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A New Model of the Tautomycin–PP1 Complex That is Not Analogous to the Corresponding Okadaic Acid Structure

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Abstract—A revised model of PP1–tautomycin (TM) complex suggests that this toxin does not bind in a conformation analogous to its structural cousin okadaic acid (OA), as has been assumed, but instead more resembles the mode of binding adopted by calyculin. This model rationalizes the unexpected potency of a truncated TM analogue lacking the bicyclic ketal common to TM and OA.

The serine/threonine protein phosphatases, which include PP1, PP2A, PP2B, PP2C, and others, comprise a group of enzymes that act in concert with kinases to regulate a large number of intracellular signaling cascades.[1] Among this group, PP1 and PP2A can be distinguished pharmacologically from all the others by their selective inhibition with natural toxins such as tautomycin, okadaic acid, calyculin, and microcystin-LR (Fig. 1), among others.[2] As a result, these toxins are important, albeit imperfect, tools in dissecting the respective roles of individual phosphatases in cellular signaling pathways. A great deal of effort has been directed toward understanding the structure–activity relationships of these compounds. One goal in particular has been to develop a pharmacophore model that might guide the design of more selective and/or structurally less complex toxin analogues.[3]

The X-ray crystallographic structure of the complex of microcystin-LR and PP1,[4] published in 1995, spawned a number of molecular modeling studies proposing structures for the other toxin–PP1 complexes,[3b,5] and from these a generalized pharmacophore model has emerged. Subsequently, X-ray structures for the PP1 complexes of okadaic acid[6] and of calyculin[7] have appeared, confirming the gross elements of the predicted structures but adding, or correcting, many important details.

The currently accepted binding model (Fig. 2) is based on interactions between inhibitor and four separate domains in the PP1 binding site: (1) the phosphate hydrolysis site containing the two metal ions, (2) the β12–β13 loop, (3) a hydrogen bonding site, and (4) the hydrophobic cleft. In the case of okadaic acid (Fig. 3, from the published X-ray structure[6]), the toxin folds into a pseudo-cyclic shape in which the C24 alcohol forms a hydrogen bond to the C1 carboxylate, which in turn occupies the phosphate hydrolysis pocket and is thus mimicking the phosphate group of normal substrates of the enzyme. The adjacent C4–C13 bicyclic ketal makes contact with the β12–β13 loop, the C24-OH forms a hydrogen bond to the protein, and finally the C30–C38 spiroketal lies in the hydrophobic cleft.

The other toxins make analogous contacts with PP1.[2,8] For example, the isoglutamyl carboxyl group of microcystin-LR is the phosphate mimic, the leucine side chain makes contact with the β12–β13 loop, and the diene side chain ('Adda') lies in the hydrophobic cleft.[4] Note that while the toxins all contain domains that make analogous contacts, the *connectivity* of the individual structural elements within the toxins can differ dramatically. Thus, the phosphate mimic and the group that occupies the hydrophobic cleft are separated by only five atoms in microcystin but by more than 25 atoms in okadaic acid. Obviously, it is the three-dimensional arrangement of the respective groups that matters, and this factor enters heavily into the design of analogues containing the pharmacophore elements.

