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Impaired local hydrophobicity, structural stability and conformational flexibility due to point mutations in SULT1 family of enzymes

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Abstract: Sulfotransferases (SULTs) are enzymes involved in phase II of the metabolism of xenobiotics. Single nucleotide polymorphisms were identified for genes encoding the SULTs leading to allozymes with modified sulfating activity. This study aims to analyze the effects of the most frequently identified amino acid mutations in the sequences of enzymes belonging to the SULT1 family on their local properties and structural stability. The outcomes reveal that single point mutations alter the local hydrophobicity and flexibility, mainly due to destabilization of the protein structures, and consequently may lead to changes in the dynamic of the active site activity reducing the affinity for the substrate. Elucidation of how the single point mutations influence the activity of enzymes contributes to understanding the molecular basis of the specificity of enzymatic activity and mitigating anomalies in the metabolism of xenobiotics.

Keywords: protein plasticity; protein stability; hydrophobicity profile; mutations; metabolism, bioinformatics.

INTRODUCTION

Sulfotransferases (SULTs) are enzymes involved in phase II of the metabolism of a wide range of both xenobiotics and endogenous compounds (hormones, bile acids, neurotransmitters, carbohydrates, proteins). They act by transferring a sulfate group from the cofactor 3′-Phosphoadenosine 5′-Phosphosulfate (PAPS) to the hydroxyl group of an acceptor substrate.[1] Sulfoconjugation increases the water solubility of chemical compounds and the formation of more excretable products contributing to detoxification, but it also may lead to potentially carcinogenic metabolites.[2]

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There were identified 13 human cytosolic sulfotransferase genes in humans conducting to proteins divided into four families differing in the tissue distribution and substrate specificity[3]: SULT1, SULT2, SULT4, and SULT6. The present study focuses on the SULT1 family, respectively on the subfamilies SULT1A1, SULT1A2, SULT1A3, SULT1B1, SULT1C2 (former SULT1C1), and SULT1E1 as they reveal frequently identified allozymes with modified biological functions. These enzymes are involved in sulfation of phenols, thyroid hormones, and numerous drugs.[4] SULT1A1 and SULT1A2 are usually active against the phenolic compounds, with SULT1A1 revealing a higher activity. Furthermore, SULT1A1 and SULT1B1 have an extensive overlap in their substrate profiles, but the sulfation efficiency of SULT1A1 is higher.[5] SULT1A3 displays selectivity for catecholamines and structurally related compounds (serotonin, dopamine).[6] SULT1C2 enzyme sulfonates thyroid hormones, and SULT1E1 is involved in the sulfation of hormones, mainly estrogens and iodothyronines.[7,8]

Single nucleotide polymorphisms (SNPs) were identified for genes encoding the SULTs conducting to SULTs allozymes with modified stability and/or sulfating activity impairing the therapeutic response of numerous drugs.[2, 9-11] In the case of SULT1A1, the frequent identified polymorphic variants are: SULT1A1*1 (the wild type, WT), SULT1A1*2 (amino acid substitution R213H), SULT1A1*3 (M223V) and SULT1A1*4 (R37Q), with SULT1A*2. SULT1A*3 and SULT1A*4 usually reveal lower catalytic activity than the WT enzyme.[2, 10,12,13] In the case of SULT1A2, the frequent allozymes are SULT1A2*1 (WT), SULT1A2*2 (I7T, N235T) and SULT1A2*3 (P19L). SULT1A2*2 displays lower thermostability and decreased catalytic activity, and SULT1A2*3 exposes higher thermostability and increased activity compared to WT.[14] The frequent variants of the SULT1A3 are SULT1A3*1 (WT), SULT1A3*2 (K234N), SULT1A3*3 (P101L), SULT1A3*4 (P101H), and SULT1A3*5 (R144C). SULT1A3*2 and SULT1A3*3 usually reveal decreased activity, and SULT1A3*4 and SULT1A3*5 reveal increased activities against numerous drugs when compared to WT.[6, 15,16] Only two allozymes are known for SULT1B1, SULT1B1 (WT) and SULT1B1-L145V, the last one showing significantly decreased sulfation of p-nitrophenol than the WT.[17] There are registered five variants for SULT1C2: SULT1C2*1 (WT), SULT1C2*2 (S255A), SULT1C2*3 (D60A), SULT1C2*4 (R73Q) and SULT1C2*5 (S111F). SULT1C2*3 and SULT1C2*4 reveal reduced activity toward p-nitrophenol when compared to the WT and SULT1C2*2, whereas SULT1C2*5 did not show detectable activity toward this substrate.[18] The frequent variants of SULT1E enzyme are SULT1E1*1 (WT), SULT1E1*2 (D22Y), SULT1E1*3 (A32V) and SULT1E1*4 (P253H). The allelic variants exhibit lower sulfation activity for estradiol compared to WT.[19,20] An up-to-date synthesis

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regarding the catalytic activities of all these variants toward various substrates and drugs has been recently published. [21]

Elucidation of the structures of the SULT1 enzymes in complex with different ligands highlights the dominant role of their structural flexibility/plasticity in controlling both the activity and specificity.[22-28] Furthermore, it is also widely presumed that structural features and biological functions of proteins are closely connected to their sequence compositions.[29] A single amino acid change in the protein sequence can disturb the network of intramolecular interactions and affect how the protein folds, its structural stability, dynamics, and, consequently, its biological function.[30] Consequently, in order to understand the molecular effects of a single point mutation, it is also necessary to consider changes in protein structural stability and dynamics.

