In silico prediction of metabolism by human carboxylesterases (hCES1 and hCES2) combining docking analyses and MD simulations

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Background

- *One of the most recent strategy in medicinal chemistry involves the PHARMACOKINETIC PROFILING of new molecules as soon as possible in the development pipeline with the clear aim to carry forward in the clinical trials only the most promising drug-like compounds.[1]*
- *Among the pharmacokinetic failures, UNSUITABLE METABOLIC FATES represent the most frequent problem that leads to high levels of attrition during development of new drugs.*
- *Hence, much effort is now devoted to in silico models to predict metabolic stability and metabolites. Such models are well known for cytochrome P450 and various conjugating enzymes, and they enjoy a relative success.*
- *In contrast, little has been done to predict the hydrolytic activity of human esterases, although they play a key role in the hydrolysis of xenobiotics and in the activation of most prodrugs. Among the esterase enzymes, the CARBOXYLESTERASES[2] play a pivotal role in the hydrolysis of a variety of drugs and prodrugs containing ester, amide or carbamate functions to the respective free acids.*

Aim of the work

The present study was then undertaken to develop PREDICTIVE MODELS of the hydrolytic activity catalysed by human carboxylesterases focusing on the hCES1 and hCES2 isozymes.

The first step of the study involved the choice of the most suitable hCES1 resolved structure as well as the generation of a reliable hCES2 model by homology techniques.

In a second step, DOCKING ANALYSES of several known hCES substrates allowed to develop predictive models for both isozymes incorporating new scoring functions designed to take hydrophobic interactions into account.

Finally, MD SIMULATIONS of the computed complexes revealed the behaviour and trajectory of substrates and products, demonstrating in particular the influence of their ionization state.

Choice of an optimal CES1 structure

Choice of some relevant structures among the 13 resolved hCES1 in PDB

↓

Choice of the best one based on preliminary docking results

↓

1YAJ: CES1 + BENZOATE, a small enzimatic product. Hence, the enzyme should be in its **apo state**

Modelling of CES2 structure

UniProt → Primary structure

↓

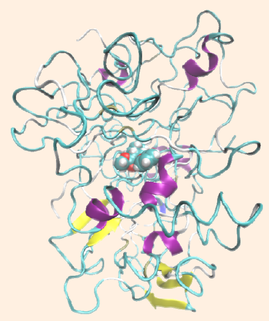
SwissModel PDB: 2H7C as the template → Folding prediction

↓

Rough 3D structure

↓

VEGA ZZ + NAMD → Refined 3D structure



Ligands

↓

Conformational analyses (Monte Carlo method as implemented in VEGA ZZ)

↓

Lowest energy structures

hCES1 hCES2

↓

Molecular docking (AutoDock 4.0, considering a 10 Å radius sphere around Ser 221/228)

↓

hCES-ligand complexes

Molecular Docking and the MLP_{InS} Score

The cross-relation between MLP_{InS} and log P is very low

Docking scores

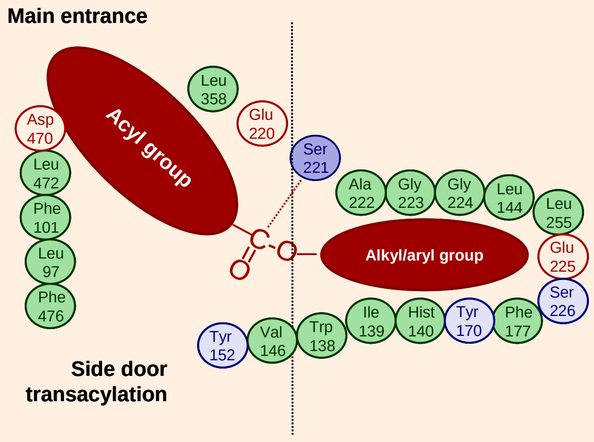
$$MLP_{InS} = \sum_p \sum_m -\frac{(f_a \cdot f_b)}{fct(r_{ab})}$$

f_a *and* f = Broto's atomic increments.
$fct(r_{ab})$ = Distance function.
Best results with $fct(r_{ab}) = (r_{ab})^3$

Overall, MLP_{InS} encodes:
- Hydrophobic contacts (f_a *and* $f_b > 0$)
- Polar interactions (fa *and* $fb < 0$)
- Repulsive forces (otherwise)

The catalytic site

hCES1 [5]



SIMILAR POLARITY PROFILE

The catalytic sites of both hCES1 and hCES2 have some common features:

1) many hydrophobic residues which determine the preference for apolar substrates;

2) negative residues which should promote the egress of negatively charged products;

3) the two subcavities harboring alkyl/aryl and acyl moieties can be easily recognized;

