Computational Estimation of the Effects of the S210R Mutation on the Structure and Enzymatic-Activity of CYP2B6 Using Molecular Dynamics Simulation

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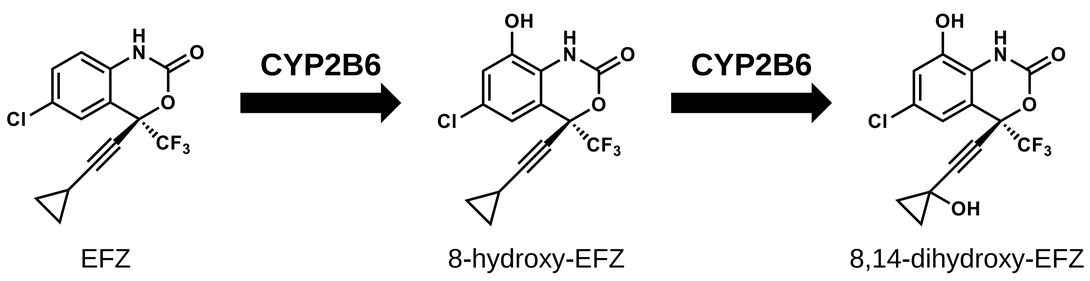
Introduction

Cytochrome P450 2B6 (CYP2B6)

- CYP2B6 is a type of phase I drug-metabolizing enzyme with a heme as an active center.
- CYP2B6 is reported to catalyze metabolic reactions for approximately 7% of clinical drugs, *e.g.* efavirenz (EFZ) anti-human immunodeficiency virus drug.

CYP2B6-catalyzed metabolic reaction of EFZ



Enzymatic activity of CYP2B6 allelic variants

- **Genetic polymorphisms of *CYP2B6* can affect their enzymatic functions,** directly contributing to individual difference in drug efficacy and toxicity.
- We recently analyzed the enzymatic activities of rare allelic variants detected in Japanese [1], and several variants exhibited remarkable reductions in enzymatic activities.
  - ✓ **The enzymatic activity was not detected in the S210R mutants.**

Aims

- As previously mentioned, our recent study has shown that the enzymatic activities of the S210R mutant is significantly reduced compared to that of the wild-type CYP2B6.
- However, the differences in the structural features between the wild-type CYP2B6 and S210R variants were unclear.

- **Molecular dynamics (MD) simulations were performed to elucidate the cause of reduction in enzymatic activity of S210R variants.**

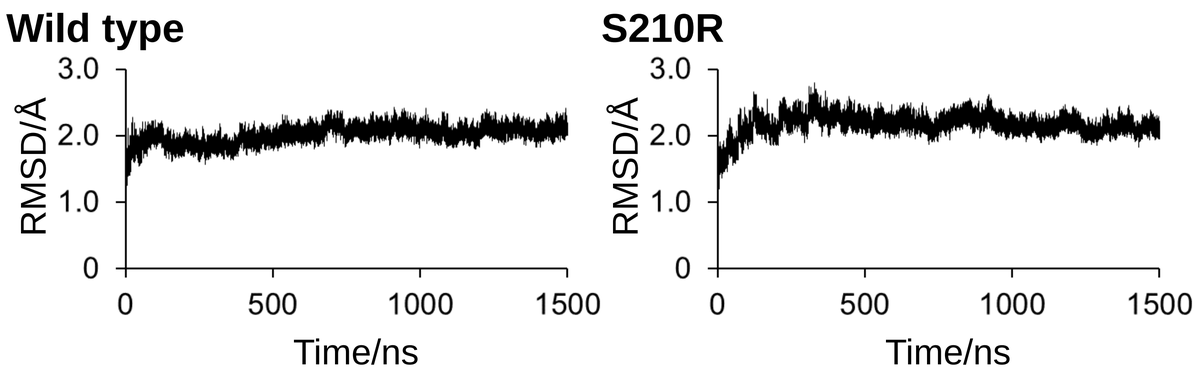
Molecular Dynamics Simulations

- Initial structures of CYP2C19 for MD simulations were constructed using crystal structures (PDB ID: 3IBD).
- Force-field parameters
  - Amino acid residues: AMBER ff14SB force field
  - Heme and ligated Cys: Our determined parameters [2]
  - Solvent water molecules: TIP3P
  - EFZ: General Amber Force Field 2
- Generation of NPT ensemble
  - Pressure: 1 bar (Monte Carlo barostat)
  - Temperature: 300 K (Langevin thermostat)
- MD production run; 1.5 μs (time step: 2 fs)
  - Snapshots were sampling every 2 ps.
  - The final 100-ns trajectories were analyzed.