This study aims to predict, compare and analyze the changes in the local hydrophobicity, structural stability and flexibility due to single point mutations in the sequences of the SULT1 enzymes using a computational approach.

EXPERIMENTAL

This study focuses on the human SULT1 enzymes having frequently identified polymorphic variants (Table I). The sequences of the wild type enzymes were extracted from the UniProt database[31] and used for further analysis.

For assessing the changes produced by the mutations present in the frequently identified allozymes of the SULT1 family in the profiles of hydrophobicity and average flexibility, the ProtScale computational tool[32] has been considered. Several parameters can be chosen when using the ProtScale computational tool: window size, the window edge relative weight value, weight variation model, and scale normalization. The window size is defined as the number of amino acids considered for determining one point of the computed property. It means that computing the value of the investigated property for a given residue *i*, the amino acids in the interval of the chosen length, positioned around residue *i*, are considered. Regarding the window edge relative weight value, the computational tools always consider that amino acid from the center of the window has a weight of 100%, and the user may choose weight values between 0 and 100% for the amino acids at the remaining positions in that window. If weight values are chosen lower than 100%, the user may select a linear or exponential decrease of the weight between the center and the edges. Furthermore, the user may choose whether to use the unmodified selected scale values or to normalize these values so that they fit into the range from 0 to 1.[32] The following settings were considered in this study: windows of 3, 5, and respectively 9 amino acids, the relative weight of the window edges compared to the window center was set to 100%, and the unmodified selected scales values were used. We also specify that for obtaining the hydrophobicity profiles, the Kyte&Doolitle scale[32] has been used.

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TABLE I. The frequently identified polymorphic variants of the enzymes belonging to SULT1 family considered in this study: WT- the wild type enzyme.

SULT1 subfamily	Polymorphic variants	Amino acid substitutions
SULT1A1	SULT1A1*1	WT
	SULT1A1*2	R213H
	SULT1A1*3	M223V
	SULT1A1*4	R37Q
SULT1A2	SULT1A2*1	WT
	SULT1A2*2	I7T, N235T
	SULT1A2*3	P19L
SULT1A3	SULT1A3*1	WT
	SULT1A3*2	K234N
	SULT1A3*3	P101L
	SULT1A3*4	P101H
	SULT1A3*5	R144C
SULT1B1	SULT1B1	WT
	SULT1B1-L145V	L145V
SULT1C2	SULT1C2*1	WT
	SULT1C2*2	S255A
	SULT1C2*3	D60A
	SULT1C2*4	R73Q
	SULT1C2*5	S111F
SULT1E	SULT1E1*1	WT
	SULT1E1*2	D22Y
	SULT1E1*3	A32V
	SULT1E1*4	P253H

In order to explore the local flexibility in the structures of the SULT1 enzymes, the PDBFlex database[33] has been considered. This database offers information on the intrinsic global and local flexibilities of protein structures based on the analysis of variations appearing between the different structural files of the same protein deposited in the Protein Data Bank (PDB). PDBFlex collects information on all depositions having at least 95% sequence identity with the sequence of the query structural file, performs the analysis of the structural differences, and clusters them according to the structural similarities.[33] Consequently, the available elucidated structural files for the investigated enzymes and their complexes with various ligands (cofactor, substrates, drugs) have been considered. These structural files are available in PDB[34], and the information regarding their active and binding sites has been also retrieved (Table II).

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TABLE II. Uniprot and Protein Data Bank (PDB) identifiers (ID) for the analyzed sequences and structures of the enzymes belonging to SULT1 family: aa – amino acid, PAPS - 3'-phosphoadenosyl-5'-phosphosulfate, the cofactor for these enzymes.

SULT1 enzyme	Uniprot ID	PDB ID	Binding site	Mutations corresponding to allozymes
SULT1A1	P50225	4GRA[a, 36], 1LS6[b], 1Z28[c], 2D06[b], 3U3J[b], 3U3K[b], 3U3M[b], 3U3O[b], 3U3R[b], 3QVU[b], 3QVV[b],	PAPS: 48-53, 106-108, 130, 138, 193, 227-232, 255-259; Substrate: 106-108	R37Q, R213H, M223V
SULT1A2	P50226	1Z29	PAPS: 48-53, 106-108, 130, 138, 193, 227-232, 255-259; Substrate: 106-108	I7T, P19L, N235T
SULT1A3	PODMM9	2A3R[a], 1CJM	PAPS: 48-53, 130, 138, 146, 193, 227-232, 255-259; Substrate: 86	P101L, P101H, R144C, K234N
SULT1B1	O43704	2Z5F, 3CKL[a]	PAPS: 48-53, 131, 139, 194, 228-233, 256-260; Substrate: 107-109	L145V
SULT1C2	O00338	3BFX[a]	PAPS: 49-54, 131, 139, 194, 228-233, 256-260; Substrate: 107-109	D60A, R73Q, S111F, S255A
SULT1E1	P49888	1G3M[a], 1HY3, 4JVL, 4JVM, 4JVN	PAPS: 47-52, 129, 137, 192, 226-231, 256-258 Substrate: 105-107	D22Y, A32V P253H

