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Bisphosphonates Target Multiple Sites in Both *Cis*- and *Trans*-prenyltransferases

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Introduction

Bisphosphonate drugs (e.g. Fosamax and Zometa) are thought to act primarily by inhibiting farnesyl diphosphate synthase (FPPS), resulting in decreased prenylation of small GTPases. Here, we show that some bisphosphonates can also inhibit geranylgeranyl diphosphate synthase (GGPPS), as well as undecaprenyl diphosphate synthase (UPPS), a cis-prenyltransferase of interest as a target for anti-bacterial therapy. Our results on GGPPS show that there are three different bisphosphonate binding sites. In UPPS, there are a total of four binding sites. These results are of general interest since they provide the first structures of GGPPS- and UPPS-inhibitor complexes, potentially important new drug targets, in addition to revealing a remarkably broad spectrum of binding modes.

Result and Discussion

We investigated the actual binding modes seen with bisphosphonate inhibitors and begin by investigating the small, "third generation" species zoledronate and minodronate, potent FPPS inhibitors that also inhibit GGPPS. This binding pattern is remarkably similar to that seen with zoledronate, minodronate (and indeed, many other bisphosphonates) binding to a variety of FPPS enzymes and strongly suggests, at least for these compounds, that they could act as carbocation transition state/reactive intermediate analogs.

We next consider the binding of three novel and more potent GGPPS inhibitors: BPH-364, BPH-629 and BPH-675, compounds that appear to be more potent GGPPS inhibitors due to addition of a large, hydrophobic moiety. With BPH-364 and BPH-675, the bisphosphonate backbones bind to the protein into the same sites as seen with GGPP bound to human GGPPS: F***PP-GG***PP. In the case of BPH-629, a neutral side-chain (dibenzofuran) containing bisphosphonate, there are two (as opposed to one) bisphosphonate binding sites, and there is no Mg^{2+} observable. In the first binding site, it represents the F***PP-F***PP binding site location seen with zoledronate, minodronate and FsPP and is shown in orange in Fig. 1 The other conformer (BPH-629-2) binds with its bisphosphonate in the I***PP*** site. As expected, there is no Mg^{2+} present in this IPP site. However, in this instance, the bulky aromatic moiety extends into the (human) ***GG***PP site, so binding is I***PP-GG***PP, using the nomenclature suggested above, and this site is shown in pink in Fig. 1.

For developing new anti-microbial therapy, we first screened a library of 29 bisphosphonates for activity against *E. coli* UPPS. We selected the five compounds (BPH-608, BPH-629, BPH-628, BPH-675, and BPH-676) for crystallographic investigation. In each of the five structures, we found that these of binding sites for bisphosphonates occupy the top of a "funnel" region while the fourth site is situated at the bottom of the funnel. The presence of a large hydrophobic cavity is not unexpected in a C_{55} prenyl synthase, and with the bisphosphonates investigated here, enables up to 4 inhibitors to bind to a central cavity (Fig. 2) The results of 2D and hologram quantitative structure activity relationship (HQSAR) in addition to a pharmacophore model are shown in Fig. 2C In the UPPS pharmacophore, there are two negative ionizable groups (blue) and three hydrophobic features (cyan) shown superimposed on the structure of BPH-629, the most potent UPPS inhibitor. The pharmacophore for GGPPS inhibition is similar, but neither pharmacophore gives evidence of the importance of the cationic feature present in the FPPS result.

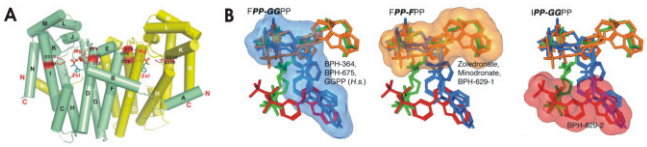

Fig. 1. (*A*) GGPPS structure containing zoledronate showing dimer structure. (*B*) F*PP-GG*PP site (in blue) occupied by BPH-364, BPH-675, and GGPP; F*PP-F*PP site (in orange) occupied by zoledronate, minodronate, and BPH-629-1; I*PP-GG*PP site (in pink) occupied by BPH-629-2.

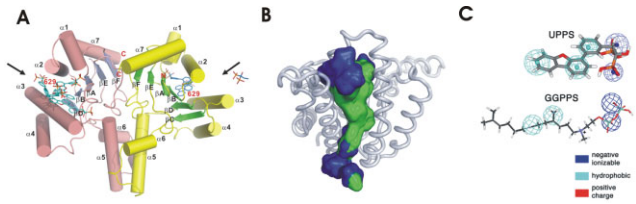

Fig. 2. (*A*) Structure of UPPS-BPH-629 complex. (*B*) Diagram showing surface structure of all five inhibitors bound to UPPS (all crystal structures). (*C*) Pharmacophores for GGPPS and UPPS.

References

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