

Inhibiting Estrogen-Dependent Breast Cancer at the Source



IN SHORT > Recent developments in breast cancer research have revealed that there are a number of different mechanisms by which this cancer may form. One of these is through inappropriate activation of cancer cell growth by naturally occurring estrogen in a woman's body. This knowledge has provided two possible targets for treatment: through inhibition of the binding of estrogen to the estrogen receptor or through inhibition of natural estrogen synthesis in the patient. Research at the SBC-CAT beamline 19-ID at the APS solved the structure of the enzyme aromatase to 2.9-Å resolution. This work provides important insights into the mechanism of action of aromatase, the specificity of its androgen binding cleft, and its membrane localization that will enable researchers to improve the existing inhibitors of this unique cytochrome P450-family enzyme.

MORE > Estrogens are synthesized in the body by an enzyme called aromatase cytochrome P450 from their cellular precursors, androgens. Inhibition of this enzyme, therefore, has provided a target for small molecules that might inhibit its synthetic activity. Three of these aromatase inhibitors have been approved by the Food and Drug Administration for treatment of estrogen-dependent breast cancer. However, these molecules have all been developed without specific structural knowledge of the aromatase enzyme's androgen binding site or catalytic mechanism of action. Aromatase belongs to a large family of membrane-bound cytochrome P450 enzymes that play important roles in cellular metabolism. Research has shown

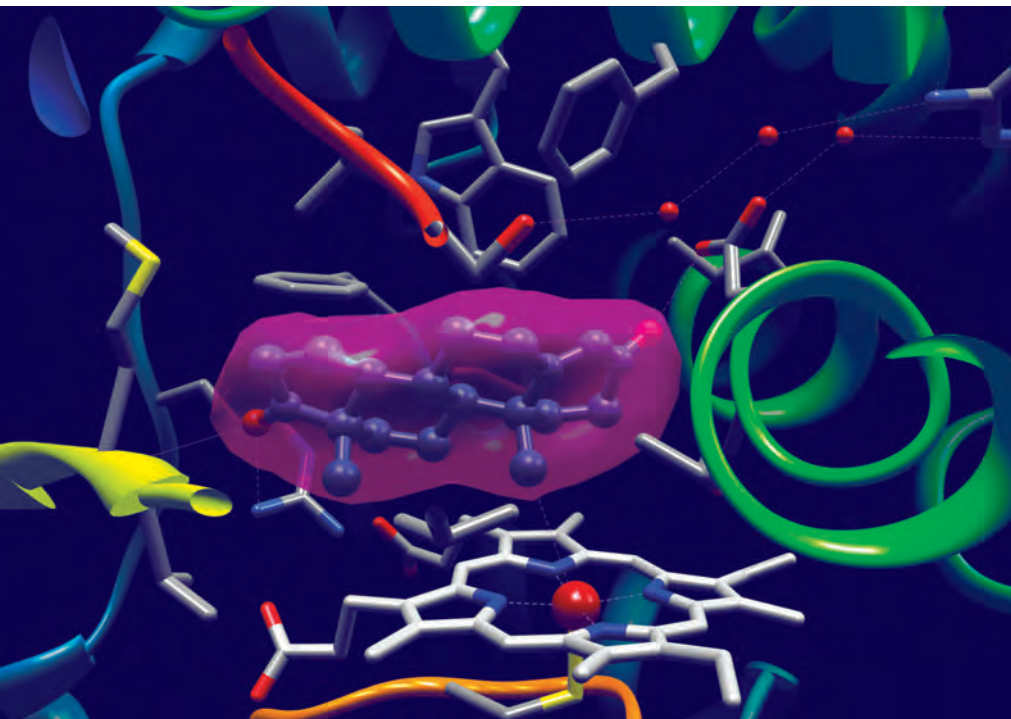
that aromatase has a unique specificity among these enzymes and that the current inhibitors cross-react with other cytochrome P450 family members causing side effects.

Now, with solution of the crystal structure by researchers from the Hauptman-Woodward Medical Research Institute and the Roswell Park Cancer Institute, the molecular basis for the specificity of aromatase has been revealed (Fig.1). Within the binding cleft for androgen, there is a unique amino acid, proline 308, which changes the structure of the pocket to accommodate androgen perfectly. This confirms earlier studies showing that proline 308 plays a crucial role in aromatase activity and that it is unique among cytochrome P450 enzymes

> Fig. 1 A view of the human placental aromatase active site showing the bound androgen molecule within its unbiased electron density surface contoured at 4.5 times the standard deviation. Amino acid side chains, the heme group, and bound water molecules that snugly enclose the bound substrate are shown. Element colors are: C: gray, N: blue, O: red, S: yellow, Fe: firebrick, and H: orange. The C atoms of androgen are colored in light blue.

when one lines up their amino acid sequences.

The next step toward understanding how to design a better aromatase inhibitor was to create a model of the structure of aromatase with one of the inhibitors bound to the active site of the enzyme. By using the information they had gained from the structure and proposed catalytic mechanism of aromatase bound to androgen, the research team was able to model an aromatase inhibitor, exemestane, into the active site of aromatase. Analysis of the differences between the way androgens bind aromatase and the way the inhibitor binds showed that exemestane can bind to the active site but does not have the chemical group available for catalysis and therefore remains tightly bound, blocking any further androgen access to the catalytic cleft. The researchers are continuing to work on the structure of aromatase bound to the other inhibitors and have already made important strides towards the design of more specific aromatase inhibitors.

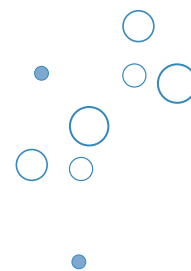


Another important aspect of cytochrome P450 enzymes is that they are bound to cellular membranes and catalyze reactions involving molecules that prefer a lipid environment to the watery environment of the cytosol. Analysis of the membrane-binding portions of aromatase allowed the researchers to hypothesize about how the orientation of the enzyme in the cellular membrane affects its interaction with its lipid-loving steroidal substrates. The analysis showed that the membrane binding portions of the enzyme bring the active site cleft of the enzyme into close proximity to the membrane, providing a channel for androgens and estrogens to get in and out of the enzyme through the lipid bilayer. This fascinating discovery provides

a clue to the reason for the crucial membrane integration requirement of aromatase and other cytochrome P450 enzymes.

For the future, the researchers plan to focus on the design of more specific inhibitors for aromatase and to gain a more complete understanding of the mechanism of aromatase's unique catalytic chemistry.

— *Sandy Field*



See > Debashis Ghosh^{1,2*}, Jennifer Griswold¹, Mary Erman¹, and Walter Pangborn¹, “Structural basis for androgen specificity and oestrogen synthesis in human aromatase,” *Nature* **457**, 219 (8 January 2009). DOI:10.1038/nature07614.

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See also > “Structural biology: Anticancer drug target pictured,” by Michael R. Waterman, *Nature News and Views*, *Nature* **457**, 159 (8 January 2009). DOI:10.1038/457159a

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