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Research Article

Multifunctional Transactivator of Transcription (Tat) Protein of Human Immunodeficiency Virus-1 as a Potential Drug Target

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Abstract

The HIV-1 transactivator of transcription (Tat) protein plays a pivotal role in the infectious lifecycle of human immunodeficiency virus-1. The objective of this study is to find the degree of conservation of the Tat protein and to detect conserved binding sites, which might be used as target sites for potential anti-Tat drugs. The conservation analysis was based on 3,365 amino acid sequences for Tat protein. The conservation analysis revealed a number of conserved and variable residues. The universally conserved residues identified in this study might be involved in either structure stabilizing or protein-protein interactions. The novel conserved residues which have been identified are Lys88, Lys89, Val91, Glu92, Glu94 and Thr95. Along with conservational analysis, structural analysis revealed novel binding sites, namely Cys22, Cys25, Cys27, Cys30, His33, Cys34, Cys37; Ile45, Lys51, Arg53, Gln54, Arg55; Gly44, Arg49, Lys50; Asp2, Val4, Ile8 and Met1, Pro3, Val4, Ile45, Tyr47, Lys50, Lys51, Arg52, Arg53. The outcome of this study provides the basis for developing anti-Tat drugs which have abridged potential to induce drug resistance through mutations.

Keywords: Tat protein, Conservation, Drugs, Mutation, Resistance, HIV-1, Binding sites

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Introduction

Acquired immunodeficiency syndrome (AIDS) is caused by the infectious agent human immunodeficiency virus (HIV), affecting estimated 2.5 million people and results in 1.5 million deaths in the year 2014 [1]. In the past few years, owing to substantial rise in the usage of anti-retroviral therapy, there has been a stable decline in the AIDS associated deaths [1]. There are two types of HIV namely, human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2) [2, 3]. Since the discovery of HIV-1 (which was way back in 1983) up till now, HIV-1 remains to be the foremost concern regarding public health globally [4]. By realizing the devastating nature of HIV, the researchers globally are unified to uncover solutions which will facilitate in fighting this elusive virus. As a result of strong and determined efforts, the highly active antiretroviral therapy (HAART) was developed [5-7]. HAART has drastically improved and raised the lifespan of patients affected with HIV; yet it is unable to eradicate the virus totally from the HIV patients. This is down to the fact that infected cells are resistant to the apoptosis which is bestowed by the HIV. Without eliminating these cells it is impractical to eradicate the virus absolutely from an infected individual. Therefore is of urgent importance to develop antiretroviral drugs against those proteins which are accountable for conferring apoptosis resistance to the infected cell.

HIV-1 is a RNA virus which belong to lentivirus class of retrovirus, whose genome encodes three structural proteins (gag, pol and env), two regulatory proteins (tat and rev) and four accessory proteins (vpu, vif, nef and vpr) [8, 9]. The transactivator of transcription (Tat) protein (M_W = 14-16 kDa) of HIV-1 is an early regulatory protein with variable length of 86-101 residues which are encoded by two exons [10]. Residues 1-72 are encoded by the first exon, while the residues 73-101 are encoded by the second exon [10]. Full length Tat protein is known to divided into six functional domains namely, the first domain is N-terminal domain (NTD; residues 1–21; acidic domain), second domain (residues 21–37; cysteine-rich domain), third domain (residues 38–48), fourth domain (residues 49–57; basic domain), fifth domain (residues 58–72) and sixth domain (amino acid 73–101) [10-12]. Residues 1-48 are enough for the Tat protein to carry out transactivation of the transcription [12]. Residues 49-57 are essential for nuclear localization of Tat protein, Tat-transactivation responsive element RNA binding and uptake of Tat protein by other cells [13-17]. Residues 48-60 are utilized by the Tat protein for functional internalization into the infected cell [18, 19]. Residues 73-101 are thought to contribute in the process of viral infectivity and in Tat-integrins binding [20-22]. Residues 78-80 form a short motif and which is ligand for integrins [20]. The residues 86-92 form another motif in the C-terminus of the protein which is related to the optimal replication of HIV-1 virus [23]. The residues E92, E94 and E96 or K88-K90 play a crucial role in activation of NF-κB, in transactivation of long-terminal repeats of HIV-1 and in enhancing the replication of HIV-1 in the T-cells [24]. Residues 30-55 were found to be involved in Tat-Sp1 binding [25]. The Tat-DNA-PK binding region was found to be in between the residues 56-101 [26]. Residues 47-67 were found to be crucial for Tat-C/EBPβ interaction [27]. Residues 1-26 is mapped as the binding site for the transcription factor NFAT1 [28]. The Tat-phosphatidylinositol (4, 5) bisphosphate binding is

fundamentally relied on the residues 11 and 49-51 [29, 30]. Tat-tubulin binding region was mapped between the residues 36-39 [31]. Tat-protein phosphatase-1 interaction is facilitated by the residues V36 and F38 [32].

