Structure of Chinese Herbal-based Medicine Captured by ATP on a Human tRNA Synthetase

For thousands of years the Chinese have been using the Chang Shan herb (*Dichroa febrifuga* Lour) to treat malaria-induced fevers (1). The active ingredient in the herb was eventually shown to be a small molecule known as febrifugine. A halogenated derivative of febrifugine, called halofuginone (HF), has been tested in clinical trials as a therapy for cancer and fibrotic diseases. Previously, investigators at Harvard University had demonstrated that HF is a suppressant of a specific part of the immune system and that its biological activity is a downstream consequence of its binding to and inhibition of prolyl-tRNA synthetase (ProRS) (2,3). Curiously, binding to ProRS requires ATP, which is a substrate of ProRS.

The 20 aminoacyl-tRNA synthetases (AARSs) realize the genetic code by catalysis of aminoacylation reactions through which each amino acid is matched with its cognate tRNA. ProRS is one of these 20 AARSs. It activates the amino acid proline (Pro) by fusing it with ATP and then reacting the resulting high-energy aminoacyl adenylate with the tRNA that is designed to hold Pro. To achieve this reaction, ProRS has 3 distinct pockets, which are grouped together and dock Pro, ATP, and the 3'-end of tRNA. When the pockets are filled, they co-operate so that Pro is passed to the acceptor end of the tRNA.

The unexplained feature of the earlier work was the idea that, in order for HF to associate with ProRS, it needed the ATP pocket to be filled. In this study, using co-crystal data collected at SSRL's Beam Line 7-1, Zhou *et al.* showed how HF, which has two pieces joined by a small linker, occupies two of the pockets on ProRS (Fig. 1). One piece of HF is in the pocket for Pro and the other fits into that for the receiving end of the tRNA. Remarkably, HF simultaneously captures ATP, which sits snugly in its pocket and forms critical bridging interactions with HF (Fig. 1). Additional biochemical experiments by Zhou *et al.* showed that, without the bridging interactions from ATP, HF is only weakly bound to ProRS.

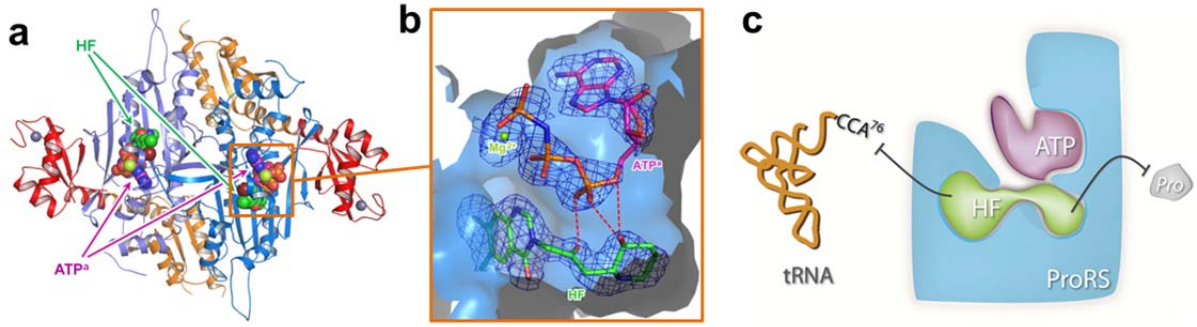

Figure 1. Structure of ProRS. a. Depiction of the dimeric ProRS structure with bound ATP (red) and HF (green). **b.** Enlarged view of the pockets on ProRS that hold ATP and HF, with ATP-HF bridging interactions. **c.** Cartoon schematic of how HF simultaneously occupies the pockets for Pro and the receiving end of the tRNA (A76) and thereby blocks access for Pro and tRNA.

The structure of this co-crystal is the first for human ProRS. However, most significantly, the co-crystal provides a highly unexpected explanation for the action of an herbal-based medicine on the atomic level: HF is an inhibitor of ProRS and yet, to achieve inhibition, it must bind to a substrate (ATP) of ProRS.

Primary Citation

H. Zhou, L. Sun, X-L. Yang and P. Schimmel, “ATP-directed Capture of Bioactive Herbal-based Medicine on Human tRNA Synthetase”, *Nature* **494**, 121 (2013), DOI: 10.1038/nature11774.

References

1. J. B. Koepfli, J. F. Mead and J. A. Brockman Jr., "An Alkaloid with High Antimalarial Activity from *Dichroa febrifuga*", *J. Am. Chem. Soc*. **69**, 1837 (1947).
2. M. S. Sundrud *et al.*, "Halofuginone Inhibits TH17 Cell Differentiation by Activating the Amino Acid Starvation Response", *Science* **324**, 1334 (2009).
3. T. L. Keller *et al*., "Halofuginone and Other Febrifugine Derivatives Inhibit Prolyl-tRNA Synthase", *Nat. Chem Biol*. **8**, 311 (2012).

Contact

Paul Schimmel, The Scripps Research Institute

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