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A missense methionine mutation augments catalytic activity but reduces thermal stability in two protein tyrosine phosphatases

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Abstract

Recent data sets that catalog the missense mutations in thousands of human genomes have revealed a vast and largely unexplored world of non-canonical protein sequences that are expressed in humans. The functional consequences of most human missense mutations, however, are unknown, and the accuracy with which their effects can be predicted by computational algorithms remains unclear. Among humans of European descent, the most common missense mutation in the catalytic domain of SH2-containing protein tyrosine phosphatase 1 (SHP-1) converts the enzyme's canonical valine 451 to methionine (V451M). The V451M mutation lies in a conserved motif adjacent to the protein tyrosine phosphatase (PTP) consensus sequence and is predicted to compromise catalytic function. In this study it is shown that, counter to prediction, V451M SHP-1 possesses increased catalytic activity as compared to the wild-type enzyme. Additionally, a PTP-wide search of missense-mutation data revealed a variant of one other PTP, Fas-associated PTP (FAP-1), that contains a methionine mutation at the position corresponding to 451 of SHP-1 (T2406M FAP-1). It is shown here that the T2406M mutation increases FAP-1's PTP activity, to a degree that is comparable to the activation deriving from the V451M mutation in SHP-1. Although the two non-canonical methionine residues confer increased activity at moderate temperatures, both V451M SHP-1 and T2406M FAP-1 are less thermally stable than their canonical counterparts, as demonstrated by the mutants' strongly reduced activities at high temperatures. These results highlight the challenges in predicting the functional consequences of missense mutations, which can differ under varying conditions, and suggest that, with regard to position 451/2406, canonical PTP domains have "chosen" stability over optimized activity during the course of evolution.

Graphical abstract

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Keywords

missense mutations; protein tyrosine phosphatases; SHP-1; FAP-1

I. Introduction

Large-scale exome-wide sequencing efforts such as the 1000 Genomes Project [1] and the Exome Aggregation Consortium (ExAC) [2] have provided the positions and frequencies of missense mutations for essentially every protein-coding gene in the human genome. Although these studies have revealed a vast world of non-canonical human protein sequences, the functional consequences of most missense mutations are not known.

Missense mutations in cell-signaling enzymes are of particular interest, as aberrant regulation of signaling is a ubiquitous contributor to the etiology of human disease. The protein tyrosine phosphatases (PTPs), which dephosphorylate phosphotyrosine residues in protein substrates, constitute a large family of signaling enzymes, and aberrant PTP activity, often arising from mutations in PTP-encoding genes, has been implicated a range of disease states [3–6]. For example, germline mutations in SH2-domain-containing PTP 2 (SHP-2, encoded by *PTPN11*) cause Noonan and LEOPARD syndromes, both of which can lead to cancer predisposition, and somatic SHP-2 mutations are the most common cause of sporadic juvenile myelomonocytic leukemia [7,8].

SH2-domain-containing protein tyrosine phosphatase 1 (SHP-1, encoded by *PTPN6*) is a phosphatase that is predominantly expressed in hematopoietic and epithelial cells and is generally viewed as negative regulator of the pathways in which it operates [9,10]. Although SHP-1 plays critical signaling roles in a range of cell-signaling processes—such as B- and T-cell receptor signaling, as well as differentiation, proliferation, and adhesion in epithelial cells—few direct connections between missense SHP-1 mutations and human disease are known [3–6].

Recently, a methionine mutation at position 451 of SHP-1 (V451M SHP-1) was reported in a targeted sequencing study of B-cell samples deriving from patients with chronic lymphocytic leukemia (CLL) [11]. The mutation is not limited to CLL patients, however, as ExAC's comprehensive analysis of protein-coding genes in over 60,000 humans recently showed that V451M is the second most common missense mutation in SHP-1 PTP's domain and the most common catalytic-domain mutation among individuals of European descent (allele frequency: 0.124%) [2]. No deleterious health effects have been described for the approximately 100 individuals in the ExAC study who carry a single germline V451M SHP-1-encoding allele [2]. (No individuals who are homozygous for the mutation were identified in the study.)