[a]When multiple structural files have been detected for an enzyme, these structural files have been chosen as they have a better resolution or a lower number of missing residues and/or missing atoms.
[b] These structural files correspond to the allelic variant SULT1A1*2 (R213H).[23, 26, 37, 38]
[c] Structural file 1Z28 corresponds to the allelic variant SULT1A1*3 (M223V).[25]

Structures of the WT variants of the SULT 1 enzymes highlighting the positions of the amino acids that suffer mutations are revealed in Figure 1. UCSF Chimera tool[35] has been used to visualize these structures.

Data presented in Table II and Figure 1 reveal that some amino acids that support mutations corresponding to allozymes situated in the regions or their close vicinity are involved in the interactions with the cofactor and/or substrate: M223V for SULT1A1*3, N235T for SULT1A2*2, K234N for SULT1A3*2, P253H for SULT1E1*4. Some residues that support mutations are missing in the structural files: I7T for SULT1A2, R73 and S255 for SULT1C2.

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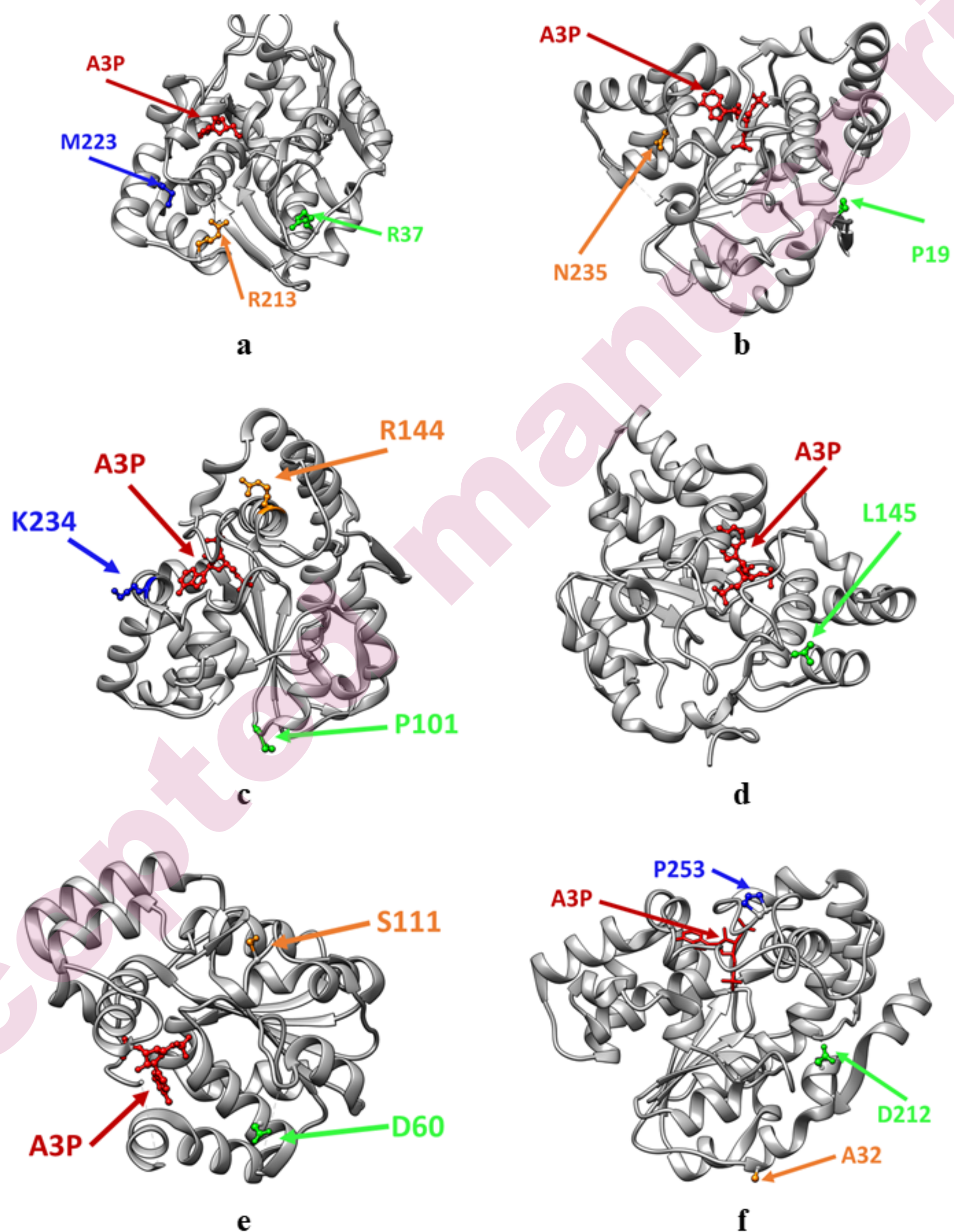

Fig. 1 Illustration of the positions of the residues that support mutations in the SULT1 enzymes: SULT1A1 (a), SULT1A2 (b), SULT1A3 (c), SULT1B1 (d), SULT1C2 (e), SULT1E1 (f). Some residues that support mutations are missing in the structural files: I7T for SULT1A2, R73 and S255 in SULT1C2. A3P - adenosine-3'-5'-diphosphate.