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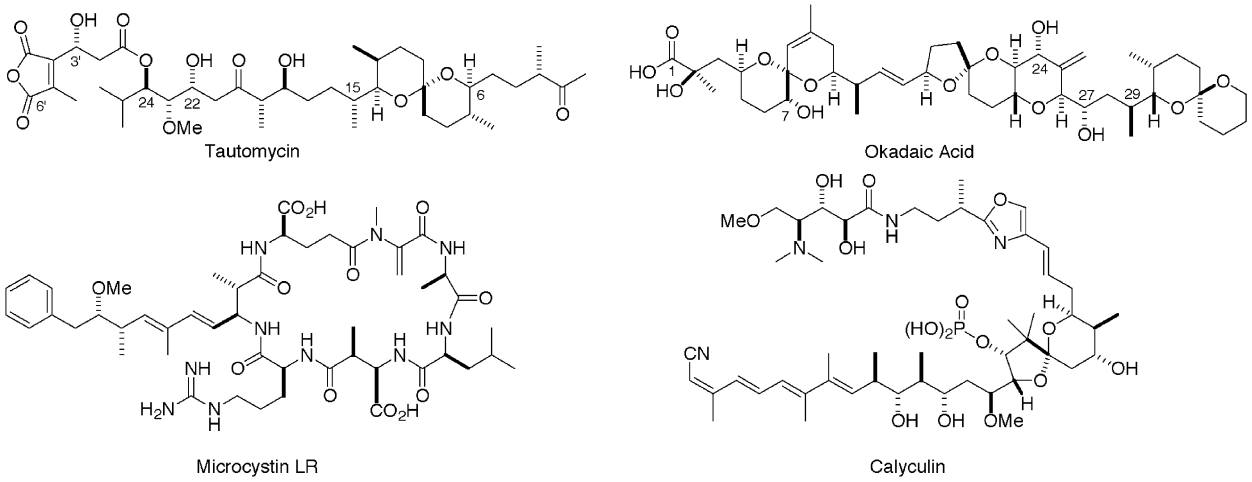

Figure 1. Structures of the natural product inhibitors of protein phosphatases, 1 and 2A.

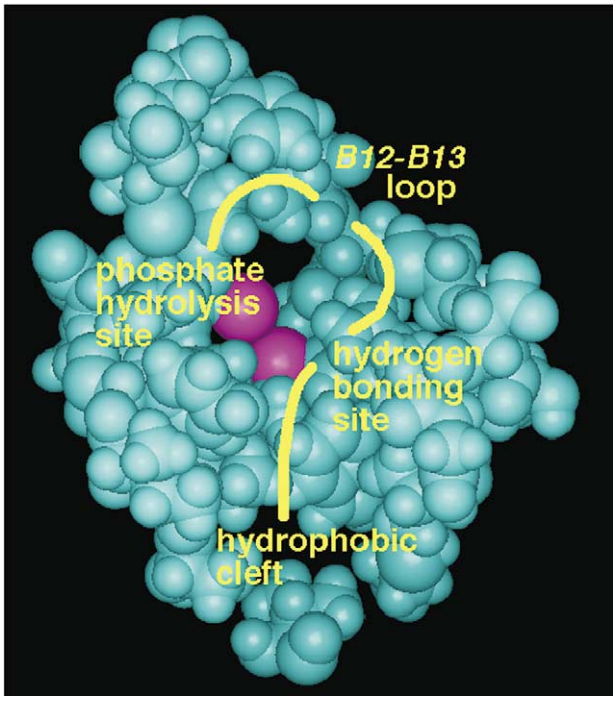

Figure 2. Current pharmacophore model for protein phosphatase binding. The binding site of PP1 is colored blue and the metals are magenta. For clarity, only residues within 5.0 Å of the active site are shown.

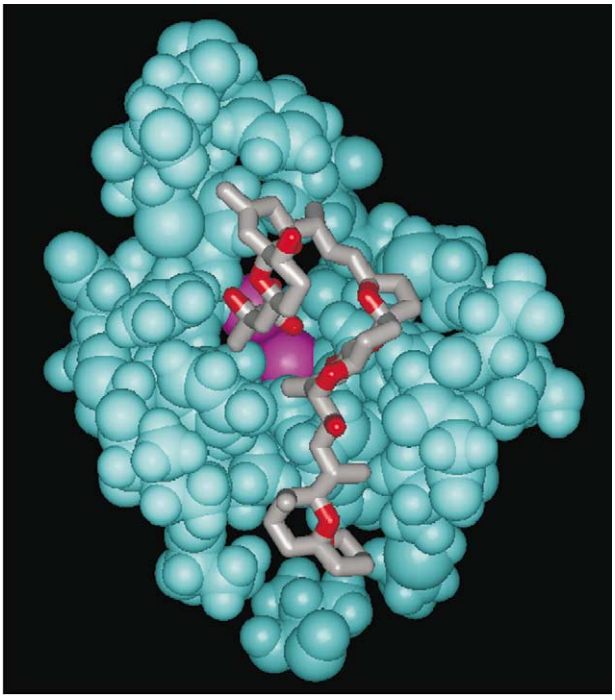

Figure 3. Conformation of binding of okadaic acid to protein phosphatase 1 according to crystal structure.[6] Okadaic acid is colored by atom type and the binding site of PP1 is blue.