Tat protein of HIV-1 is a multifunctional protein whose function include: (i) chromatin remodelling (ii) RNA polymerase II phosphorylation (iii) viral genes transactivation (iv) binding to viral mRNA specific structures (v) modulation of cellular gene expression (vi) up regulation of non-viral genes (vii) associated with reverse transcription (viii) immune suppression (ix) induction of neuronal apoptosis (x) associated with anti-apoptosis activities in latent infected cells [33-51].

The emergence of HIV-1 mutants which are drug resistant and the spreading of these mutants globally are serious cause of concern. To overcome this problem one has to exploit HIV-1 proteins as a potential drug targets. In order to achieve this target one has to understand the conservation pattern of these proteins. The Tat-based therapeutics available which targets the Tat protein are Stilbene (CGA137053) and Suramin along with its derivatives [52, 53]. But the main problem with the above said drugs are that these drugs get ineffective as a result of mutations in the HIV-1 Tat protein. To overcome this problem one has to exploit the conserved residues in the HIV-1 Tat protein. The aims of this study are to identify the degree of conservation of Tat protein to facilitate the detection of regions with universal conservation. By mapping the obtained conservational scores on to the protein structure in conjunction with binding site analysis suggests binding sites for antiviral compounds which are resistant to mutations that may arise in the future.

Methods

Sequence analysis and protein structure

The full length sequences of the Tat protein were obtained from UniProt [54]. Sequences thus collected belong to all the known subtypes of HIV-1. For alignment of the collected protein sequences, MUSCLE version 3.8 [55] was used with default parameters. Multiple refinements of the obtained alignment were carried out resulting in 26-30 iterations, until no further improvement was attained. The structures of Tat protein (residues 1-86) was obtained from Protein Data Bank (PDB) [56] with the entries 1TIV [57].

Conservation analysis

By providing multiple sequence alignment (MSA) and experimental protein structure files as an input, conserved regions were identified using ConSurf server (http://consurf.tau.ac.il/) [58-61]. By taking evolutionary relationships between protein sequences into account, ConSurf algorithm generates resultant conservation scores. ConSurf algorithm emphasizes more on those protein sequences, which are evolutionarily distant, thus calculating conservation scores which are significant [58-61]. The resultant conservation scores are criterion scores (0, 1). The residues with score lesser than 0 indicate higher conservation, while those with score greater than 0 are variable residues [58-61]. The Bayesian algorithm is utilized to appraise the confidence intervals of calculated conservation scores [58-61]. The conservation scores given by ConSurf server are separated into scale of nine grades, which are given with the intention for visualization [58-61]. Most variable positions are placed in grade one (turquoise), intermediately

conserved positions are placed in grade five (white), and most conserved positions are placed in grade nine (maroon) [58-61].

Binding site analysis

Ligand binding sites (LBS) on Tat protein structure were identified using COFACTOR (http://zhanglab.ccmb.med.umichedu/COFACTOR/), identifies the LBS using both global and local with templates from PDB and match local motifs of the identified template with that of query structure [62-64]; TM-site (http://zhanglab.ccmb.med.umich.edu/COACH/), identifies the LBS using intermediary approach if both local and global alignments [64, 65]; S-site (http://zhanglab.ccmb.med.umich.edu/COACH/), identifies the LBS by explicitly comparing binding site specific sequence profiles [64, 65] and SiteHound (http://scbx.mssm.edu/sitehound/sitehound-web/Input.html) which uses energy based method to find regions with high potential for ligand interactions [66].

Results

Multiple alignments of protein sequences and Tat protein structure

For the HIV-1 Tat protein, a total of 3,365 sequences were obtained from UniProt [54]. None of the templates identified in the Protein Data Bank (PDB) [56] has covered the full length Tat protein. The region between the residues Met1-Glu86 of the Tat protein is the only one whose structures are experimentally solved [57].