In wild-type SHP-1, valine 451 lies in a highly conserved motif (motif 9) at the beginning of the PTP signature sequence in an amino-acid position that is not occupied by methionine in any wild-type human PTP (Figure 1A) [12]. The hydrophobic side chain of Val451 extends from a β -strand (Figure 1B) to pack against side chains that are presented from a nearby α -helix [13]. The relative spacing of these two secondary structural elements helps to

determine the position of the catalytically critical PTP loop [14], and it possible that a larger methionine side chain would not be tolerated at the 451 position. Consistent with this analysis, multiple computational algorithms for analyzing the probable effects of amino-acid substitutions (Mutation Assessor [15], PROVEAN [16], and PolyPhen-2 [17]) unanimously predict that the V451M would be damaging to SHP-1's function [11]. If these predictions are correct, then individuals who carry the V451M-encoding allele would carry the equivalent of a heterozygous SHP-1 knockout, at least with regard to the enzyme's PTP activity.

The potential functional consequences of the V451M substitution, coupled with the high degree of sequence and structural homology between PTP domains, led us to ask whether any other classical PTPs carry methionine mutations at the corresponding position. Indeed, the ExAC study found the homologous mutation (T2406M) in a non-canonical allele of Fas-associated PTP 1 (FAP-1 [18,19], encoded by *PTPN13*; see Figure 1A) [2]. Interestingly, T2406M FAP-1 has also been detected in a sequencing study of endometrial carcinomas [20] and therefore appears in the Catalogue of Somatic Mutations in Cancer (COSMIC) database [21]. (Due to existence of multiple FAP-1 isoforms, the COSMIC mutation is listed as T2411M.) The provenance of the T2406M mutation in the endometrial-carcinoma sample is unclear, however; the mutation may have been somatic or it may have derived from an individual who carried the mutation in his or her germline, as did seven individuals in the ExAC cohort (all heterozygous) [2].

The possibility of functional and/or disease-related consequences led us to investigate the effects of V451M and T2406M on the catalytic activities of SHP-1 and FAP-1, respectively. We show here that, surprisingly, both methionine mutations increase PTP activities at low-to-moderate temperatures. However, at temperatures above 37 °C, the activities of the mutants drop dramatically, in comparison to their canonical counterparts. These data show that the functional consequences of missense mutations can vary dramatically under different conditions and suggest that, during the course of evolution, canonical PTP domain sequences have been optimized for stability, rather than activity, at position 451/2406.

2. Material and methods

2.1. General

All assays were performed in triplicate; error bars and “±” values represent the standard deviations of at least three independent measurements.

2.2. Cloning and mutagenesis of PTP-encoding genes

The plasmids encoding His₆-tagged catalytic domains of SHP-1 (SHP-1cat) and FAP-1 (FAP-1cat) have been previously described [22,23]. The plasmid encoding His₆-tagged full-length SHP-1 (SHP-1fl) was ordered from VectorBuilder. Site-directed mutations were introduced using the Quikchange mutagenesis kit (Stratagene) according to the manufacturer's instructions. Desired mutations were confirmed by sequencing.

2.3. Protein expression and purification

His₆-tagged PTPs (SHP-1cat, SHP-1fl, and FAP-1cat) were expressed and purified using HisPur Ni-NTA (Thermo Scientific) according to the manufacturer's instructions and as described [22], with one change: IPTG (0.5 mM) inductions were carried out at 18°C for 20 hours. After purification, proteins were exchanged into storage buffer (50 mM Tris at pH 8.0, 150 mM NaCl, 25 mM CaCl₂, 1 mM TCEP), concentrated, flash-frozen in liquid nitrogen, and stored at -80 °C. Bradford assays and SDS-PAGE were used to measure enzyme concentrations.

2.4. Phosphatase activity using *para*-nitrophenylphosphate (pNPP)

PTP assays using pNPP as substrate were carried out in a total volume of 200 µL, containing PTP buffer (50 mM 3,3-dimethylglutarate at pH 7.0, 1 mM EDTA, 50 mM NaCl), enzyme (varying concentrations: see figure legends), and pNPP (varying concentrations: see figure legends) at 22 °C. PTP reactions were quenched by the addition of 40 µL of 5 M NaOH, and the absorbances (405 nm) of 200 µL of the resulting solutions were measured. Kinetic constants were determined by fitting the data to the Michaelis-Menten equation using SigmaPlot 12.3. For assays at elevated temperatures, all components were pre-incubated in a water bath for 10 minutes at the relevant temperature before the PTP reactions were started.

2.5. Phosphatase activity with 6,8-Difluoro-4-Methylumbelliferyl Phosphate (DiFMUP)

PTP assays using DiFMUP as substrate were carried out in a total volume of 200 µL, containing PTP buffer (see section 2.4), enzyme (varying concentrations: see figure legends), and DiFMUP (varying concentrations: see figure legends) at 22 °C. The increase in fluorescence signal over time was measured using a fluorescence plate reader (excitation: 360 nm, emission 450 nm), and the slopes of the resulting lines from methionine-mutant enzymes were normalized to the corresponding wild-type controls.