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UCSF Chimera tool has been also considered for illustrating the changes in the hydrophobicity and Coulombic electrostatic potential of the regions of SULT1A1 containing the point mutations R213H and M223V compared to the WT enzyme. This analysis has been made only for SULT1A1, as this enzyme has solved structures of mutants deposited in PDB.

DynaMut2 web server (http://biosig.unimelb.edu.au/dynamut2/) has been considered for predicting changes in stability caused by single point mutations in the sequences of investigated enzymes.[30] The changes in the enzymes stability are assessed by predicting the variations in folding free energy values ($\Delta\Delta G$, expressed in kJ mol^{-1}) for single point mutations: $\Delta\Delta G<0.0$ corresponds to mutations destabilizing the structure and $\Delta\Delta G>0.0$ to mutations contributing to the stabilization of the structure. The predictions are based on an experimental data set collected for 4633 mutations (2640 destabilizing and 1993 stabilizing) that were divided into 4022 entries for the training set and 611 entries for the test set. DynaMut2 has a good accuracy of predictions achieving Pearson's correlation of up to 0.72 for single point mutations across 10-fold cross-validation and independent blind tests.[30]

RESULTS AND DISCUSSION

Analysis of the influence of mutations present in the frequently identified allozymes belonging to SULT1 family on their average flexibility and hydrophobicity profiles.

The effect of the mutations corresponding to the main allelic variants of the SULT1 family of enzymes on their local hydrophobicity are shown in Figure 2 for a window of 3 amino acids and in the Supplementary Tables S-I – S-VI for windows of 5 and 9 amino acids, respectively. Figure 2 and data presented in Supplementary Tables S-I – S-VI reveal that the punctual amino acid mutations corresponding to the frequently identified allozymes of the SULT1 enzymes conduct to altered local hydrophobicity profiles. Some mutations cause decreased local hydrophobicity, but others produce increased local hydrophobicity. Even if only one amino acid is changed, this point mutation affects the local hydrophobic profile over a range of at least 9 residues. A molecular dynamics study revealed that in the case of SULT1A1*3, the M223V mutation led to the loss of a hydrophobic contact between M223 and M60 and may be responsible for the altered sulfonation activity of the SULT1A1*3.[21]

The flexibility profiles of the enzymes belonging to the SULT1 family and of their frequently identified allozymes are shown in Figure 3 for a window of 3 amino acids and in the Supplementary Tables S-VII – S-XII for windows of 5 and 9 amino acids, respectively. Figure 3 and Supplementary Tables S-VII – S-XII reveal that the amino acid mutations corresponding to the frequently identified allozymes of the SULT1 enzymes also alter the local flexibility profiles.

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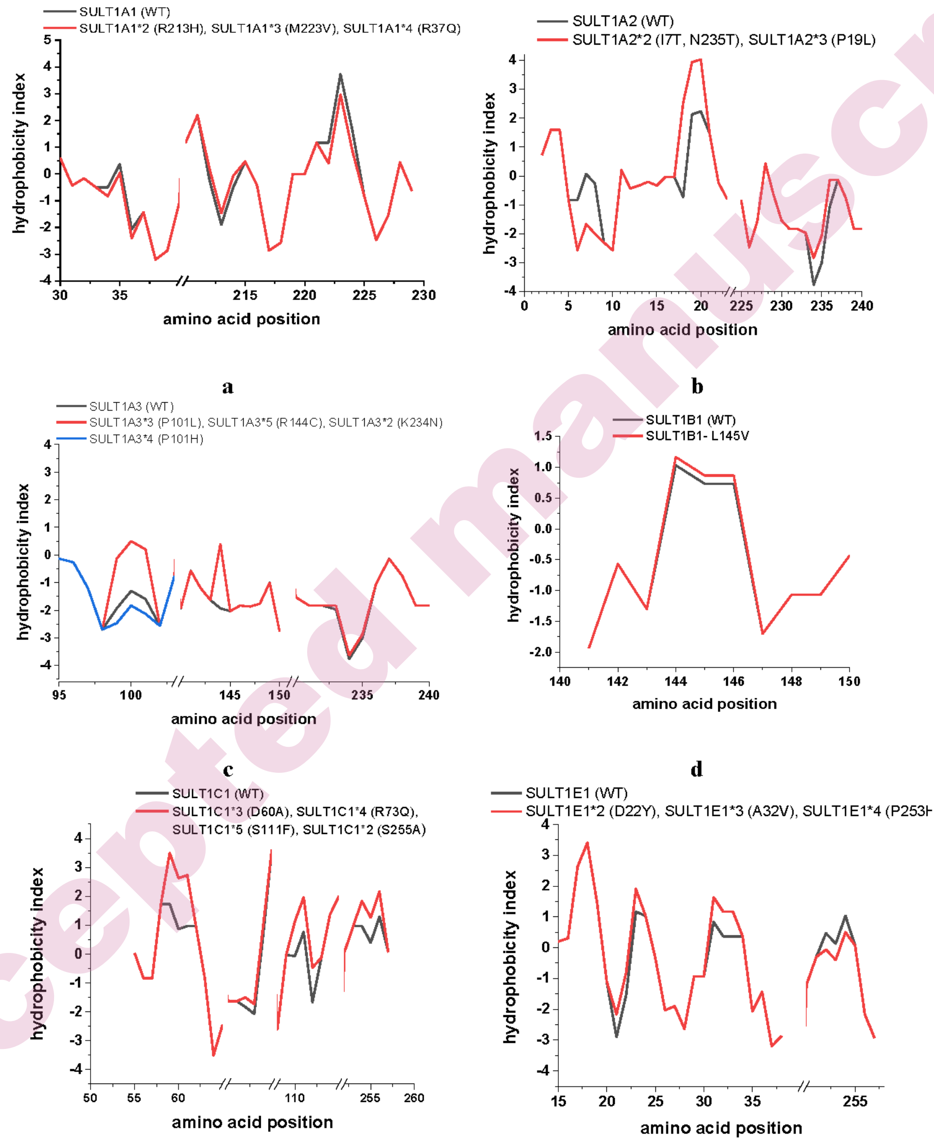

