BMC Research Notes

DATA NOTE

Open Access

Molecular dynamics simulations of the interaction of wild type and mutant human CYP2J2 with polyunsaturated fatty acids

K. K. Abelak[1], D. Bishop-Bailey[1] and I. Nobeli[2*]

Abstract

Objectives: The data presented here is part of a study that was aimed at characterizing the molecular mechanisms of polyunsaturated fatty acid metabolism by CYP2J2, the main cytochrome P450 enzyme active in the human cardiovasculature. This part comprises the molecular dynamics simulations of the binding of three eicosanoid substrates to wild type and mutant forms of the enzyme. These simulations were carried out with the aim of dissecting the importance of individual residues in the active site and the roles they might play in dictating the binding and catalytic specificity exhibited by CYP2J2.

Data description: The data comprise: (a) a new homology model of CYP2J2, (b) a number of predicted low-energy complexes of CYP2J2 with arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid, produced with molecular docking and (c) a series of molecular dynamics simulations of the wild type and four mutants interacting with arachidonic acid as well as simulations of the wild type interacting with the two other eicosanoid ligands. The simulations may be helpful in identifying the determinants of substrate specificity of this enzyme and in unraveling the role of individual mutations on its function. They may also help guide the generation of mutants with altered substrate preferences.

Keywords: CYP2J2, Cytochrome P450, Polyunsaturated fatty acids, Molecular dynamics, Homology model, Docking, Arachidonic acid, Docosahexaenoic acid, Eicosapentaenoic acid, Polyunsaturated fatty acids

Objective

The polyunsaturated fatty acids (PUFAs) arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are oxidised by cytochrome P450 (CYP) enzymes to produce metabolically active products that play significant roles in inflammation pathways [1, 2]. Due to the absence of a crystal structure of the main such enzyme in the human cardiovasculature (CYP2J2), the precise mechanism by which it metabolises PUFAs into specific stereo- and regio-epoxyisomers is not fully understood. Consequently, the effect of mutations in the protein sequence arising from non-synonymous single nucleotide polymorphisms found in the population cannot be predicted, hindering our ability to link genomic information to dysregulation of inflammatory responses and thus successful prognoses of cardiovascular health. In this project, we aimed to understand binding of PUFAs in the active site of CYP2J2 using computational methods and leverage this information to investigate the residues essential for ligand positioning and metabolism. In previous work, our groups investigated the interaction of AA with human CYP2J2 and revealed Arg117 as a key player in the recognition of this substrate [3], although these simulations were relatively short (50 ns). Simulations from other studies have come to diverse conclusions about the role of individual residues in the active site [4–6]. Here, we tried to investigate further using much more extensive simulations of both wild type and mutant forms of the enzyme. These new simulations confirmed the

*Correspondence: i.nobeli@bbk.ac.uk
[2] Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, Malet Street, London WC1E 7HX, UK
Full list of author information is available at the end of the article

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1: Homology modelling, molecular docking and molecular dynamics simulations of wild type and mutant human CYP2J2 with three polyunsaturated fatty acids	Abelak_etal_Methods.pdf C2J2_min3_mod_noH.pdb create_sim4_repeats.sh docking_wildtype_C2J2.zip	PDF document PDB file (.pdb) Shell script (.sh) Zipped file of 9 pdb files (.zip)	Zenodo https://doi.org/10.5281/zenodo.3465884
Data set 2: Molecular dynamics simulations of the interaction of wild type human CYP2J2 with arachidonic acid	MD_wt_CYP2J2_AA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 6$; Y = repeat number, $1 \leq Y \leq 4$)	Zipped files (.zip)	Zenodo Poses 1 and 2 https://doi.org/10.5281/zenodo.3465590 Poses 3 and 4 https://doi.org/10.5281/zenodo.3466692 Poses 5 and 6 https://doi.org/10.5281/zenodo.3473886
Data set 3: Molecular dynamics simulations of the interaction of wild type human CYP2J2 with DHA (POSES 1–4)	MD_wt_CYP2J2_DHA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 4$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip)	Zenodo https://doi.org/10.5281/zenodo.3473909
Data set 4: Molecular dynamics simulations of the interaction of wild type human CYP2J2 with EPA (POSES 1–4)	MD_wt_CYP2J2_EPA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 4$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip)	Zenodo https://doi.org/10.5281/zenodo.3473927
Data set 5: Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R111A) with arachidonic acid	MD_mutR111A_CYP2J2_AA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 6$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip)	Zenodo Poses 1–3 https://doi.org/10.5281/zenodo.3483594 Poses 4-6: https://doi.org/10.5281/zenodo.3483966
Data set 6: Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R117A) with arachidonic acid	MD_mutR117A_CYP2J2_AA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 6$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip),	Zenodo Poses 1–4 https://doi.org/10.5281/zenodo.3482943 Poses 5-6: https://doi.org/10.5281/zenodo.3483493
Data set 7: Molecular dynamics simulations of the interaction of double mutant human CYP2J2 (R111A, R117A) with arachidonic acid	MD_mutR111A_R117A_CYP2J2_AA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 6$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip)	Zenodo Poses 1–3 https://doi.org/10.5281/zenodo.3484029 Poses 4-6: https://doi.org/10.5281/zenodo.3484124
Data set 8: Molecular dynamics simulations of the interaction of quadruple mutant human CYP2J2 (R111A, R117A, R382A, R446A) with arachidonic acid	MD_quadmut_CYP2J2_AA_StateX_repeatY.zip (X = pose number, $1 \leq X \leq 6$; Y = repeat number, $1 \leq Y \leq 3$)	Zipped files (.zip)	Zenodo Poses 1–3 https://doi.org/10.5281/zenodo.3484437 Poses 4-6: https://doi.org/10.5281/zenodo.3484448