2.6. Phosphatase activity with phosphopeptide substrate (DiFMUP)

PTP kinetic assays with the phosphopeptide DADEpYLIPQQG were carried out by measuring increasing absorbance at 282 nm, essentially as described [24]. Assays were performed at 22 °C in a total reaction volume of 200 µL in PTP buffer (see section 2.4) with 100 µM DADEpYLIPQQG (Biomatik) and the appropriate PTP (varying concentrations: see figures).

3. Results

3.1. Catalytic activity of V451M SHP-1

To investigate the effect of a methionine mutation on activity of the SHP-1 catalytic domain we expressed both wild-type and V451M SHP-1 as isolated catalytic domains (SHP-1cat). (The full-length SHP-1 protein also contains two SH2 domains; see below.) We found that the V451M mutation was well tolerated, as wild-type and V451M SHP-1cat expressed at comparable levels from *E. coli*. In light of computational algorithms' prediction of compromised function in V451M SHP-1 [15–17], we were surprised to find that V451M SHP-1cat demonstrated heightened catalytic activity, as compared with the wild-type protein

(Figure 2 and Table 1). When assayed with *para*-nitrophenyl phosphatase (*p*NPP) as substrate, V451M showed higher activity regardless of the *p*NPP substrate concentration used (Figure 2A). Overall, the mutant possesses an increased catalytic rate constant (k_{cat}) and a decreased Michaelis constant (K_M), which combine to give a catalytic efficiency (k_{cat}/K_M) that is increased by approximately 2.5-fold over the canonical SHP-1cat (Table 1). To test whether the increased activity in V451M SHP-1 is substrate-dependent, we further tested the activity of the mutant with two more PTP substrates—6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, Figure 2B) and a phosphopeptide (DADEpYLIPQQG, Figure 2C)—and found that in each case V451M SHP-1cat had higher activity than its canonical counterpart.

Unlike SHP-1cat, full-length SHP-1 is a multi-domain enzyme that contains two Src-homology 2 (SH2) domains in addition to its PTP domain [25]. In human cells, SHP-1's phosphatase activity is regulated through an autoinhibitory interaction between its catalytic PTP domain and one of its SH2 domains; in SHP-1's autoinhibited state, the amino-terminal SH2 domain blocks the PTP-domain's active site. To investigate whether or not the heightened activity of V451M SHP-1cat is an artifact of expressing the catalytic domain independently, we generated a “full-length” SHP-1 construct (SHP-1fl, comprising amino acids 1–542), and we expressed and purified both canonical and V451M SHP-1fl. (For the purposes of efficient expression, SHP-1fl lacks the unstructured C-terminal portion of the protein, residues 543–595.) As shown in Table 1, both SHP-1fl enzymes are much less active than their SHP-1cat counterparts, indicating that the full-length enzymes' SH2-mediated autoinhibition is functioning as expected. More to the point, the catalytic activity of V451M SHP-1fl is substantially higher than that of wild-type SHP-1fl over a range of *p*NPP substrate concentrations (Table 1, Figure 2D). These data suggest that V451M-conferred SHP-1 activation is not an artifact of catalytic-domain expression and that the mutation could potentially increase SHP-1 activity in human cells that carry the V451M-encoding allele.

3.2. Catalytic activity of T2406M FAP-1

Classical PTP domains share a high degree of structural and sequence homology to one another, making it likely that the biochemical effects of a particular mutation in one PTP domain will be similar to the effects of the corresponding mutation in a second PTP [12]. We therefore asked whether methionine mutants that correspond to V451M SHP-1 occur in other human PTPs. Indeed, a missense-mutant FAP-1 that has been identified in both healthy individuals [2] and an endometrial-carcinoma patient [20] contains a threonine to methionine mutation at position 2406 (T2406M FAP-1), which corresponds to amino acid 451 in SHP-1 (Figure 1A). To investigate the effects of the mutation, we expressed the catalytic domains of canonical FAP-1 (FAP-1cat) and its T2406M counterpart (T2406M FAP-1cat). We found, in strong correspondence to the data on V451M SHP-1cat, that the T2406M mutation increases the catalytic activity of the FAP-1 catalytic domain over all *p*NPP concentrations tested (Figure 3A). Also, the activation conferred by T2406M appears to be independent of substrate, as comparable levels of activation are observed with three different substrates: *p*NPP (Figure 3A), DiFMUP (Figure 3B), and a phosphopeptide (Figure 3C). Although the increase in catalytic efficiency conferred by the T2406M mutation (1.5-

fold, Table 2) is somewhat smaller than that of V451M in SHP-1, the totality of the activity data of T2406M FAP-1cat present a consistent biochemical picture of very similar activating effects of the two mutations.