Fig. 2 Hydrophobicity profiles obtained using ProtScale tool for a window of 3 amino acids for the enzymes belonging to the SULT1 family and for their frequently identified allozymes: WT – wild type protein.

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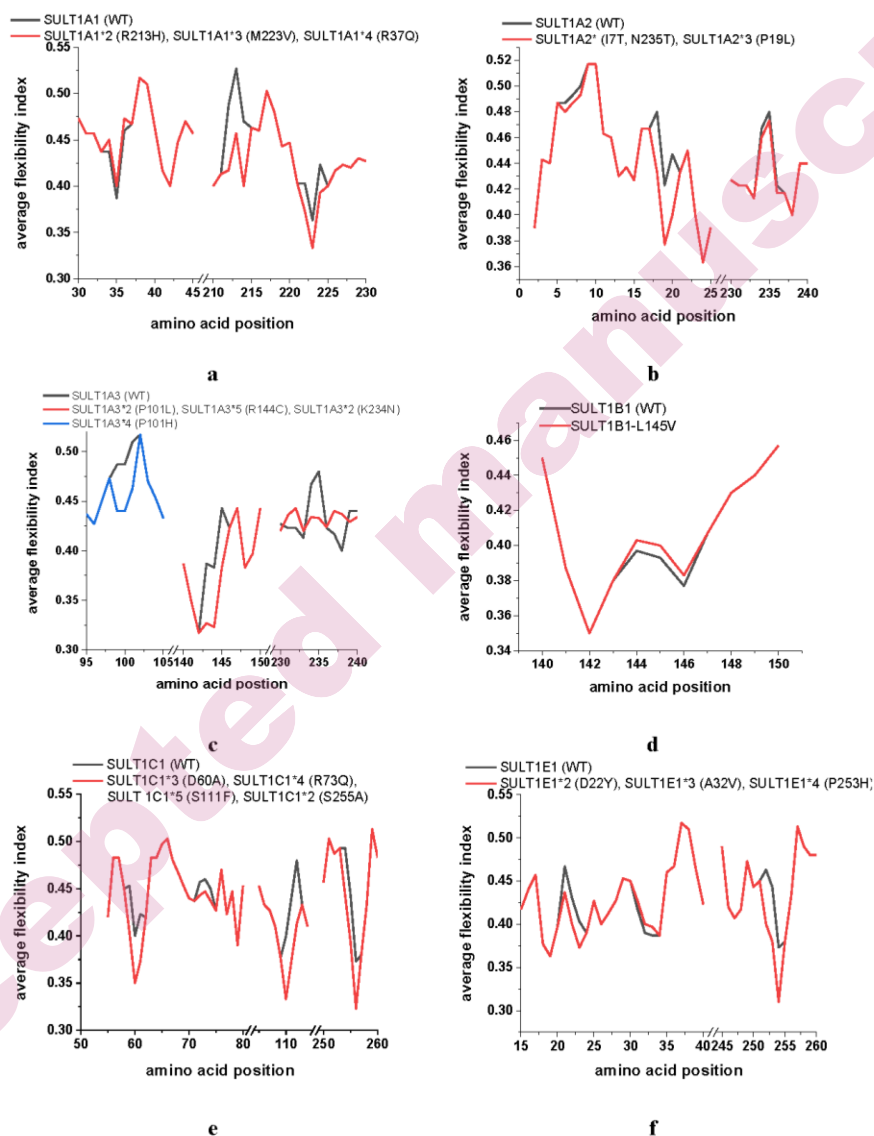

Fig. 3 Average flexibility profiles obtained using ProtScale tool for a window of 3 amino acids for the enzymes belonging to the SULT1 family and for their frequently identified allozymes: WT – wild type protein.