While secure structural information is available for the complexes of PP1 with microcystin, okadaic acid, and calyculin, a corresponding X-ray structure for tautomycin–PP1 complex has not yet been reported. This is unfortunate because until recently, tautomycin was the only known small molecule inhibitor selective for PP1,[9] and knowing the details of binding to PP1 (and PP2A) might provide significant insight to the factors controlling PP1 selectivity within this group of toxins. Because of its obvious structural similarity to okadaic acid, it has often been assumed that tautomycin binds to PP1 in a pseudo-cyclic conformation as okadaic acid does.[3b,10] However, in refining our previous model for this complex, using the recently published X-ray structure of okadaic acid as a starting point, we have discovered a new possible binding motif for tautomycin that is distinctly *unlike* okadaic acid, and which rationalizes the surprising activity of truncated tautomycins relative to similarly truncated okadaic acids.

As a starting point, tautomycin was overlaid on the bound conformation of okadaic acid (from the X-ray structure) and minimized using the Discover_3 module of Insight II.[11] Assuming that the C4′–C7′ anhydride of tautomycin binds in its hydrolyzed form, as originally suggested by Isobe and co-workers,[10a] five different low energy conformations of tautomycin were docked into the active site of PP1. For subsequent dynamics calculations, the C6′ carboxy group was constrained to the