3.3. Thermal stabilities of the V451M SHP-1 and T2406M FAP-1 catalytic domains

The activity data presented in Figures 2 and 3 raise an interesting evolutionary question: If a methionine at position 451/2406 increases the catalytic efficiency of a PTP domain, why did no canonical PTP acquire methionine at this position during the course of human evolution? One possibility is that the methionine mutation only increases activity at the expense of another “desirable” characteristic, such as thermal stability. To investigate whether the heat resistance of the methionine mutants is compromised, we measured the activities of V451M SHP-1cat and T2406M FAP-1cat over a range of temperatures at a fixed substrate concentration (Figure 4). Consistent with earlier results, both methionine mutants displayed heightened activity at 22 °C, as compared to the corresponding wild-type controls. At higher temperatures, however, it was found that the degree of activation decreased as the temperature increased, with both enzymes reaching similar thresholds above which the methionine mutant demonstrated lower activity than its wild-type counterpart: between 39 and 42 °C for V451M SHP-1cat (Figure 4A) and between 37 and 42 °C T2406M FAP-1cat (Figure 4B). Above 42 °C, the activity of both mutants decreases substantially, dropping off to only a fraction of that of the corresponding wild-type enzymes (Figure 4). Taken together, these data show that 451/2406 methionine mutations significantly reduce the thermal resistance of PTP domains and suggest that, in canonical PTP sequences, methionine may have been excluded due to the thermal instability that is conferred by its side chain.

4. Discussion

In the current study, we have investigated the biochemical effects of a missense mutation that occurs naturally in two PTPs, SHP-1 and FAP-1, and has been found in patients with CLL (SHP-1) and endometrial carcinoma (FAP-1), as well as healthy individuals (both SHP-1 and FAP-1). Our data show that, despite computational predictions of generally compromised function [15–17], both methionine mutations lead to increases in PTP activity at low-to-moderate temperatures. At high temperatures, however, the activities of the mutants fall off dramatically, in comparison to their wild-type counterparts.

While the structural basis for the methionine-mutant behavior has yet to be elucidated, a potential explanation can be inferred from crystal structures of wild-type PTPs. In wild-type SHP-1 the V451 side chain packs close to two isoleucine side chains (I463 and I464) that extend from an adjacent α -helix [25]. A mutation to a longer, straight-chain amino acid such as methionine could disturb these interactions, increasing the conformational flexibility of the PTP loop, and therefore augmenting the low-temperature activity of the enzyme. The cost of this increased flexibility is potentially the disruption of the PTP domain’s “jigsaw-like” packing of its hydrophobic core, a loss that could lead to the decreased thermal stability. In short, the temperature-dependent behavior of V451M SHP-1 and T2406M FAP-1 is reminiscent of enzymes from psychrophilic organisms, which often display activities greater than those of their mesophilic counterparts at low-to-moderate

temperatures, but are heat inactivated more readily than “normal” enzymes from mesophiles [26].

While the biochemical effects of V451M and T2406M mutations are presented here, the physiological consequences of the non-canonical methionine remain to be explored. It is difficult to infer from the current data whether the mutations would augment, reduce, or have little effect on PTP activity in living cells. Although V451M SHP-1 exceeds and T2406M FAP-1 matches wild-type activity at the temperature of human physiology (37 °C) in *in vitro* assays (Figure 4), it is possible that the mutations compromise enzymatic activity and/or protein stability under cellular conditions. Nevertheless, these data show that the functional consequences of missense mutations can vary dramatically under different conditions and suggest that, during the course of human evolution, canonical PTP domain sequences have been optimized for stability, rather than activity, at position 451/2406. More broadly, this study highlights the difficulty in making functional predictions from mutational data, a phenomenon that will become increasingly prevalent as more individuals are identified as harboring alleles that are predicted—possibly inaccurately—to encode proteins that are defective and/or pathogenic [27].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A rare methionine mutation occurs at the same position in two PTP domains
- The mutation augments catalytic activity for both PTPs, SHP-1 and FAP-1
- The mutation decreases the resistance to increased temperature in both PTP domains
- Stability may have been selected over optimized activity in wild-type PTP sequences

A

SHP-1	(448–461)	PIIVHCSAGIGRTG
FAP-1	(2403–2416)	PIITHCSAGIGRSG
PTP1B	(210–223)	PVVVHCSAGIGRSG
Lyp	(222–235)	PICIHCSAGCGRTG
MEG2	(510–523)	PIVVHCSAGIGRTG

B

Figure 1.

