BIOTRANS
LA ROCHELLE 2023

N°136 / OC
TOPIC(s) : Enzyme discovery and engineering / Artifical intelligence / computational methods

Rational design of cyclohexanone dehydrogenase for enhanced α, β-desaturation and substrate specificity

AUTHORS

Gary BLACK / NORTHUMBRIA UNIVERSITY, FACULTY OF HEALTH AND LIFE SCIENCES, NEWCASTLE UPON TYNE
Kshatresh DUBEYE / SHIV NADAR UNIVERSITY, DEPARTMENT OF CHEMISTRY, UTTAR PRADESH
Warispreet SINGH / NORTHUMBRIA UNIVERSITY, FACULTY OF HEALTH AND LIFE SCIENCES, NEWCASTLE UPON TYNE
Nicola BROWN / NORTHUMBRIA UNIVERSITY, FACULTY OF HEALTH AND LIFE SCIENCES, NEWCASTSTLE UPON TYNE
Hannah MCCUE / UNIVERSITY OF LIVERPOOL, INSTITUTE OF SYSTEMS, LIVERPOOL
Sophie MARRIOTT / UNIVERSITY OF LINCOLN, DEPARTMENT OF LIFE SCIENCES, LINCOLN
Richard WILSON / UNIVERSITY OF YORK, DEPARTMENT OF COMPUTER SCIENCE, YORK
Justin PERRY / NORTHUMBRIA UNIVERSITY, FACULTY OF HELTH AND LIFE SCIENCES, NEWCASTLE UPON TYNE
Andrew CARNELL / UNIVERSITY OF LIVERPOOL, DEPARTMENT OF CHEMISTRY, LIVERPOOL
Johan TURKENBURG / UNIVERSITY OF YORK, DEPARTMENT OF CHEMISTRY, YORK
Stephen PRIOR / UNIVERSITY OF LINCOLN, SCHOOL OF CHEMISTRY, LINCOLN
Edward TAYLOR / UNIVERSITY OF LINCOLN, DEPARTMENT OF LIFE SCIENCES, LINCOLN

PURPOSE OF THE ABSTRACT

The selective α, β-desaturation of cyclic carbonyl compounds, which are found in the core of many steroid and bioactive molecules, using green chemistry is highly desirable. To achieve this task, we have for the first time described and solved the de novo structure of a member of a new enzyme class, cyclohexanone dehydrogenases (Figure 1). The breadth of substrate specificity was investigated by assaying the cyclohexanone dehydrogenase against several cyclic ketones, lactones and lactams. To investigate substrate binding, a catalytic mutant was generated and used to obtain a crystallographic complex with the natural substrate, cyclohexanone. This revealed substrate-active site interactions, as well as the proximity of the cofactor, flavin adenine dinucleotide, and enabled us to propose a mechanistic function to key amino acids. We then used molecular dynamic simulations to guide design to add functionality to the cyclohexanone dehydrogenase enzyme. The resulting mutant had overall improved enzyme activity and substrate scope, i.e., accepting the bulkier carbonyl compound, lactone dihydrocoumarin. Structural analysis of the mutant revealed a broader, more open active site, which helped explain the modified substrate specificity. This work paves the way for future bespoke regioselective α, β-desaturation in the synthesis of important bioactive molecules via rational enzyme engineering.

FIGURES

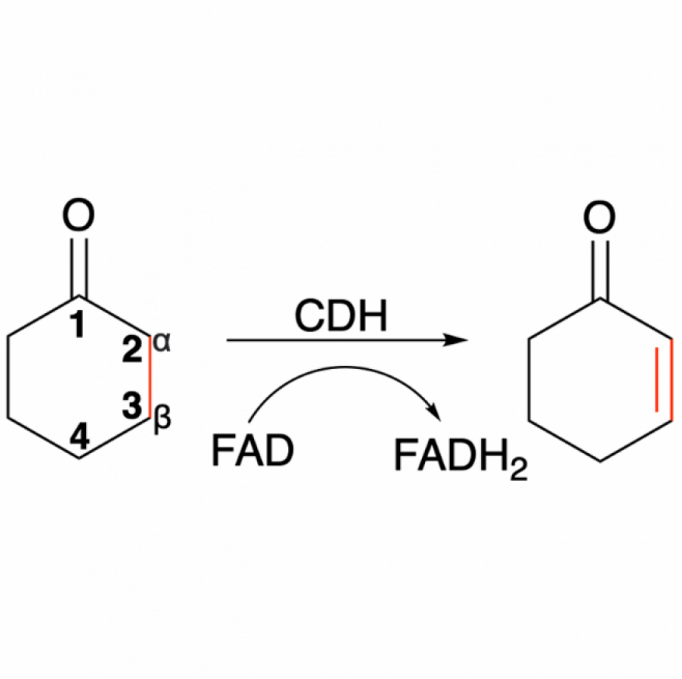

FIGURE 1

Figure 1

FIGURE 2

KEYWORDS

cyclic ketones | molecular dynamics | rational engineering | X-ray crystallography

BIBLIOGRAPHY