Conserved and variable residues

The variable and conserved residues in the Tat protein were identified using ConSurf server [58-61] and are illustrated in figure 1. The variable residues of grades 1-3; conserved residues of grades 7-8 and highly conserved residues of grade 9 are clustered together in Table 1. The conservation scores were projected onto the spacefill model of the tat protein Figure 1.

Table 1 Variable and conserved residues of Tat protein of HIV-1 identified using the ConSurf server [58-61].

Residues	Transactivator of transcription (Tat) protein
Highly Conserved (grade 9)	Met1, Asp5, Pro10, Trp11, His13, Pro14, Gly15, Ser16, Pro18, Thr20, Cys22, Cys25, Cys27, Ser28, Cys30, His33, Cys34, Cys37, Phe38, Lys41, Leu43, Gly44, Ile45, Gly48, Arg49, Lys50, Lys51, Arg55, Arg56, Gln66, Gln72, Val91
Conserved (grades 7-8)	Val4, Pro6, Leu8, Glu9, Gln17, Gly42, Ser46, Arg52, Thr82, Gly83, Glu86, Lys88, Lys89, Glu92
Variable (grades 1-3)	Lys7, Lys19, Ala21, Asn24, Ser29, Phe32, Val36, Thr39, Arg40, Ala58, Pro59, Arg60, Asp61, Gln63, Thr64, Ala67, Ser68, Leu69, Ser70, Ala74, Ser75, Pro77, Pro81, Lys85, Glu96, Thr97, Pro99, Glu100, Asp101

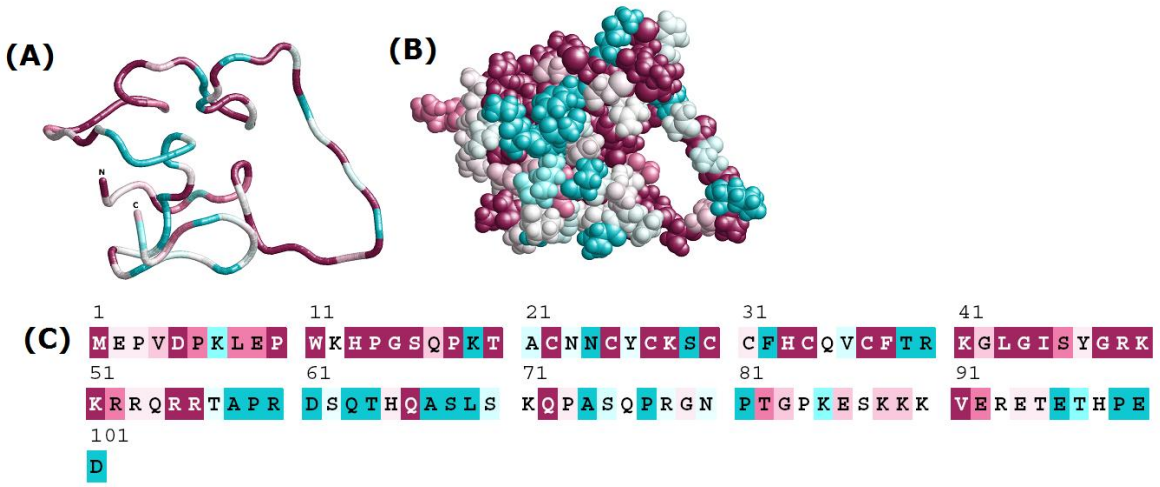

Figure 1 The conservation obtained by projecting conservation scores onto the ribbon structure of the Tat protein (A); onto the spacefill structure of the Tat protein (A) and the residue conservation of the Tat protein (C) obtained from ConSurf server [58-61].

Conservation scores for the Tat protein were obtained between the values -0.963 (maximum conservation) and 4.530 (maximum variability) by the ConSurf server [59-62]. In general the Tat protein is poorly conserved with 38.7% of the residues belongs to grades 8-9 (conserved), while 27.7% of the residues belong to grades 1-2 (variable). Altogether, thirty two residue positions (31.7% of total residues) were found to be highly conserved (grade 9). In total, hundred and twenty seven residues (21.8% of total residues) were found to be highly variable (grade 1).