importance of Arg117 but in addition suggested Arg111 as a residue necessary for epoxidation and pointed to the role of two more arginine residues in the active site that allow some redundancy in substrate tethering and contribute to the flexibility of the catalytic capabilities of the system. Expression trials in HEK293T cells to produce CYP2J2 and its mutants were unsuccessful so the computationally derived hypotheses could not be validated in the lifetime of this project.

Data description

The data presented here comprise the results of homology modeling of the human wild type CYP2J2 and generation of models for a series of mutants [7]; molecular

docking of three eicosanoid ligands (AA, DHA and EPA) to wild type CYP2J2 [7]; finally, a series of molecular dynamics simulations of the wild type and mutant enzyme with the three ligands [8–20]. Below is a brief description of each part of the data. More details are available in the Methods document on the top Zenodo repository [7].

Homology model of CYP2J2

The homology model [7] is based on the UniProt [21] protein sequence with UID P51589. A model of the sequence with the N-terminal transmembrane domain (residues 1–43) trimmed was built using MODELLER version 9.14 [22], using as templates the PDB structures: 1SUO [23], 2P85 [24], 3EBS [25] and 1Z10 [26]. A haem molecule was incorporated into the model building using the HETATM records from PDB structure 1SUO.

Structure models of mutants of CYP2J2 were produced using the homology model of the wild type enzyme as the starting point and changing residues 111, 117, 382 and 446 from arginine to alanine. The expectation was that mutating these residues to a non-charged amino acid would have a noticeable impact on the binding of fatty acid substrates.

Docking of PUFAs to CYP2J2

The fatty acids arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were investigated in this study. The structure of AA was obtained from the Zinc Dock database version 12 [27]. Structures for DHA and EPA were derived using the Automated Topology Builder version 2.2 [28]. Docking of all ligands to CYP2J2 models was carried out using Autodock VINA version 1.1.2 [29]. Five independent docking runs were carried out for each ligand.

Molecular dynamics simulations

MD simulations were carried out using AMBER14 [30] as described in the Methods document (data set 1 [7]). The simulations included the standard minimization, heating, equilibration and production phases. Six docked wild type CYP2J2-AA complexes were simulated in four independent runs, each lasting 1 μs [8–10]. Simulations of the mutant enzymes started from the same six docked poses of AA but each pose was simulated in three repeats, each lasting 500 ns. Two single mutants were investigated (Arg111Ala [13, 14], Arg117Ala [15, 16]) followed by a double mutant (Arg111Ala and Arg117Ala [17, 18]) and finally a quadruple mutant (Arg111Ala, Arg117Ala, Arg382Ala and Arg446Ala [19, 20]). Simulations of DHA [12] and EPA [11] were carried out starting from four docked poses, each simulation repeated three times and lasting 300 ns.

The simulations highlighted two residues in the active site (Arg111 and Arg117) that appear to play important roles in anchoring the carboxylate group of the substrate. Simulations also suggested that mutating any one of these two residues, results in enhancing the role of the other one as a hydrogen-bond donor, and that if both are mutated, two more arginine residues (Arg382 and Arg446) can partially make up for the missing charged groups in the active site.