Being well known that the flexibility of SULTs is responsible for recognizing the diverse types of substrates[39], this outcome becomes important. A molecular dynamics simulation study involving the major allozymes of SULT1A1 (WT, R213H, and M223V) revealed increased flexibility in the region of the binding site

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for the mutants compared to the WT enzyme and alteration of the protein dynamics.[21] It should be noted that neither R213 nor M223 are in the substrate-binding loops,[39] but their mutations induce conformational changes affecting the flexibility of at least one of these loops.[40] These changes may be responsible for the observed alternation of sulfation activities of mutants compared with WT toward numerous endogenous compounds and drugs.[2, 10, 12, 13]

These outcomes revealing the altered local hydrophobicity and flexibility for the allozymes are not unexpected, being known that polar or charged residues are more flexible and less hydrophobic, whereas nonpolar residues are more hydrophobic and quite inflexible.[41] Furthermore, the alteration of the local hydrophobicity and/or flexibility may cause the proteins dysfunctionalities, as long-range correlations concerning hydrophobicity and flexibility along the proteins chains have been observed for sequences of numerous proteins.[42-45] As the protein hydrophobicity and flexibility are closely related to the primary structure, it is expected that the amino acid mutations affect not only the spatial structure of the protein but also the structural flexibility of the protein and its biological function.[46] Consequently, a better understanding of the relationship between the local hydrophobicity and flexibility of SULT1 enzymes and their functional properties is essential for understanding the metabolism of numerous drugs.

Analysis of the flexibility of enzymes belonging to the SULT1 family taking into account structural data.

For the investigated SULT1 enzymes, their local structural flexibility has been analysed using PDBFlex. The identified clusters for the structural files corresponding to these enzymes are revealed in Supplementary Table S-XIII. SULT1A1 and SULT1A2 enzymes are considered as members of the same cluster due to their high sequence similarity, about 95%.[47] The regions with local flexibility identified in the structures of SULT1 enzymes are presented in Table 3.

The N- and C-terminal regions are not mentioned in this table as it is known that they can be disordered and flexible in many proteins.[48] As expected, the regions with higher structural flexibility involve amino acids that interact with the cofactor and/or the substrate. Several mutations corresponding to allozymes of the investigated SULTs correspond to the identified flexible regions revealing their possible effects on the local structural stability and flexibility of the proteins.

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TABLE III. Regions with local structural flexibility in SULT1 enzymes identified using PDBFlex computational utility.

SULT1 member	Maximum RSMD in the cluster (Å)	Average RSMD in the cluster (Å)	Region (amino acid interval)	Average RMSD (Å)	Ligand interacting with amino acids belonging to this region	Amino acid mutation corresponding to allelic variant that is present in the flexible region
SULT1A1, SULT1A2	0.596 for 3U3K and 1Z28	0.363	110-122	0.053	p-nitrophenol, 3-hydroxy-7,7-dimethyl-2-phenyl-4-(thiophen-2-yl)-2,6,7,8-tetrahydro-5H-pyrazolo[3,4-b]quinolin-5-one, 7-hydroxy-2-oxo-2H-chromene-3-carbonitrile, estradiol	R213H, M223V
			210-225	0.034		
SULT1A3	1.167 for 2A3R and 1CJM[a]	0.793	24 - 63	0.072	adenosine-3'-5'-diphosphate, L-dopamine	
			94 -104	0.182	L-dopamine	P101L in SULT1A3*3, P101H in SULT1A3*4
			141 – 156	0.036	adenosine-3'-5'-diphosphate, L-dopamine	R144C in SULT1A3*5
SULT1B1	0.562 for 2Z5F and 3CKL	0.395	83 - 89	0.282	L-dopamine	-
SULT1E1	0.625 for 1HY3 and 4JVL	0.384	79 - 94	0.362	adenosine-3'-5'-diphosphate	
			141-157	0.036	L-dopamine	
			210 - 229	0.027	resveratrol	
			240-252	0.041		P243H în SULT1E1*4

[a]Many regions are missing in the structure of SULT1A3 with the PDB ID 1CJM, and these regions were not considered for the local flexibility analysis.

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Analysis of the influence of mutations that are present in the frequently identified allozymes belonging to SULT1 family on their local hydrophobicity and electrostatic potential

For SULT1A1, the output of the PDBFlex tool reveals local structural flexibility for the region 210-225, taking into account the different structural files of this protein deposited in the PDB. This region incorporates two mutations corresponding to SULT1A1*2 (R213H) and SULT1A1*3 (M223V), respectively. The output of the ProtScale tool reveals that mutations corresponding to SULT1A1*2 and SULT1A1*3 reduce the local hydrophobicity and flexibility and may facilitate the electrostatic interactions with the solvent. This output strongly correlates with data presented in Figure 4 obtained using Chimera software for the regions 210-225 of the SULT1A1 WT and of the enzyme containing the mutations R213H and M223V. Figure 4 reveals changes in both the local hydrophobicity and Coulombic electrostatic potential of this region for the allozymes compared with the WT enzyme.