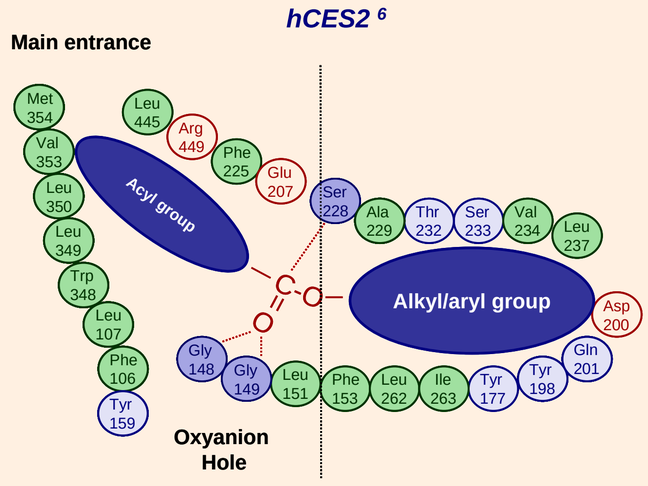
4) the subcavities for alkyl/aryl groups are less flexible and slightly more polar then the other subcavity, thus explaining the preference for substrates with alkyl/aryl moieties relatively more polar then the acyls.

OPPOSITE OPTIMAL SIZE

The hCES1 alkyl/aryl subcavity is smaller then the acyl one. Thus, hCES1 prefers substrates with acyl moieties bulkier then the alkyl/aryl ones.

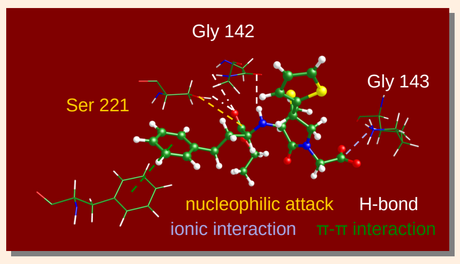
The hCES2 alkyl/aryl subcavity is larger then the acyl one. The enzyme prefers substrates with alkyl/aryl moieties bulkier then the acyl ones.

hCES2 [6]



Legend for residues: **Green = apolar** **Red = negative** **Blue = H-bonding**

Docking simulations



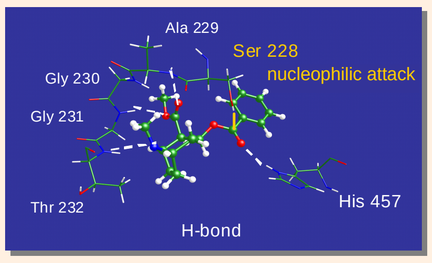
hCES1-temocapril

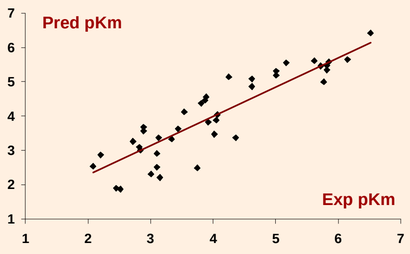
The weak interactions stabilizing the optimized complex are in line with the experimental data indicating that temocapril is a poor substrate of hCES1 (K_m = 786 μM). However, the distance between the oxygen atom of catalytic Ser221 and the ester carbon atom is conducive to the catalytic mechanism.

- *Docking simulations were performed considering a set of 40 known substrates;*
- *The basic substrates were docked in both their neutral and ionized forms;*
- *The two best relationships include docking scores and ligand-based properties.*
- *Substrates with unprotonated basic groups correlate markedly better;*
- *Both equations include the same parameters even though with different coefficients.*

hCES2-cocaine

hCES2 is fairly active on cocaine as confirmed by Km = 390 μM. The complex is stabilized by a network of H-bonding and the catalytic Ser228 contacts the carbonyl carbon atom in a pose suitable for the catalytic mechanism.





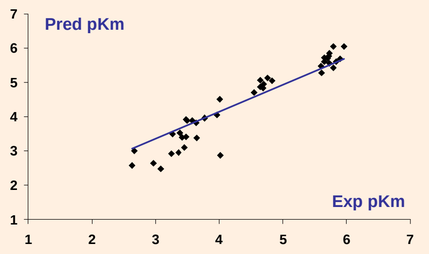
$Dist_{Ser}$
It defines the distance between the catalytic serine and the labile group and encodes the substrate capacity to assume productive poses.

MLP_{InS}
It describes the apolar interactions elicited by the substrate and emphasize their relevance in the recognition of these enzymes.

Volume
The contrasting sign of this parameter in the two equations suggests that the hCES2 cavity is generally narrower.