Limitations

As with all computational studies, the data here should be interpreted with care. The starting CYP2J2 structure used in these simulations is a homology model, i.e. a structure built in silico using information from related proteins whose structures have been deposited in the PDB. Although we have built the model using an alignment of multiple, carefully selected structures, it is possible that inaccuracies in the initial structure have affected the final simulations. Our molecular dynamics simulations (ranging from 900 ns to 4 μs) are, to the best of our knowledge, the longest carried out on human CYP2J2 and, in addition, multiple repeats using the same starting docked pose of the ligand were used to assess the robustness of observations to differences introduced by the random nature of the algorithm. Despite the length of these simulations and the evidence pointing to reasonable convergence in energy terms, simulations appeared to sample different conformations of the system, even when the same starting pose was used (in different repeats). These MD runs thus point towards a very flexible system that is better described as an ensemble of possible states, whose probability is affected by the substrate nature or mutations in the active site. Longer simulation times would have been useful in revealing whether convergence of the system to a few distinct conformations is possible, given enough simulation time. The haem molecule plays an important role in these simulations. Haem was modeled here in its penta-coordinated high-spin ferric form but the alternative highly reactive iron-oxygen species complex should be considered too. Finally, modeling a restricted part of this system around the haem molecule using a quantum mechanical (QM) model would be advisable. A joint QM/MM system could be setup that would offer a more realistic representation of how the intermediate complex between haem and substrate is formed.

Abbreviations

AA: arachidonic acid; CYP: cytochrome P450; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MD: molecular dynamics; MM: molecular mechanics; PDB: Protein Data Bank; PUFA: polyunsaturated fatty acid; QM: quantum mechanical.

Acknowledgements
The authors would like to acknowledge that the work presented here made use of Emerald, a GPU-accelerated High Performance Computer, made available by the Science & Engineering South Consortium operated in partnership with the STFC Rutherford-Appleton Laboratory.

Authors' contributions
DBB and IN conceived the study. IN and KKA designed the simulations and KKA carried out all computational work. IN and KKA wrote the manuscript. All authors read and approved the final manuscript.

Funding
KKA was supported by a PhD studentship from the Bloomsbury Colleges. The funding body played no role in the design of the study, collection, analysis and interpretation of data or in the writing of the manuscript.

Availability of data materials
The data described in this Data Note can be freely and openly accessed on Zenodo.
Please see Table 1 and reference list for details. The list of doi links is given below:
Data set 1: https://doi.org/10.5281/zenodo.3465884
Data set 2:https://doi.org/10.5281/zenodo.3465590; https://doi.org/10.5281/zenodo.3466692; https://doi.org/10.5281/zenodo.3473886
Data set 3: https://doi.org/10.5281/zenodo.3473909
Data set 4: https://doi.org/10.5281/zenodo.3473927
Data set 5: https://doi.org/10.5281/zenodo.3483594; https://doi.org/10.5281/zenodo.3483966
Data set 6: https://doi.org/10.5281/zenodo.3482943; https://doi.org/10.5281/zenodo.3483493
Data set 7: https://doi.org/10.5281/zenodo.3484029; https://doi.org/10.5281/zenodo.3484124
Data set 8: https://doi.org/10.5281/zenodo.3484437; https://doi.org/10.5281/zenodo.3484448.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Comparative Biomedical Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, UK. [2] Department of Biological Sciences, Institute of Structural and Molecular Biology, Birkbeck, Malet Street, London WC1E 7HX, UK.

Received: 17 October 2019 Accepted: 11 November 2019
Published online: 21 November 2019