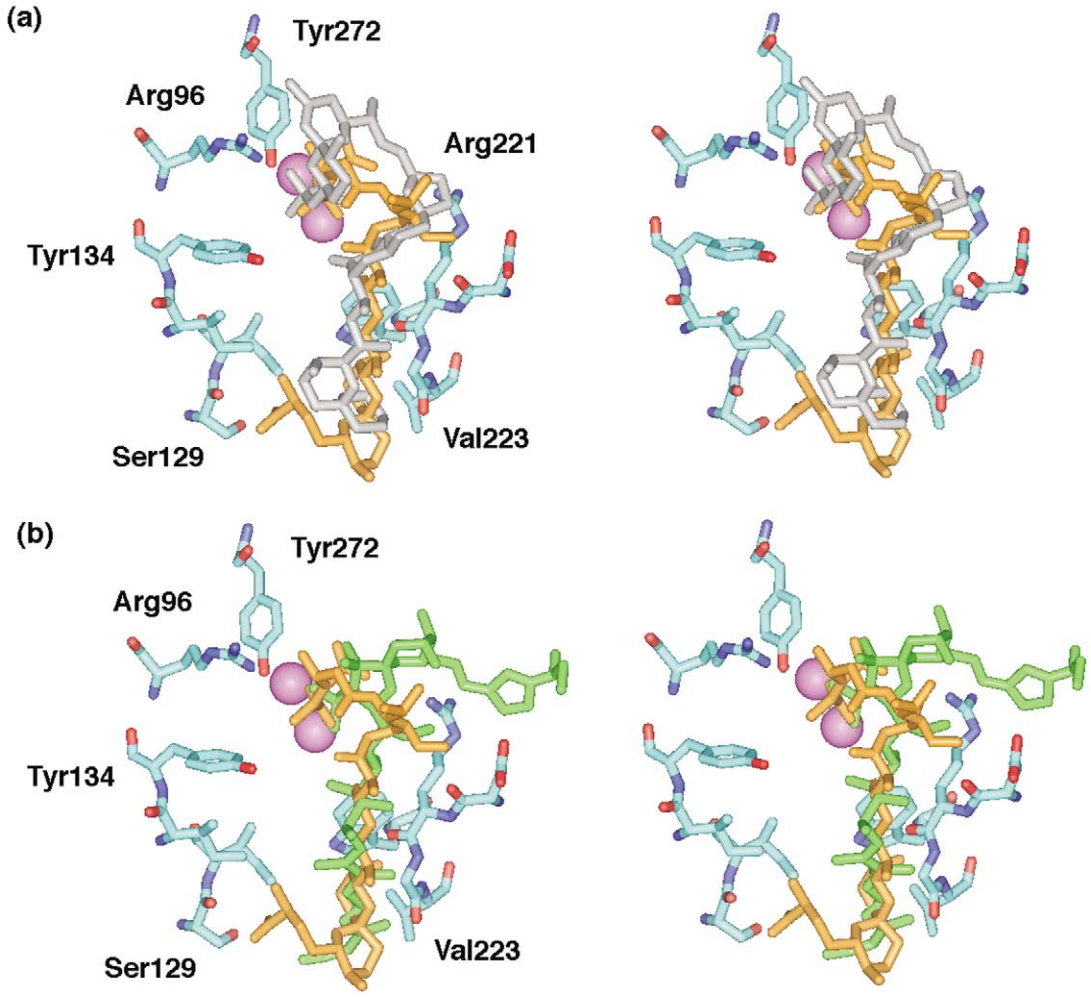

Figure 4. (a) Stereoview of tautomycin model with superimposition of okadaic acid. Tautomycin is colored gold, okadaic acid is silver, and the contacting residues of PP1 are blue. (b) Stereoview of tautomycin model with superimposition of calyculin. Tautomycin is colored gold, calyculin is green, and the contacting residues of PP1 are blue.

vicinity of the metal ions in the active site, and the C3′ hydroxy group was constrained within hydrogen bonding distance of Arg96 (which has been shown to hydrogen bound with the C2-OH of okadaic acid[6]). Similarly, the C22 hydroxy group of tautomycin was constrained to within hydrogen bonding distance of Arg221 for two reasons: (1) mutagenesis studies have shown that the Arg221 residue is important for the activity of okadaic acid, calyculin, and microcystin,[12] and (2) structure–activity studies have shown that the C22-OH of tautomycin is required for activity.[10a] No further constraints were imposed.

A molecular dynamics conformational search[11] provided several low energy candidates similar to previously calculated structures, that is, pseudo-cyclic conformations similar to okadaic acid. However, the lowest energy conformation was markedly different in that it was not pseudo-cyclic. Instead, tautomycin had pulled away from the β12–β13 loop to extend farther down the hydrophobic cleft so that the spiroketal came in contact with Val223 (Fig. 4a). This arrangement is quite unlike that of the corresponding okadaic acid complex (and of previous models of the tautomycin complex) but it is instead similar to the binding motif recently reported for the calyculin–PP1 complex (Fig. 4b),[7] excluding of course the additional peptide-like side chain of calyculin.

These similarities are visually evident from the pair-wise comparisons in Figure 4, but to be more specific, okadaic acid makes multiple contacts with the β12–β13 loop, while tautomycin—like calyculin[7]—makes only a single contact in this domain, with Tyr272. This new model is also consistent with mutagenesis studies by Lee in which mutations to Tyr272 in the β12–β13 loop of PP1 lead to over 1000-fold increases in IC_{50} for both calyculin and tautomycin, compared to only a 200-fold increase observed for okadaic acid.[13]

Besides rationalizing literature data, this new model suggests some interesting and perhaps unrecognized possibilities for tautomycin analogue design and SAR studies. The accepted paradigm for PP1 inhibition, based primarily on microcystin and okadaic acid SAR studies, requires both a phosphate mimic and a hydrophobic cleft binder for potent inhibition. Microcystin derivatives lacking either of these groups are extremely

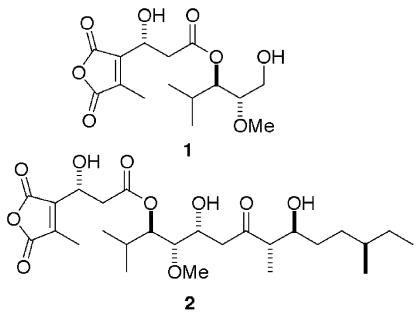

Figure 5. Structures of truncated tautomycin derivatives.[16]

