In silico prediction of metabolism by human carboxylesterase-1 (hCES1) combining docking analyses and MD simulations.

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Background

- *The more recent strategies in medicinal chemistry involve the pharmacokinetic profiling of new molecules as soon as possible in the development pipeline with the clear aim to develop only drug-like compounds.[1]*
- *Among the pharmacokinetic failures, unsuitable metabolic fates represent the most frequent and impeding problems during clinical trials.*
- *Hence, much effort is now devoted to in silico models to predict metabolic stability and metabolites. Such models are well known for cytochromes P450 and various conjugating enzymes, and they enjoy a relative success.*
- *In contrast, little has been done to predict the hydrolyzing activity of human esterases, although these play a key role in the hydrolytic metabolism 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 or prodrugs containing ester, amide or carbamate functions to the respective free acids. CES isozymes can be classified into five groups (CES1-CES5) and the majority of CESs, which have been identified, belong to the CES1 or CES2 family.*

❖ *They are members of the serine hydrolase family (α,β hydrolase fold). The catalytic triad is composed by a serine (Ser221 in hCES1) which contacts the substrates, plus two residues (His468 and Glu354 in hCES1) which increase the polarity of Ser221.*

❖ *CES1 and CES2 differ from their specificity. CES1 hydrolyzes substrates with small alcohol groups and large acyl groups; CES2 recognizes substrates with large alcohols and small acyls. The CES2 specificity is much more constrained.*

Aim of the work

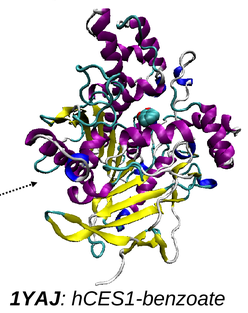
Aim of this work was to develop a computational approach able to predict and rationalize the hydrolysis of new chemical entities by the human carboxylesterase hCES1.

Choice of an optimal CES1 structure for docking

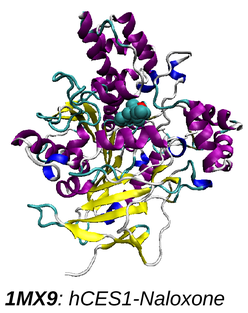
- *Up to now, 13 experimental structures for hCES1 have been deposited in PDB, but the apo-enzyme has not been yet resolved.*
- *The structures of the enzyme in complex with non competitive and covalently bound inhibitors were discarded.*
- *The similarity between the nine remaining hCES1 structures was analyzed by the pair-wise rmsd values.*

The structures are very similar and the main differences concern the catalytic cavity. The greatest rmsd value is afforded by CES1 in complex with benzoate (**1YAJ**), an enzymatic product which derives from degradation of the *benzil* inhibitor, *versus* CES1 in complex with naloxone methiodide (**1MX9**), a stable analogue of heroin.

PDB	Bound Ligand	1YA8	1YAH	1YAJ	1MX5	1MX9	2DQY	2DQZ	2DR0
1YA8	Mevastatin								
1YAH	Ethylacetate	0.01							
1YAJ	Benzoate	0.46	0.46						
1MX5	Homatropine	0.29	0.29	0.55					
1MX9	Naloxone	0.52	0.52	***0.71***	0.52				
2DQY	Cholate	0.26	0.26	0.48	0.35	0.54			
2DQZ	Palmitate	0.32	0.32	0.44	0.34	0.56	0.35		
2DR0	Taurocholate	0.32	0.32	0.45	0.34	0.53	0.34	0.32	
2H7C	Coenzyme A	0.45	0.45	0.52	0.46	0.62	0.41	0.44	0.46



***1YAJ**: hCES1-benzoate*



***1MX9**: hCES1-Naloxone*

1YAJ may represent the enzyme in the *apo* state since the small size of the enzymatic product cannot markedly distort the catalytic cavity during its egress.

1MX9 may correspond to the *holo* state since the enzyme is accommodating a large substrate and its binding cavity should be enlarged enough to encompass any substrate.

Docking analyses involved both experimental structures (**1YAJ** and **1MX9**) as they should represent two different states of the enzyme. The training set was then used to uncover which CES1 structure affords the best predictions.