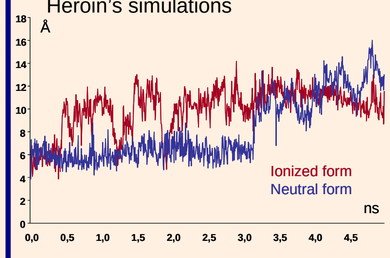
$pKm = -0{,}25\ dist_{Ser221} - 4{,}17\ MLP_{InS} + 2{,}12 \cdot 10^{-3}\ V + 3{,}43$
n = 40 $r^2 = 0{,}91$ S = 0,36 F = 122,09

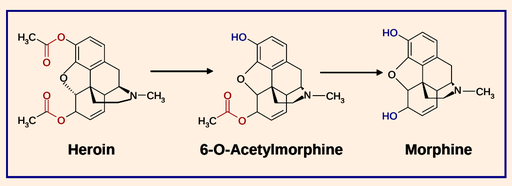
$pKm = -0{,}168\ dist_{Ser228} - 1{,}26\ MLP_{InS} - 2{,}54 \cdot 10^{-3}\ V + 4{,}35$
n = 40 $r^2 = 0{,}87$ S = 0,38 F = 44,96

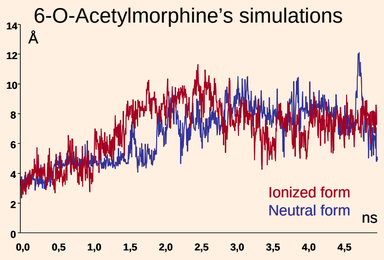


MDsimulation

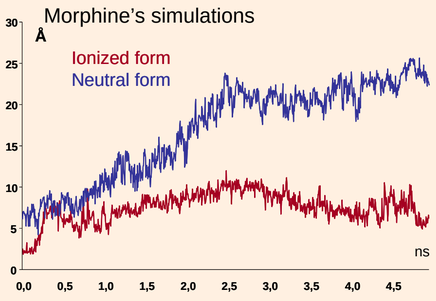
Basic substrates and hCES1 activity: the case of heroin hydrolysis [4]







The two substrates stably remain in the catalytic site irrespective of their ionization state.



The neutral form leaves the site and allows the catalytic turn-over.
The ionized form remains into the site behaving as a competitive inhibitor.

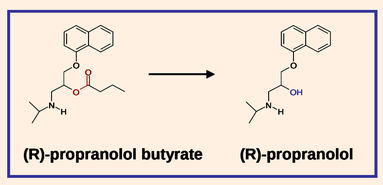
To explore the influence of ionization on CES and CES2 activity, the stability of some complexes was analyzed by MD runs of **5-ns** for CES1 and **10-ns** for CES2. All plots report the distance between Ser221/Ser228 and the ester groups.

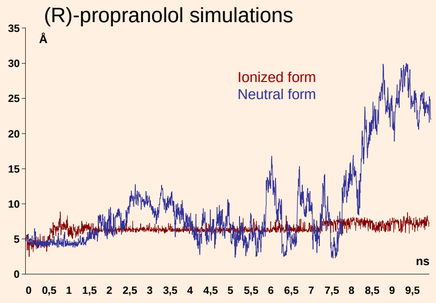
The reasonable stability of the simulated complexes affords an encouraging validation of their reliability.

MD simulation confirm that enzymes stabilize better interactions with unprotonated basic ligands.

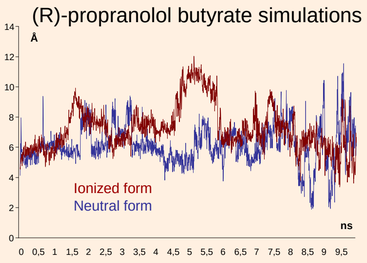
MD simulation are able to successfully discriminate between substrates and products even when the structural differences are modest.

Basic substrates and hCES2 activity: the case of R-propanolol butyrate hydrolysis





The neutral product shows a progressive exit from the enzymatic cavity.
The protonated form remained stable within the catalytic cavity.



The substrates remain in the catalytic site irrespective of their ionization state.

The Product exit in hCES2 is slower than in hCES1: the different velocity can impact on catalytic efficiency as confirmed by the kinetic parameters for hydrolysis which are on average lower in hCES2 then in hCES1.

Conclusion

The congruity of the obteined complexes and the correlations between docking scores and the enzymatic data afford an ancouraging validation for the described docking results, which can be used to predict the hydrolytic metabolism of new molecules. In detail, the simulation reveal that:

1) MLP_{InS} scores proved successful to accoount for lipophilic interactions in binding;
2) an optimal hCES1 substrate should possess the alkyl/aryl group smaller then the acyl one while. On the contrary, an optimal hCES2 substrate should possess the acyl group smaller the alkyl/aryl one;
3) both isozymes prefer neutral or anionic substrates, while the cationic ligand can behave as inhibitors;
4) the products egress can be simulated by simple all-atoms MD runs.

References

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