These changes may influence the activity of these allozymes as their sulfation activity toward both endogenous and numerous xenobiotic compounds is decreased compared with the sulfation activity of the WT.[21] Furthermore, literature data reveal that other two mutations in the SULT1A1 sequence, D249G and Y240C (both amino acids located in the vicinity of the active site), conduct to lower affinity for 3-cyano-7-hydroxycumarin and p-nitrophenol. Analysis of the D249G mutant structure shows an increase in the local flexibility of this region and a significant change in the charge distribution around the active site.[26] The importance of the local electrostatic interactions for determining the structure, stability, and conformational adaptabilities has been revealed for numerous proteins.[49, 50]

There are no structures determined for the allozymes of the others SULT1 enzymes considered in this study, and consequently, the changes in the local hydrophobicity and electrostatic potential could not be analyzed for these enzymes by using structural data. Taking into account the very good correlation between the information obtained using ProtScale and PDBFlex computational tools for the SULT1A1 subfamily, we may extrapolate that the punctual amino acid mutations that appear in the allozymes of the SULT1 enzymes conduct to alteration of the local structural flexibility, local hydrophobicity, and consequently on the electrostatic potential.

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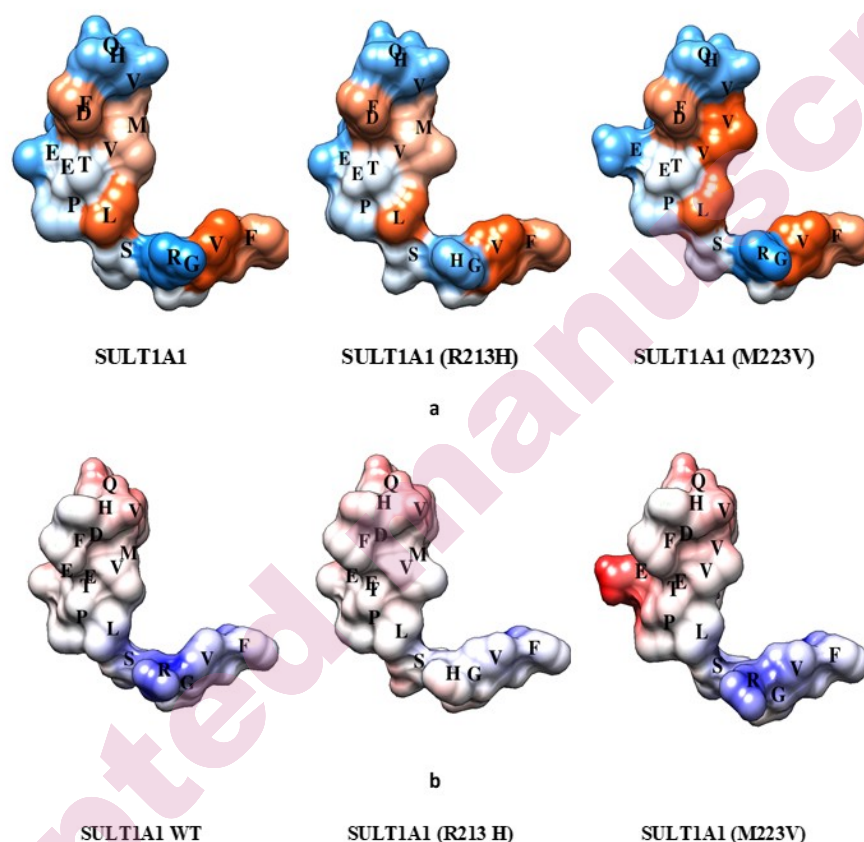

Fig. 4 Changes in the local hydrophobicity (a) and Coulombic electrostatic potential (b) of the 210-225 region of SULT1A1 due to the point mutations R213H and M223V compared to the wild type (WT) enzyme. The following structural files have been considered when mapping the hydrophobicity and electrostatic potential: 4GRA (WT), 1LS6 (R213H), and 1Z28 (M223V). Blue regions in figure 4a correspond to the hydrophilic surface, and orange regions correspond to the hydrophobic surface. As the color is more intense, the higher is hydrophilicity/hydrophobicity of the surface. In Figure 4b, red regions illustrate the negative potential, and blue regions correspond to the positive potential.

Analysis of the influence of mutations that are present in the frequently identified allozymes belonging to SULT1 family on their structural stability

It is already known that even small reductions in protein stability can lead to dysfunctional proteins.[51] The stabilization/destabilization effects of the single point mutations in the sequences of SULT1 enzymes conducting to the most frequently identified allozymes have been analyzed using DynaMut2 webserver, and the results are presented in Table IV.

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TABLE IV. Illustration of the ΔΔG values produced by the single point mutations corresponding to the frequently identified allozymes of the SULT1 enzymes. ΔΔG<0.0 values correspond to mutations destabilizing the structure, and ΔΔG>0.0 values to mutations contributing to the stabilization of the structure.