Small molecule binding potential

One of the important objectives of the present study was to detect potential drug binding sites which are spatially in close proximity to the conserved regions. Putative ligand binding sites were identified using COFACTOR (detects LBS by global and local alignments with template structures in PDB), TM-site (detects LBS by transitional approach balancing global and local alignments), S-site (detects LBS by comparing sequence profiles which are binding site specific) and SiteHound (detects LBS by positive interaction between a chemical probe and protein structure) algorithms [62-66]. By amalgamating the binding site results with that of evolutionary analysis, binding sites which are in the regions of conservation were identified on Tat protein, which are shown inTable 2.

In total, thirteen potential binding sites with high conservation were found on the HIV-1 Tat protein. Binding sites 1-4 and site 13 were found to be completely conserved. Site 1, site 4, site 5 and site 12 were found to be located in the second domain of Tat protein. Site 2 and site 3 were found to be located in the fourth domain of the protein. Site 6 was found to span second, third, fourth and fifth domains of the protein. Site 7 was found to span the first, fifth and sixth domains of the protein. Site 8 was found to span the second and third domain of the protein. Site 9, site 10 and site 13 are found to span first, third and fourth domains of the protein. Site 11 was found to span first and third domains of the protein.

Table 2 The putative conserved ligand binding sites identified on Tat protein using COFACTOR, TM-site, S-site and SiteHound algorithms [62-66].

Site	Binding sites located in conserved regions of Tat protein
1	Cys22, Cys25, Cys27, Cys30, His33, Cys34, Cys37
2	Ile45, Lys51, Arg53, Gln54, Arg55
3	Gly44, Arg49, Lys50
4	Asp2, Val4, Ile8
5	CYS25, HIS26, CYS27, LYS28, CYS34
6	TYR32, GLY42, LEU43, GLY44, ILE45, SER46, LYS50, GLN54, ARG56, GLN63
7	ASP5, PRO6, ASN7, ILE8, PRO14, PRO70, GLN76
8	TYR32, HIS33, CYS34, LYS40, LYS41, GLY42, SER46
9	VAL4, PRO6, ASN7, PHE38, ILE39, TYR47, LYS51,
10	PRO3, VAL4, ASP5, PRO6, ILE39, TYR47, GLY48, ARG49, LYS51
11	VAL4, PRO6, ASN7, GLU9, PRO10, PHE38, ILE39, TYR47, GLY48
12	ASP5, PRO6, ASN7, ILE8, GLU9, HIS13, PRO14
13	MET1, PRO3, VAL4, ILE45, TYR47, LYS50, LYS51, ARG52, ARG53

Note: Conserved residues (grade 7-8) are shown in bold face while highly conserved residues (grade 9) are shown in bold face and underlined.

Discussion

Sequence conservation

The objective of the present study was to determine the degree of conservation of the Tat protein among all the subtypes of human immunodeficiency viruses. The Tat protein from all subtypes were analyzed together to facilitate the identification of universally conserved residues of potential pandemic HIV viruses that might arise in future due to either a event of mutation. The conserved residues detected on the Tat protein may have either functional importance or structural importance [67]. On the contrary, variable sites arise as a result of either adaptation or evolutionary pressure to evade the host immune system [67].

In the established functionally important regions of the Tat protein high conservation was found, for example in the region (residues 49-57) essential for nuclear localization of Tat protein, Tat-transactivation responsive element RNA binding and uptake of Tat protein by other cells, residues Arg49, Lys50, Lys51, Arg55 and Arg56 were found to be highly conserved (grade 9). In the region (residues 48-60) utilized by