Predicting the CES1 activity

❖ Selecting best docking conditions (training set) (i.e. CES structure and ionization state)

❖ Assessing statistical robustness (test set)

- *Docking simulations were performed considering the 11 basic substrates included in training set both neutral and ionized.*
- *Since the study initially involved two CES1 structures, four sets of docking simulations were globally carried out.*
- *The four best relationships were derived including constantly 2 variables taken from docking scores and/or ligand properties.*

CES1 1YAJ

Eq. 1: Ionized basic substrates

$pKm = -0.0073\ PSA - 0.34\ MLP_{InS} + 4.39$

$n = 25;\ r^2 = 0.61;\ q^2 = 0.49;\ SE = 0.72;\ F = 17.52$

Eq. 2: Neutral basic substrates

$pKm = -0.38\ dist_{Ser221} - 1.66\ MLP_{InS} + 4.18$

$n = 25;\ \mathbf{r^2 = 0.85};\ q^2 = 0.73;\ SE = 0.49;\ F = 64.06$

CES1 1MX9

Eq. 3: Ionized basic substrates

$pKm = -0.0067\ PSA - 0.12\ MLP_{InS} + 4.22$

$n = 25;\ r^2 = 0.38;\ q^2 = 0.32;\ SE = 0.92;\ F = 6.62$

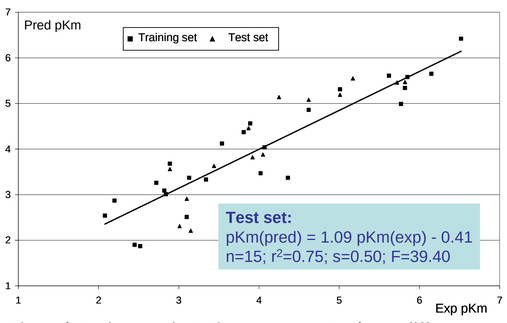
Eq. 4: Neutral basic substrates

$pKm = 0.0039\ Volume - 1.29\ MLP_{InS} + 1.76$

$n = 25;\ r^2 = 0.64;\ q^2 = 0.51;\ SE = 0.65;\ F = 19.38$

- *Obtained relationships unveil that (i) docking analyses performed using **1YAJ** structure afford always better correlations and (ii) in both cases the neutral substrates correlate better than the ionized forms suggesting that the formers are more probably involved in the enzyme recognition. The Eq. 2 was thus exploited to investigate its predictivity power.*

Is the Eq. 2 truly robust ? Can Eq. 2 predict external test set ?



The plot shows that there are not clear differences between training (squares) and test (triangles) substrates as confirmed by good relation between experimental and computed values in test set.

Eq. 2

$pKm = -0.38\ dist_{Ser221} - 1.66\ MLP_{InS} + 4.18$

$n = 25;\ \mathbf{r^2 = 0.85};\ q^2 = 0.73;\ SE = 0.49;\ F = 64.06$

Considerations:

- $dist_{Ser221}$ encodes the ability of a ligand to assume a pose conducive to the catalysis.
- All relations include the MLP_{InS} score, confirming that:
  - CES1 recognition is driven by hydrophobic contacts.
  - MLP_{InS} is fruitful to account for lipophilicity in binding.

Accounting for lipophilicity in docking: the MLP_{InS} score[3]

- *In general, the lipophilicity plays a crucial role in ligand recognition, but little has been done to quantify specifically its effect in docking scores.*
- *In particular, the substrate recognition by hCES1 appears largely driven by hydrophobic contacts emphasizing the need for an appropriate and specific lipophilic score.*

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

f_a and f_b = Broto's atomic increments.

$fct(r_{ab})$ = Distance function. Many functions were tested.

Best results are obtained by $fct(r_{ab}) = (r_{ab})^3$

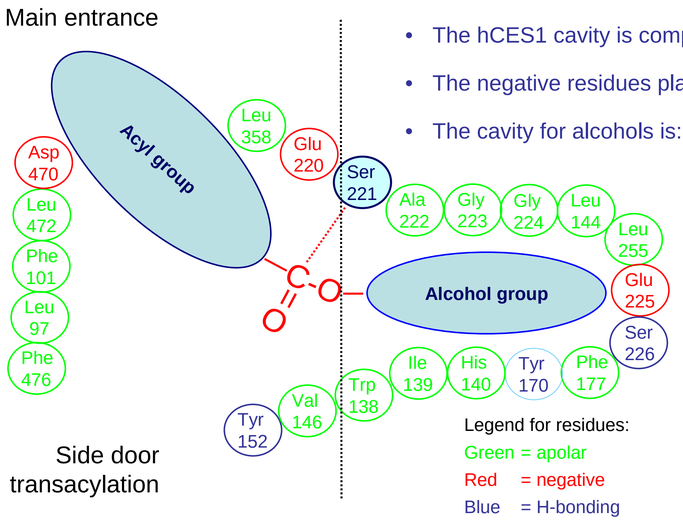
The sums involve all ligand (p) and enzyme (m) atoms.