SULT1 enzyme	Single point mutation	ΔΔG / kJ mol^{-1}	Effect	Observations
SULT1A1	R37Q	-2.18	destabilizing	
	R213H	-0.54	destabilizing	
	M223V	-1.92	destabilizing	
SULT1A2	I7T	-	-	T7 residue is missing in the crystallographic structure
	P19L	-1.84	destabilizing	
	N235T	0.09	stabilizing	
SULT1A3	P101L	-2.05	destabilizing	
	P101H	-1.04	destabilizing	
	R144C	-3.93	destabilizing	
	K234N	0.29	stabilizing	
SULT1B1	L145V	-9.07	destabilizing	
SULT1C2	D60A	1.04	stabilizing	
	R73Q			Q73 residue is missing in the crystallographic structure
	S111F	-1.71	destabilizing	
	S255A			A225 residue is missing in the crystallographic structure
SULT1E1	D22Y	5.68	stabilizing	
	A32V	-2.38	destabilizing	
	P253H	-5.85	destabilizing	

Data presented in Table IV reveal that the typical point mutations corresponding to allozymes usually destabilize the structure. It is in good correlation with known data revealing that the allozymes containing point mutations that destabilize the structure usually have lower sulfation activity compared to the WT enzymes. In the case of SULT1A1, it was shown that the position of residue 213 precedes a flexible region, whereby mutation of this residue affects both stability and flexibility of the enzyme.[38] Among the frequent point mutations appearing in the SULT1A1 enzyme, R213H produces the lower destabilizing effect, which is also in correlation with published data revealing that R213H induces local conformational changes affecting the substrate-binding loop and has only a low impairing effect on the overall stability of the protein structure.[40]

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It should be noted that several amino acids that correspond to the mutants of SULT1 enzymes have ionizable sidechains. The frequently identified polymorphic variants of SULT1A are R37Q and R213H, with arginine and histidine being among the amino acids having ionizable sidechains. Histidine is neutral, and arginine is protonated under physiological conditions. Consequently, the standard protonation states have been considered for arginine and histidine residues. Both histidine and arginine play essential structural and functional roles in proteins, which correlate with the ionization state of their side chains. It emphasizes that a more accurate approach should include electrostatic calculations by solving the Poisson-Boltzmann equation with subsequent Monte Carlo titration[52] or employing DFT/solvation electrostatic calculations.[53]

The mutation L145V corresponding to SULT1B1 conducts to a substantial destabilization of the structure ($\Delta\Delta G$=-9.07 kJ mol^{-1}), and literature data show that this mutation results in a significantly decreased sulfation of p-nitrophenol compared to the WT.[17]

Single amino acid mutations in the sequences of the enzymes belonging to the C2 family of the human cytochromes (CYP2C) have also been suggested to be structurally destabilizing in close connection with the observed interindividual differences in CYP2C-mediated drug metabolism.[54]

CONCLUSIONS

Data obtained in the current study reveal that the point mutations present in the most frequently observed polymorphic variants of the enzymes belonging to the SULT1 family result in altering the local hydrophobicity and flexibility and usually conduct to destabilize the protein structure. Such changes may be responsible for the reduced affinity for the substrate due to possible effects on the dynamics and flexibility of the binding region of the protein. The outcomes of this study contribute to elucidating how SULT SNPs may influence the metabolism of drugs and endogenous compounds and may allow for the improvement of strategies for mitigating anomalies in the metabolism of xenobiotics. Furthermore, these results may contribute to understanding the molecular basis for the altered specificity of other enzymes having polymorphic variants.

SUPPLEMENTARY MATERIAL

Supplementary tables S-I - S-XIII. Hydrophobicity and average flexibility profiles of the SULT1A1 (Table S-I and S-VII); SULT1A2 (Table S-II and S-VIII); SULT1A3 (Table S-III and S-IX); SULT1B1 (Table S-IV and S-X); SULT1C1 (Table S-V and S-XI); SULT1E1 (Table S-VI and S-XII) enzyme (WT) and of its frequently identified allozymes obtained using ProtScale tool for a window of 5 and 9 amino acids; The clusters identified by PDBFlex computational tool for assessing the structural flexibility for the SULT1 enzymes that reveal frequently identified allozymes (Table S-XIII).

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Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12271, or from the corresponding author on request.

ИЗВОД

СМАЊЕНА ЛОКАЛНА ХОДРОФОБНОСТ, СТРУКТУРНА СТАБИЛНОСТ И ФЛЕКСИБИЛНОСТ КОНФОРМАЦИЈЕ УСЛЕД ТАЧКАСТИХ МУТАЦИЈА SULT1 ФАМИЛИЈЕ ЕНЗИМА

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Сулфотрансферазе (SULT) су ензими укључени и фазу II метаболизма ксенобиотика. Идентификовани су полиморфизми појединачних нуклеотида за гене који кодирају SULT ензиме, доводећи до синтезе алоензима са измењеном сулфатационом активношћу. У овој студији су испитани ефекти најчешћих мутација амино киселина у секвенци ензима SULT1 фамилије на њихове локалне особине и структурну стабилност. Показано је да тачкасте мутације мењају локалну хидрофобност и флексибилност, претежно због дестабилизације структуре протеина, што може довести до промене у активности активног места и смањења афинитета за супстрат. Сазнања о начину на који тачкасте мутације утичу на активност ензима доприносе разумевању молекулских основа специфичности ензимске активности и ублажавање аномалија у метаболизму ксенобиотика.

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