

Think Pink: The Role of Cytochrome P450 Aromatase in Estrogen Production and Breast Cancer Risk

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Abstract

According to the American Cancer Society (2012), postmenopausal women with high levels of endogenous hormones have about twice the risk of developing cancer compared to women with the lowest levels. A key protein for estrogen biosynthesis from androstenedione (AD), and possibly linked to development of breast cancer, is cytochrome P450 aromatase (CYP19A1) found in adipose breast tissue. CYP19A1 converts AD to an aromatic C18 estrone through two consecutive hydroxylations at the C19 methyl group and catalyzing a third lyase step, culminating in cleavage of the C10–C19 bond of the C19-aldehyde, with concurrent aromatization of the A ring of the steroid framework. AD is attracted to the active site by Arg192, Asp309, and Glu483. A heme group, bound in the CYP19A1 active site by Cys437, is responsible for these 3 oxidation steps. The key residues were modeled by the Cudahy SMART (Students Modeling A Research Topic) Team using 3D printing technology. The heme group binds molecular oxygen and then forms strong oxidizing intermediates that achieve these difficult oxidation reactions. The resonance Raman technique provides detailed structural insight into these important but unstable heme intermediates. Gaining an understanding of the reaction mechanism of CYP19A1 is important. If it can be learned how CYP19A1 functions, a suppression treatment to disable local estrogen production in breast adipose tissue by CYP19A1 could be developed by scientists to control estrogen levels, possibly reducing tumor growth or diminishing the risk of development of breast cancer.

I. Cytochrome link to breast cancer

A genetic component of breast cancer has long been established by the scientific community. However, a link between a cytochrome P450 aromatase and estrogen production also may exist. Estrogen production can occur in breast adipose tissue, the principle site of estrogen production in postmenopausal women, by the cytochrome P450 enzyme, CYP19A1. CYP19A1 is responsible for the production of estrone, an estrogen precursor, from androstenedione. When estrogen is present in the adipose cell, this leads to an increase in growth factor which in turn increases the risk of the formation and proliferation of cancer cells. These growth factors also participate in a positive feedback loop, causing more estrogen production, stimulating further growth factor release and tumor cell growth.

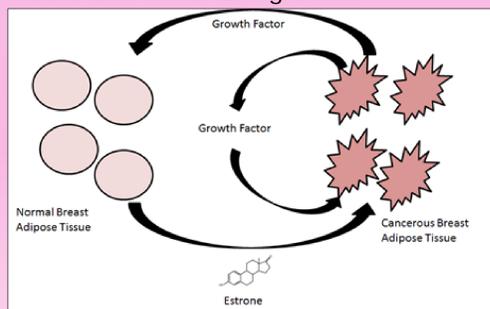


Figure 1: Estrogen production and tumor growth

A study by Bulun, et.al. (1993) showed the link between CYP19A1 and tumor production and proliferation by using tumors from patients who had their whole breast removed. The highest CYP19A1 transcript levels were found in quadrants bearing tumors. Normal breast tissue did not reveal elevated CYP19A1 levels.

II. CYP19A1 Structural Components

The crystal structure of CYP19A1 (Figure 2: Crystal Structure) reveals the presence of a heme group deep in the active site; the substrate is bound, attracted to the active site by the Glu483, Arg192, Asp309 residues. Another residue, Cys437, is responsible for binding the heme to the main structure of the protein. The heme group performs two consecutive hydroxylations of the C19 methyl group, followed by a so-called lyase reaction, which results in cleavage of the C19 methyl group and the formation of an aromatic (A) ring in the product, estrone.

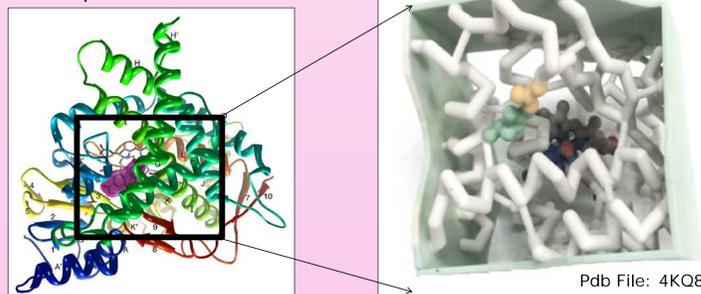


Figure 2: CYP19A1 Crystal Structure with binding pocket and heme

III. Enzymatic Cycle of CYP19A1

CYP19A1 is a membrane-bound protein. When unbound, CYP19A1 aggregates into a non-native state, making it difficult to study. By using the nanodisc sampling system to effectively act as a natural membrane, studies on the structure and function of CYP19A1 had become feasible. Nanodiscs are constructed using a membrane scaffold protein (MSP) to surround and stabilize a phospholipid bilayer (Figure 3).



Figure 3: Nanodisc with CYP19A1 bound

The steps of the enzymatic cycle are depicted in Figure 4.