the Tat protein for functional internalization into the infected cell, residues Gly48, Arg49, Lys50, Lys51, Arg55 and Arg56 were found to be highly conserved (grade 9). The region (residues 73-101) thought to contribute in the process of viral infectivity and in Tat-integrins binding, a lone residue Val91 was found to be highly conserved (grade 9), while residues Thr82, Gly83, Glu86, Lys88, Lys89 and Glu92 were found to be conserved (grades 8-7). The region (residues 78-80) which form a short motif and which is ligand for integrins, was found to be variable. The region (residues 86-92) of the protein which is related to the optimal replication of HIV-1 virus, a lone residue Val91 was found to be highly conserved (grade 9), while residues Thr82, Gly83, Glu86, Lys88, Lys89 and Glu92 were found to be conserved (grades 8-7). Among the residues (Glu92, Glu94 and Glu96 or Lys88-Lys90) which play a crucial role in activation of NF-κB, in transactivation of long-terminal repeats of HIV-1 and in enhancing the replication of HIV-1 in the T-cells, only residues Lys88, Lys89 and Glu92 were found to be conserved (grades 8-7). In the Tat-Sp1 binding region (residues 30-55), residues Cys30, His33, Cys34, Cys37, Phe38, Lys41, Leu43, Gly44, Ile45, Gly48, Arg49, Lys50, Lys51 and Lys55 were found to be highly conserved (grade 9). In the Tat-DNA-PK binding region (residues 56-101), residues Arg56, Gln66, Gln72 and Val91 were found to be highly conserved (grade 9) while residues Thr82, Gly83, Glu86, Lys88, Lys89 and Glu92 were found to be conserved (grades 8-7). In the Tat-C/EBPβ interaction region (residues 47-67), residues Gly48, Arg49, Lys50, Lys51, Arg55, Arg56 and Gln66 were found to be highly conserved (grade 9). In the Tat- NFAT1 interaction region (residues 1-26), residues Met1, Asp5, Pro10, Trp11, His13, Pro14, Gly15, Ser16, Pro18, Thr20, Cys22 and Cys25 were found to be highly conserved (grade 9). The residues (11 and 49-51) on which Tat-phosphatidylinositol (4, 5) bisphosphate binding is fundamentally relied were found to be highly conserved (grade 9). In the Tat-tubulin binding region (residues 36-39), residues Cys37 and Phe38 were found to be highly conserved (grade 9). Among the residues (Val36 and Phe38) which facilitate the Tat-protein phosphatase-1 interaction, only Phe38 was found to be highly conserved.

Small molecule binding potential

Most of the predicted LBS identified on the Tat protein are surrounded by conserved residues. Targeting these LBS using small drug molecule will most probably result in disruption of Tat function. Among the predicted binding sites identified by different methods, few novel binding sites were detected on the Tat protein. These novel sites may be either involved in new protein-protein interactions or sites of known protein-protein interactions. The novel binding sites identified on Tat protein are binding site 1 to binding site 4 and binding site 13. These novel sites are completely conserved. Targeting these sites using small ligand molecule would inhibit the functions of the Tat protein. These sites might be functionally important which can be explained by the degree of conservation found in these sites and the functions of these sites are not elucidated yet.

In order to significantly inhibit the nuclear localization of Tat protein, Tat-transactivation responsive element RNA binding and uptake of Tat protein by other cells, binding sites- site 2, site 3, site 6, site 9, site 10 and site 13 should be targeted with small drug like molecules. In order to inhibit the functional internalization of Tat protein into the infected cell, binding sites- site 2, site 3, site 6, site 9, site 10, site 11 and site 13 should be targeted with small drug like molecules. By targeting the binding site 7 with small drug like molecules the process of viral infectivity and in Tat-integrins binding can be inhibited. In order to inhibit the Tat-Sp1 binding, binding sites- site 1-site 3, site 5, site 6, site 8-site 11 and site 13 should be targeted with small drug like molecules. By targeting the binding site 6 and binding site 7 with small drug

like molecules, Tat-DNA-PK binding can be inhibited. In order to inhibit Tat-C/EBPβ interaction, binding sites- site 2, site 3, site 6, site 9, site 10, site 11 and site 13 should be targeted with small drug like molecules. . In order to inhibit Tat- NFAT1 interaction, binding sites- site 4, site 5, site 7, site 9- site 13 should be targeted with small drug like molecules. In order to significantly inhibit the Tat-phosphatidylinositol (4, 5) bisphosphate binding, binding sites- site 2, site 3, site 6, site 9, site 10 and site 13 should be targeted with small drug like molecules. By targeting the binding site 1 and binding sites 9-11 with small drug like molecules, Tat-tubulin binding can be inhibited. By targeting the binding site 9 and binding site 11 with small drug like molecules, Tat-protein phosphatase-1 interaction can be inhibited.

Conclusion

In conclusion the study of 3,365 Tat protein sequences divulge an elevated level of sequence conservation pattern that intersects with prospective ligand binding site analysis rendering the Tat protein as an exceptional drug target. Targeting the conserved binding sites identified in this study using small drug molecule will effectively diminish the activity of the protein. Moreover, anti-Tat drugs targeting these conserved sites are less likely to become ineffective due to drug resistance in the future.

Abbreviations

Tat, Transactivator of transcription; HIV, Human immunodeficiency virus; HAART, highly active antiretroviral therapy; RNA, ribonucleic acid; MSA, Multiple Sequence Alignment; PDB, Protein Data Bank; LBS, Ligand binding sites.

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