(A) Primary sequence alignment of motif 9 in human SHP-1 with that of human FAP-1 and, for reference, several other human classical PTPs. The position corresponding to 451 of SHP-1 (2406 of FAP-1) is highlighted. Note that the amino-acid numbering of the SHP-1 and FAP-1 PTP domains is highly divergent because the two proteins include different complements of non-catalytic domains N-terminal to their homologous PTP domains. (B) Three-dimensional structure of SHP-1's catalytic domain (PDB ID: 4hjp) [13]. SHP-1 is shown as a ribbon, with the PTP loop colored yellow and the flanking β -strand and α -helix colored green and blue, respectively. The side chain of V451 is shown in gray.

Figure 2.

The V451M mutation increases the catalytic activity of SHP-1. (A) Wild-type SHP-1cat (closed circles, 50 nM) and V451M SHP-1cat (open circles, 50 nM) were assayed for PTP activity with the substrate *p*NPP at the indicated concentrations. The initial rates of the resulting reactions were fit to the Michaelis-Menten equation to derive the kinetic constants shown in Table 1. (B) Wild-type SHP-1cat (black bar, 2.5 nM) and V451M SHP-1cat (white bar, 2.5 nM) were assayed for PTP activity with the substrate DiFMUP (6.25 μM). The initial rates of the resulting reactions were normalized to the average wild-type rate. (C) Wild-type SHP-1cat (closed circles, 15 nM) and V451M SHP-1cat (open circles, 15 nM) were assayed for PTP activity with the phosphopeptide DADEpYLIPQQG as substrate. (D) Wild-type SHP-1fl (closed circles, 500 nM) and V451M SHP-1fl (open circles, 500 nM) were assayed for PTP activity with the substrate *p*NPP at the indicated concentrations. The data were fit as described for Figure 2A.

Figure 3.

The T2406M FAP-1 catalytic domain possesses increased catalytic activity. (A) Wild-type FAP-1cat (closed circles, 50 nM) and T2406M FAP-1cat (open circles, 50 nM) were assayed for PTP activity with the substrate *p*NPP at the indicated concentrations. The initial rates of the resulting reactions were fit to the Michaelis-Menten equation to derive the kinetic constants shown in Table 2. (B) Wild-type FAP-1cat (black bar, 5 nM) and T2406M FAP-1cat (white bar, 5 nM) were assayed for PTP activity with the substrate DiFMUP (50 μM). The initial rates of the resulting reactions were normalized to the average wild-type rate. (C) Wild-type SHP-1cat (closed circles, 50 nM) and V451M SHP-1cat (open circles, 50 nM) were assayed for PTP activity with the phosphopeptide DADEpYLIPQQG as substrate.

Figure 4.

The activities of V451M SHP-1cat and T2406M FAP-1cat are less resistant to heat than their canonical counterparts. Wild-type and V451M SHP-1cat (50 nM, panel A) or wild-type and T2406M FAP-1cat (50 nM, panel B) were assayed for PTP activity with the substrate *p*NPP (10 mM) at the indicated temperatures. The initial rates of the V451M SHP-1cat and T2406M FAP-1cat reactions were normalized to the rates of their respective wild-type enzymes at each temperature.

Table 1

Kinetic constants of wild-type and V451M SHP-1 constructs assayed with *p*NPP at 22 °C.

enzyme	k_{cat} (s ⁻¹)	K_M (mM)	k_{cat}/K_M (mM ⁻¹ s ⁻¹)
Wild-type SHP-1 cat	6.2 ± 0.34	4.5 ± 0.42	1.4
V451MSHP-1 cat	9.8 ± 0.46	2.8 ± 0.11	3.5
Wild-type SHP-1 fl	0.80 ± 0.078	9.5 ± 1.4	0.084
V451MSHP-1 fl	1.3 ± 0.97	10.3 ± 0.68	0.13

Table 2

Kinetic constants of wild-type and T2406M FAP-1 cat assayed with *p*NPP at 22 °C.

enzyme	k_{cat} (s ⁻¹)	K_M (mM)	k_{cat}/K_M (mM ⁻¹ s ⁻¹)
Wild-type FAP-1 cat	5.8 ± 0.29	2.4 ± 0.22	2.4
T2406M FAP-1 cat	8.3 ± 0.81	2.4 ± 0.37	3.5