1. Substrate binds to ferric heme, displacing a water ligand; iron can gain an electron
2. Ferrous heme iron can bind dioxygen, forming an Fe(III)-(O-O⁻) fragment
3. Reductase delivers a 2nd electron, forming a "peroxy" intermediate, Fe(III)-(O-O²⁻)
4. "Peroxy" gains a proton to form a hydroperoxy intermediate, Fe(III)-(O-OH⁻)
5. Intermediate gains another proton, O-O bond is cleaved, water is released, Compound I forms (Fe(IV)=O)
6. Compound I is a ferryl heme π Cation radical, (Fe(IV)=O)
7. The substrate C-H bond changes to a C-OH fragment, completes hydroxylation reaction.
8. Enzyme spontaneously return to resting states.

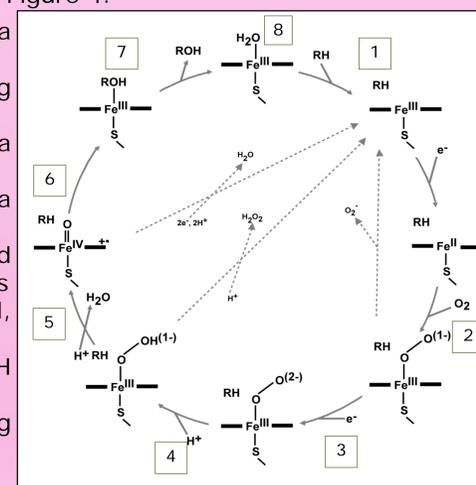


Figure 4: Enzymatic Cycle

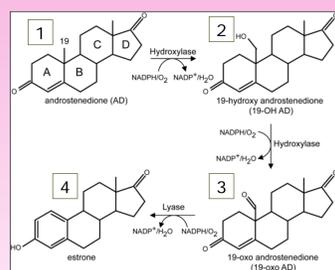


Figure 5: Aromatization of Androstenedione

The enzymatic cycle is repeated 3 times (Figure 5). CYP19A1 converts AD to an aromatic C18 estrone through two consecutive hydroxylations (1 and 2) at the C19 methyl group and catalyzing a third lyase step (3), cleaving the C10–C19 bond of the C19-aldehyde, forming an aromatic A ring of the steroid framework (4).

IV. Experimental evidence for involvement of Compound I in the lyase reaction

After understanding the enzymatic cycle of CYP19A1, it is important to note the third substrate, containing the aldehyde group at C19, is susceptible to attack by the peroxy intermediate (Figure 4, step 3). Therefore it had been speculated that the relevant intermediate in the 3rd step of estrone formation (Figure 5) is the ferric peroxy intermediate. The Resonance Raman (rR) spectroscopy can easily detect changes in the Fe-O and O-O stretching modes, as seen in Figure 6.

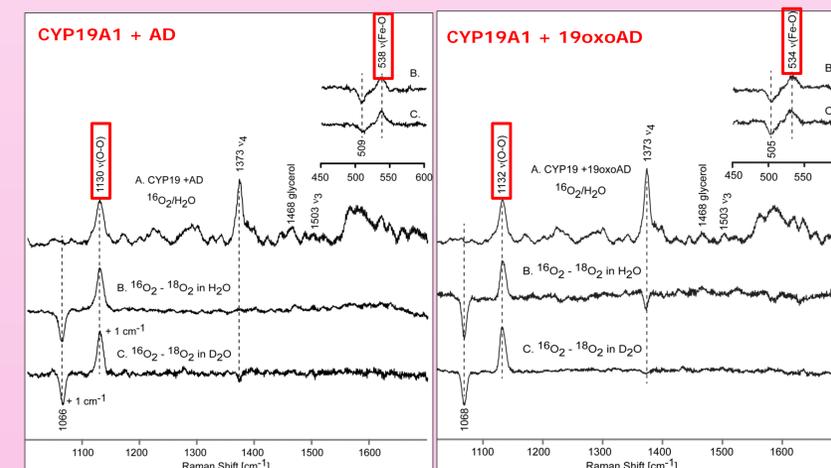


Figure 6a: ¹⁶O₂ adduct of CYP19A1

Figure 6b: ¹⁹O₂ adduct of CYP19A1

The $\nu(\text{O-O})$ stretching frequencies are similar for H-bonding interactions to the terminal and proximal oxygen atoms of the Fe-O_p-O_t fragment. However, the $\nu(\text{Fe-O})$ modes behave quite differently for these two cases, with the $\nu(\text{Fe-O})$ frequency for H-bonding to the O_t being ~15-20 cm⁻¹ higher than that for H-bonding to O_p. The fact that the spectral patterns for the 3rd substrate are so similar to those for the 1st substrate, implies both participate in similar H-bonding interactions. Since the 1st substrate undergoes hydroxylation via a Compound I oxidation, it's reasonable to assume that the lyase chemistry in the 19oxoAD-bound (3rd substrate) CYP19A1 is also catalyzed by a Compound I intermediate, as the rR spectra of both AD and 19oxoAD are almost identical.

V. Conclusions

By understanding the mechanisms involved in the reactions catalyzed by cytochrome P450 aromatase (CYP19A1), one can learn how CYP19A1 functions (e.g., through the rR studies). Studies suggest that locally produced estrogens may influence growth and development of breast cancer tumors. In breast cancer, estrogen is over-produced thus promoting local growth of tumors. Armed with this understanding, a suppression treatment to disable local estrogen production in breast adipose tissue by CYP19A1 could be developed to control estrogen levels. The treatment could result in reduced tumor growth or diminished risk of development of breast cancer.

References

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