UV (dashed) curves of demethoxyrapamycin **2**. Peak positions of the CD (filled) and UV (unfilled) curves are indicated in cm^{-1}: (a) free and (b) bound.

free drugs and protein in two aspects: (i) the negative CE at *ca.* 210 nm is stronger and (ii) the CE of the triene at *ca.* 270 nm shows enhanced vibrational fine structure and undergoes sign inversion (Figures 3 and 4). Similar spectra changes are observed in the case of compound **4**, although to a less extent (Figure 3d). In contrast, for analogs **5** and **6**, the CD of coincubated solutions of drugs and protein are superimposable on the summation spectra of the drugs and protein (Figure 3e,f). Trends (i) and (ii) demonstrate a tight binding of **1–4** to FKBP. The difference among rapamycins **1–6** in the CD spectra is reflected in the bioassays; **1–3** strongly inhibit peptidyl-prolyl *cis–trans* isomerase activity of FKBP and suppress thymocyte proliferation (see K_i for PPIase assay[15a] and IC_{50} for LAF assay,[15b] Chart 1). Compound **4** displays a similar inhibitory effect but is less potent (Chart 1). Compounds **5** and **6**, on the other hand, show a greatly decreased activity in both assays.

In the CD of **1–4** adducts with FKBP, the more negative 210-nm CE cannot be fully interpreted due to overlap in the CD of the protein and drugs; however, the changes in the CD at *ca.* 270 nm are assignable to the drug moiety since FKBP lacks absorption in this region. An analysis of 270-nm CD changes due to protein–drug complexation becomes feasible by second derivatization, as illustrated for 32-demethoxyrapamycin **2** (Figure 4; peak signs invert). The second-derivative analysis was developed during studies on the CD library of oligosaccharides.[16–18] Since wavenumbers of positive peaks in the second derivative of the *ca.* 270-nm CD band of free **2** (Figure 4a) match those of the negative UV peaks, they represent positions of the fine-structured peaks; moreover, the 1600–1650-cm^{-1} intervals between these peaks correspond to the IR frequency of conjugated C=C bonds. The fine structure therefore arises from the triene moiety, the CE sign of which is *negative*. Wavenumbers of the peaks at 270 nm in the second-derivative CD of **2**–FKBP adduct (Figure 4b), again match those of the UV, with intervals of 1600–1650 cm^{-1} in both spectra. The second derivative CD peaks are negative, and hence the CE sign is *positive*. The enhanced fine structure in the **1–4** and FKBP complexes (Figures 3a–d), relative to free compounds, demonstrates that the triene moiety adopts a more planar and rigid conformation; the enhancement of vibrational fine structure in planar, rigid polyene systems is well documented (e.g., retinoids).[19] The influence of FKBP on the triene is substantial, as seen in the sign inversion at 270 nm from negative (free drug) to positive (bound drug).

Crystallographic and NMR data indicate that the triene protrudes from FKBP binding pocket with little change between free and bound rapamycin;[5] on the other hand, CD suggests that the protruding triene undergoes subtle conformational changes upon binding to FKBP. It is possible that *this conformational change* is important in the binding of the rapamycin–FKBP adduct to its effector protein, as reflected in the LAF assay. Such information gained from the CD of drug–protein complexes should be valuable in elucidating the interaction on a molecular structural basis. CD spectroscopy, coupled with second derivative analysis method, offers a unique tool for studying subtle conformational changes arising from ligand–receptor interactions.

Acknowledgment. The authors wish to thank their colleagues at Wyeth Ayerst Research: S. Lee, W. Hum, and C. Hsiao for providing the FKBP12, W. Baeder and R. Caccese for the LAF assay data, and D. Longhi for helpful discussions. The studies were supported, in part, by NIH Grant GM 34509 (to K.N.) and NSF Grant INT-90-15531 (to K.N. and N.B.) and a Wyeth-Ayerst fellowship (to Y.C.).

(14) The 200–180-nm region was not measured for FKBP in the presence of **1–6** because the focus was on the 270-nm rapamycin triene moiety; moreover, the presence of other rapamycin transitions make interpretation of this region too complex.

(15) (a) The K_i values were measured using a modification of the method: Kofron, J. L.; Kuzmic, P.; Kishore, V.; Colon-Bonilla, E.; Rich. D. H. *Biochemistry* **1991**, *30*, 6127. (b) The LAF assay is a comitogen induced thymocyte proliferation procedure: Staruch, M. J.; Wood, D. D. *J. Leuk. Biol.* **1985**, *37*, 193.

(16) The smoothing program is based on DFT (discrete Fourier transform) or FIR (finite-duration impulse reponse filter).

(17) Wiesler, W. T.; Berova, N.; Ojika, M.; Meyers, H. V.; Chang, M.; Zhou, P.; Lo, L. C.; Niwa, M.; Takeda, R.; Nakanishi, K. *Helv. Chim. Acta* **1990**, *73*, 509.

(18) (a) Savitzky, A.; Golay, M. J. E. *Anal. Chem.* **1964**, *36*, 1627. (b) Baedecker, P. A. *Anal. Chem.* **1985**, *57*, 1477. (c) Gorry, P. A. *Anal. Chem.* **1990**, *62*, 570.

(19) (a) Sheves, M.; Kohne, B.; Friedman, N.; Mazur, Y. *J. Am. Chem. Soc.* **1984**, *106*, 5000. (b) Takahashi, T.; Yan, B.; Mazur, P.; Derguini, F.; Nakanishi, K.; Spudich, J. L. *Biochemistry* **1990**, *29*, 8467.