References

1. Harizi H, Corcuff J-B, Gualde N. Arachidonic-acid-derived eicosanoids: roles in biology and immunopathology. Trends Mol Med. 2008;14:461–9.
2. Lone AM, Taskén K. Proinflammatory and immunoregulatory roles of eicosanoids in T cells. Front Immunol. 2013. https://doi.org/10.3389/fimmu.2013.00130.
3. Proietti G, Abelak KK, Bishop-Bailey D, Macchiarulo A, Nobeli I. Computational modelling of the binding of arachidonic acid to the human monooxygenase CYP2J2. J Mol Model. 2016;22:279.
4. Lafite P, André F, Zeldin DC, Dansette PM, Mansuy D. Unusual regioselectivity and active site topology of human cytochrome P450 2J2. Biochemistry. 2007;46:10237–47.
5. Arnold WR, Baylon JL, Tajkhorshid E, Das A. Asymmetric binding and metabolism of polyunsaturated fatty acids (PUFAs) by CYP2J2 epoxygenase. Biochemistry. 2016;55:6969–80.
6. Lafite P, André F, Graves JP, Zeldin DC, Dansette PM, Mansuy D. Role of Arginine 117 in substrate recognition by human cytochrome P450 2J2. Int J Mol Sci. 2018;19:2066.
7. Abelak K, Bishop-Bailey D, Nobeli I. Homology modelling, molecular docking and molecular dynamics simulations of wild type and mutant human CYP2J2 with three polyunsaturated fatty acids. Zenodo. 2019. https://doi.org/10.5281/zenodo.3465884.
8. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of wild type human CYP2J2 with arachidonic acid (POSES 1 and 2). Zenodo. 2019. https://doi.org/10.5281/zenodo.3465590.
9. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of wild type human CYP2J2 with arachidonic acid (POSES 3 and 4). Zenodo. 2019. https://doi.org/10.5281/zenodo.3466692.
10. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of wild type human CYP2J2 with arachidonic acid (POSES 5 and 6). Zenodo. 2019. https://doi.org/10.5281/zenodo.3473886.
11. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of wild type human CYP2J2 with EPA (POSES 1–4). Zenodo. 2019. https://doi.org/10.5281/zenodo.3473927.
12. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of wild type human CYP2J2 with DHA (POSES 1–4). Zenodo. 2019. https://doi.org/10.5281/zenodo.3473909.
13. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R111A) with arachidonic acid (POSES 1–3). Zenodo. 2019. https://doi.org/10.5281/zenodo.3483594.
14. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R111A) with arachidonic acid (POSES 4–6). Zenodo. 2019. https://doi.org/10.5281/zenodo.3483966.
15. Abelak K, Bishop-Bailey, Nobeli. Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R117A) with arachidonic acid (POSES 1–4). Zenodo. 2019. https://doi.org/10.5281/zenodo.3482943.
16. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of mutant human CYP2J2 (R117A) with arachidonic acid (POSES 5–6). Zenodo. 2019. https://doi.org/10.5281/zenodo.3483493.
17. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of the double mutant human CYP2J2 (R117A and R111A) with arachidonic acid (POSES 1–3). Zenodo. 2019. https://doi.org/10.5281/zenodo.3484029.
18. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of the double mutant human CYP2J2 (R117A and R111A) with arachidonic acid (POSES 4–6). Zenodo. 2019. https://doi.org/10.5281/zenodo.3484124.
19. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of the quadruple mutant human CYP2J2 (R111A + R117A + R382A + R446A) with arachidonic acid (POSES 1–3). Zenodo. 2019. https://doi.org/10.5281/zenodo.3484437.
20. Abelak K, Bishop-Bailey D, Nobeli I. Molecular dynamics simulations of the interaction of the quadruple mutant human CYP2J2 (R111A + R117A + R382A + R446A) with arachidonic acid (POSES 4–6). Zenodo. 2019. https://doi.org/10.5281/zenodo.3484448.
21. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019;47:D506–15.
22. Webb B, Sali A. Comparative protein structure modeling using MODELLER. Curr Protoc Bioinform. 2016;54:5.6.1–6.37.
23. Scott EE, White MA, He YA, Johnson EF, Stout CD, Halpert JR. Structure of mammalian cytochrome P450 2B4 complexed with 4-(4-chlorophenyl) imidazole at 1.9-A resolution: insight into the range of P450 conformations and the coordination of redox partner binding. J Biol Chem. 2004;279:27294–301.
24. Smith BD, Sanders JL, Porubsky PR, Lushington GH, Stout CD, Scott EE. Structure of the human lung cytochrome P450 2A13. J Biol Chem. 2007;282:17306–13.
25. DeVore NM, Smith BD, Urban MJ, Scott EE. Key residues controlling phenacetin metabolism by human cytochrome P450 2A enzymes. Drug Metab Dispos. 2008;36:2582–90.
26. Yano JK, Hsu M-H, Griffin KJ, Stout CD, Johnson EF. Structures of human microsomal cytochrome P450 2A6 complexed with coumarin and methoxsalen. Nat Struct Mol Biol. 2005;12:822–3.
27. Sterling T, Irwin JJ. ZINC 15–ligand discovery for everyone. J Chem Inf Model. 2015;55:2324–37.

28. Malde AK, Zuo L, Breeze M, Stroet M, Poger D, Nair PC, et al. An automated force field topology builder (ATB) and repository: version 1.0. J Chem Theory Comput. 2011;7:4026–37.
29. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 2010;31:455–61.
30. Case DA, Babin V, Berryman JT, Betz RM, Cai Q, Cerutti DS, et al. AMBER14. San Francisco: University of California; 2014.

Publisher's Note