Globally MLP_{InS} encodes:

- **Hydrophobic contacts (f_a and f_b > 0)**
- **Polar interactions (fa and fb < 0)**
- **Repulsive forces (otherwise)**

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

Docking analyses: unveiling the CES1 catalytic site



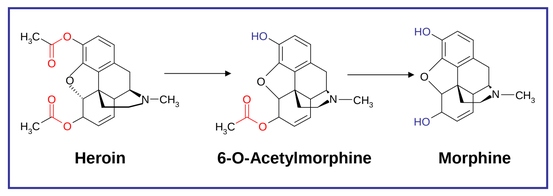
- The hCES1 cavity is completely lined by hydrophobic residues.
- The negative residues play a key role in product egress.
- The cavity for alcohols is: smaller, less flexible and slightly more polar.

The differences between the two sub-cavities reflect on the average properties for alcohol and acyl groups of the substrates.

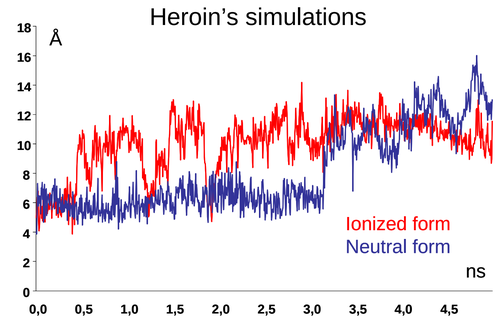
Property average	Acyl groups	Alcohol groups
Volume (Å³)	204.2	102.3
log P	2.19	1.48

Basic substrates and CES1 activity the case of heroin hydrolysis[4]

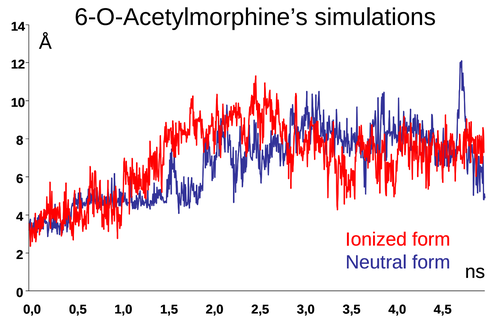
To explore the influence of ionization on CES1 activity, the stability of the complexes of heroin, 6-O-acetylmorphine and morphine in both ionization states was analyzed by 5-ns MD runs All plots report the distance between Ser221 and ester group.



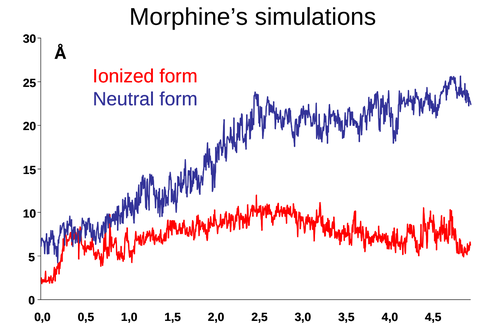
Heroin's simulations



6-O-Acetylmorphine's simulations



Morphine's simulations



Considerations:

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

✓ The neutral form of morphine leaves the site and allows the catalytic turn-over.

✓ The ionized form of morphine remains docked into the site behaving as competitive inhibitor.

✓ The reasonable behavior of simulated complexes affords an encouraging validation of their stability.

Conclusions

The congruity of the obtained complexes and the correlations between docking scores and enzymatic data afford an encouraging validation for the described docking results which can be used to predict the hydrolytic metabolism of new molecules. In detail, the simulations reveal that (i) MLP_{InS} scores proved successful to account for lipophilicity in binding; (iii) an optimal CES1 substrate should possess the alcohol group smaller and more polar; (iii) CES1 prefers neutral or anionic substrates, while the cationic ligands can behave as inhibitors; (iv) the product egress can be simulated by simple all-atoms MD runs.

References

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