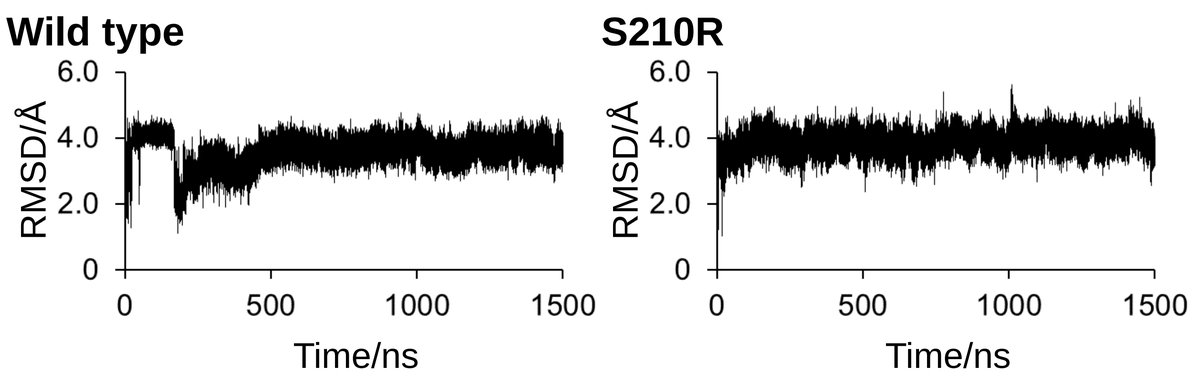
Results and Discussion

Simulation stability

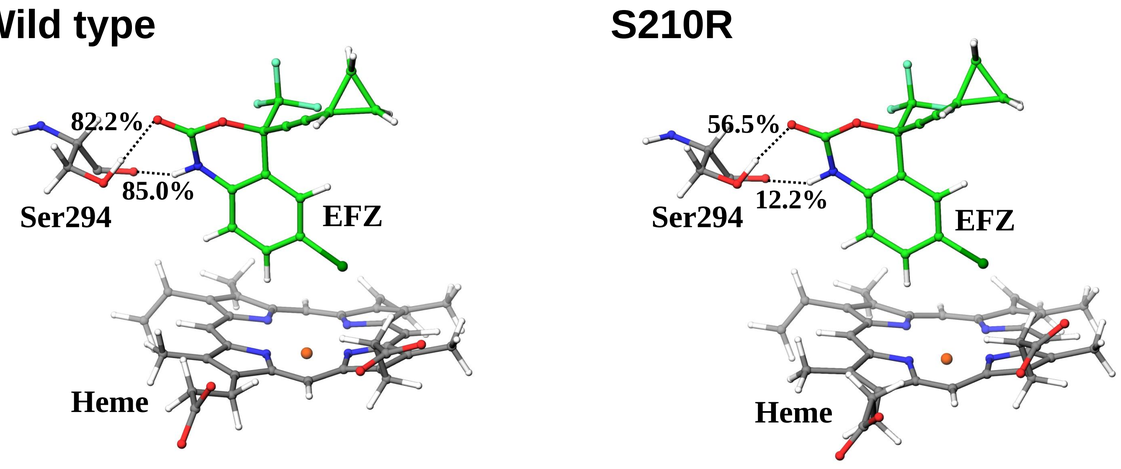
RMSDs for C_α atoms of CYP2B6



RMSDs for heavy atoms of EFZ

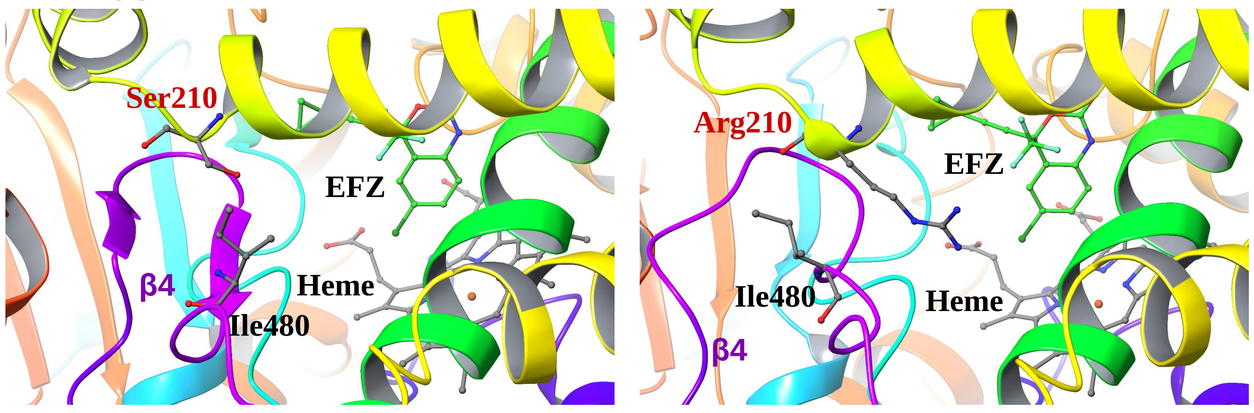


Differences in the hydrogen bonds with



- S210R mutation did not affect the relative position of EFZ, but it remarkably reduced the occurrence frequencies of hydrogen bonds between Ser294 and EFZ.

Structural differences near the mutation site



- In the β4 region in wild type, β-sheet structure formed with a high frequency of 95.1%. In contrast, in the β4 region in S210R mutant, β-sheet structure did not formed at all.
- Side chain of Arg residue is more bulky than that of Ser, and it is considered to be because side-chain orientation of Ile480 significantly differs between wild type and S210R mutant due to steric hindrance.

Conclusions

- S210R mutation disrupted β-sheet formation in β4 region that forms the active site and hydrogen-bond formation between EFZ and Ser294. This can explain why the S210R mutant exhibits significantly lower enzyme activity.

References

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2. A. Oda, N. Yamaotsu, S. Hirono, *J. Comput. Chem.*, 26, 818–826 (2005)