Table 1. Phosphatase inhibition by tautomycin and analogues[a]

Compd	IC_{50} (nM)	
	PP1	PP2A
1	4.8×10^5	3.3×10^5
2	130	100
Tautomycin	0.19	0.90

[a]Values from assay are based on phosphorylase-*a* as substrate.

weak inhibitors, and conversely, derivatives containing *only* these two structural elements are reasonably potent (as low as 170 nM IC_{50} for inhibition of PP2A).[14] Similarly, okadaic acid analogues generated by removing the hydrophobic spiroketal have little activity.[15] This new, 'extended' binding model suggests that truncated tautomycin analogues lacking the bicyclic ketal might retain significant activity—in direct contrast to analogous truncated okadaic acids[15]—because of previously unrecognized favorable contacts in the H-bonding domain and the hydrophobic cleft that could compensate for the missing bicyclic ketal.

To test this possibility, the truncated analogues **1** and **2** were prepared (Fig. 5),[16] based closely on our published synthesis of tautomycin.[17] The more highly truncated analogue **1** is a negative control, in that it cannot extend into the hydrophobic cleft, unlike **2** that possesses an additional six carbons and can occupy the hydrophobic cleft of PP1 (Fig. 6). The respective IC_{50} values for the inhibition of PP1 and PP2A were determined in the standard phosphorylase-*a* inhibition assay[18] (Table 1). Synthetic tautomycin was used as a positive control and gave IC_{50} values of 0.190 nM and 0.940 nM for PP1 and PP2A, respectively, consistent with literature values.[19] As expected, the negative control analogue **1** showed little inhibitory activity for either PP1 or PP2A. On the other hand, the analogue **2**, while less active than the parent compound, showed surprisingly high inhibitory activity, in the 100 nM range. It is, in fact, the most potent truncated analogue of any of these four toxins yet discovered, providing experimental support for the proposed binding mode of tautomycin, itself.

The activity of **2** is particularly striking considering that the C6–C14 spiroketal group of tautomycin, previously viewed as likely to be essential for activity, is completely absent. Studies are underway to further apply this model to the design of simplified protein phosphatase inhibitors.

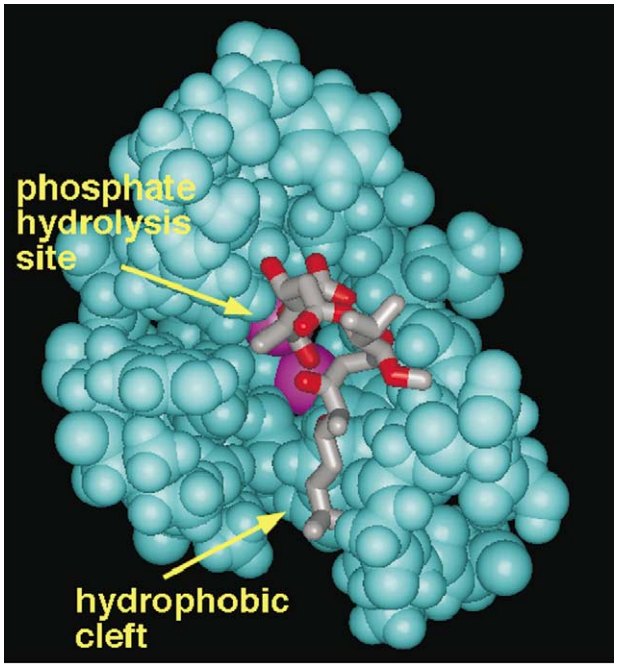

Figure 6. Conformation of the analogue **2** bound to PP1 according to molecular modeling.[11]

Acknowledgements

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References and Notes

1. (a) Cohen, P. T. W. *J. Cell. Sci.* **2002**, *115*, 241. (b) Shenolikar, S.; Nairn, A. C. *Adv. Second Messenger Phosphoprotein Res.* **1991**, *23*, 1.
2. (a) McCluskey, A.; Sim, A. T. R.; Sakoff, J. *A. J. Med. Chem.* **2002**, *45*, 1151. (b) Sheppeck, J. E.; Gauss, C.-M.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1739.
3. (a) Wipf, P.; Cunningham, A.; Rice, R. L.; Lazo, J. S. *Bioorg. Med. Chem.* **1997**, *5*, 165. (b) Guass, C.-M.; Sheppeck, J. E.; Nairn, A. C.; Chamberlin, A. R. *Bioorg. Med. Chem.* **1997**, *5*, 1751.
4. Goldberg, J.; Huang, H. B.; Kwon, Y. G.; Greengard, P.; Nairn, A. C.; Kuriyan, J. *Nature* **1995**, *376*, 745.
5. (a) Gupta, V.; Ogawa, A. K.; Du, X.; Houk, K. N.; Armstrong, R. W. *J. Med. Chem.* **1997**, *40*, 3199. (b) Bagu, J. R.; Sykes, B. D.; Craig, M. M.; Holmes, C. F. B. *J. Biol. Chem.* **1997**, *272*, 5087. (c) Lindvall, M. K.; Pihko, P. M.; Koskinen, A. M. P. *J. Biol. Chem.* **1997**, *272*, 23312. (d) Quinn, R. J.; Taylor, C.; Suganuma, M.; Fujiki, H. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1029.
6. Maynes, J. T.; Bateman, K. S.; Cherney, M. M.; Das, A. K.; Luu, H. A.; Holmes, C. F. B.; James, M. N. G. *J. Biol. Chem.* **2001**, *276*, 44078.
7. Kita, A.; Matsunaga, S.; Takai, A.; Kataiwa, H.; Wakimoto, T.; Fusetani, N.; Isobe, M.; Miki, K. *Structure* **2002**, *10*, 715.
8. Takai, A.; Saski, K.; Naga, H.; Mieskes, G.; Isobe, M.; Isono, K.; Yasumoto, T. *Biochem. J.* **1995**, *306*, 657.
9. Recently the natural product tautomycetin has been shown to be PP1 selective, as well. Mitsuhashi, S.; Matsuura, N.; Ubukata, M.; Oikawa, H.; Shima, H.; Kikuchi, K. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 328.

10. (a) Sugiyama, Y.; Ohtani, I. I.; Isobe, M.; Takai, A.; Ubukata, M.; Isono, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3. (b) Ubukata, M.; Koshino, H.; Yamasaki, C.; Fujita, K.; Isono, C. *J. Antiobiot.* **1997**, *50*, 801.
11. Molecular dynamics and minimizations were carried out on a Silicon Graphics Octane 2 computer using the Discover_3 module on Insight II version 2000. Dynamics were carried out at 300, 400, and 600 K. The residues of PP1 within 7.0 Å of tautomycin were allowed to relax.
12. Huang, H. B.; Horiuchi, A.; Goldberg, J.; Greengard, P.; Nairn, A. C. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3530.
13. Zhang, L.; Zhang, Z.; Long, F.; Lee, E. Y. C. *Biochemistry* **1996**, *35*, 1606.
14. Gulledge, B. M.; Aggen, J. B.; Huang, H. B.; Nairn, A. C.; Chamberlin, A. R. *Curr. Med. Chem.* **2002**, *9*, 1991.
15. Frydrychowski, V. A.; Urbanek, R. A.; Forsyth, C. *J. Bioorg. Med. Chem. Lett.* **2001**, *11*, 647.
16. Compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, and HRMS.
17. Sheppeck, J. E.; Liu, W.; Chamberlin, A. R. *J. Org. Chem.* **1997**, *62*, 387.
18. Cohen, P.; Alemany, S.; Hemmings, B. A.; Resink, T. J.; Stralfors, P.; Tung, H. Y. L. *Methods Enzymol.* **1988**, *159*, 390.
19. (a) MacKintosh, C.; Klumpp, S. *FEBS Lett.* **1990**, *277*, 137. (b) Hori, M.; Magae, J.; Han, Y. G.; Hartshorne, D. J.; Karak, H. A. *FEBS Lett.* **1991**, *285*, 145.