

COMPUTATIONAL CHARACTERISTICS OF VALPROIC ACID BINDING TO HISTONE DEACETYLASE

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أثبتت الدراسات الحديثة التي أجريت على دواء حمض فالبرويك المعروف بفاعليته في علاج الصرع، بأن له تأثير فاعل في علاج السرطان وذلك عن طريق تثبيط فاعلية إنزيم هيستون دياسيتيلاز. ويعد حمض فالبرويك ذو فاعلية عالية التأثير على الخلايا السرطانية دون التأثير على الخلايا العادية وذلك بالمقارنة بدواء ترايكوستاتين - أ المعروف بفاعليته في تثبيط هذا الإنزيم. وحتى الآن لم يتم اكتشاف الاختلاف الجوهري بين الدوائين على المستوى البيولوجي، ولذا تعد الدراسة الحالية بمثابة وسيلة للتعرف على الاختلاف في الارتباط بين كل منهما مع إنزيم هيستون دياسيتيلاز باستخدام الدراسات النظرية الكومبيوترية التي تتناول قياس الروابط الهيدروجينية ودرجة ثبوت الارتباط بين المادة المختبرة والإنزيم. وقد تناولت هذه الدراسة العديد من الحسابات الكومبيوترية للطاقة الناتجة من التغيرات في الشكل البنائي للدواء المستخدم التي أثبتت أن حمض فالبرويك ذو قوة فائقة في الارتباط بالإنزيم عن طريق الروابط الأيونية والهيدروجينية بطريقة مختلفة ومتميزة عن تلك المعروفة من قبل ترايكوستاتين. وذلك يؤيد الإثباتات الحديثة لتأثير حمض فالبرويك في درجة تخصصه العالية في تثبيط نمو الخلايا السرطانية. ولذا تعد استراتيجية التصميم لمشتقات جديدة من حمض فالبرويك من المهام الفعالة للحصول على مركبات جديدة ذات درجة عالية من الفاعلية وأقل درجة من السمية.

Recently, the antiepileptic drug valproic acid (VPA) has also demonstrated efficacy in the management of cancer and bipolar disorders. These actions are largely mediated by inhibition of the HDAC enzyme/induction of certain genes. Relative to other HDAC inhibitors such as trichostatin-A (TSA), VPA offers higher selectivity on cancer cells with virtually no detrimental effects on normal cells. The molecular underpinnings of these biological profiles for VPA remain undefined. We currently propose for and attempt to identify differences in the binding of VPA and TSA to HDAC. In this paper, conformational changes and energy calculations have been derived. VPA had to accomplish conformational changes in its structure for best accommodation at the HDAC binding site. Energy computations showed that VPA has a lower binding affinity than TSA (-53.80 vs. -66.30 Kcal/mol). These findings demonstrate that VPA binding to HDAC confers catalytic, conformational, and computational characteristics that are distinct from those of TSA. These findings for VPA are consistent with a moderate inhibition of HDAC, a low toxicity on normal cells, and a higher selectivity on cancer cells than TSA. Accordingly, these newly identified binding properties of VPA can state a framework strategy for the rational design of VPA-related anticancer drugs with superior cytodifferentiating- and/or safety-profiles.

Key words: Computational, valproic-acid (VPA), chemoprevention, histone-deacetylase (HDAC)-inhibitors, anti-cancer drugs, ligand-binding mechanism.

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Introduction

Cancer ranks second only to cardiovascular disease as a cause of mortality. Unfortunately, chemotherapeutic drugs tend to indiscriminately destroy both normal and tumor cells, as a consequence of their remarkably low therapeutic windows. Moreover, the frequent eruption of resistance to these drugs poses a serious challenge to their efficacy in cancer management. Hence, the search has unfailingly continued to identify more promising anti-tumor agents (1-3). It is largely accepted that agents, which stimulate the differentiation and/or apoptosis of cancer cells can hold much efficacy, selectivity, and safety than conventional chemotherapeutic agents (4,5). In this context, a growing number of natural products have been found to promote differentiation and limit proliferation of tumor cells. These include retinoic acid, antineoplastons, and short-chain fatty acids, such as butyric acid and valproic acid (VPA) (Figure 1) (6-9). In carcinoma cells and leukemic blasts from acute myeloid leukemia patients, VPA was shown to appreciably promote cellular differentiation (10-12). Likewise, VPA significantly reduced tumor growth and metastasis in a variety of *in vivo* animal models (12). Accordingly, there has been a great interest in identifying biological targets of the VPA-evoked anti-tumor effects, hoping to rationalize for newer generations of anti-cancer drugs. In this direction, a breakthrough was the finding that VPA can inhibit the nuclear enzyme histone deacetylase (HDAC), a negative regulator of gene expression whose inhibition can disrupt the DNA machinery to promote differentiation of tumor cells (12-14). Not surprisingly, therefore, HDAC has become a milestone target in cancer treatment and prevention. Mechanistically, VPA is thought to, somehow, mimic the actions of the HDAC-inhibitor (HDACi), trichostatin-A (TSA) (Figure 1), a fungistatic antibiotic from *Streptomyces platensis* that is known to activate transcription from diverse exogenous and endogenous promoters (13). The exact mode of interaction of VPA with the HDAC active site has, nevertheless, remained ambiguous. By far, ligand binding studies performed with radiolabeled VPA revealed that TSA efficiently competes for the binding of VPA, thus suggesting that both compounds may have a common or overlapping binding sites whereby they could block the utilization of the substrate acetyl-lysine" to HDAC (Figure 2). These

observations, however, largely failed to account for a number of biological and catalytic differences in the actions of VPA and TSA. For instance, VPA was found to be a 2.5-fold less efficacious inhibitor of HDAC than TSA; as evident from the extent of substrate "histone-H4" acetylation observed after treatment with both agents (12). Moreover, whereas VPA was as efficient as TSA in inducing differentiation of the neoplastic Kasumi-1 cells, unlike TSA, it had no effect on the viability of normal hematopoietic stem cells, even at relatively higher concentration (absence of cytotoxicity and apoptosis) (12). Altogether, these observations imply a possibly altered mode of binding of VPA to the active site. To that end, we performed a comparative molecular modeling computational study for binding of both VPA and TSA. Our findings demonstrate for the first time appreciable conformational and computational-differences in the binding of these ligands to HDAC.

Computational methods

All molecular modeling calculations and manipulations were performed using the software package HyperChem6 (15).

Enzyme structure:

Starting coordinates of the bacterial homolog of histone deacetylase (histone deacetylase-like protein) in complex with its reversible inhibitor hydroxamic acid trichostatin (TSA) (entry code ID 1C3R) was obtained from the Protein Data Bank of Brookhaven National Laboratory (16). This enzyme is an HDAC homolog that is commonly used to represent various HDAC isozymes, as it retains the active site core residues (~390 aa) known to be conserved across the eukaryotic HDAC family of enzymes (16).

Molecular structure of valproic acid:

The starting chemical structure of VPA was constructed and the partial atomic charges were assigned with the semiempirical mechanical calculation method "AM1" using HyperChem6 (15, 17).

The lowest energy conformer of VPA was derived from conformational search method where all the rotatable bonds of VPA were searched with an increment of 10^0 using conformational search module as implemented in HyperChem6.

Molecular docking:

The lowest energy conformer of VPA was pre-positioned on TSA at the HDAC active site and the TSA structure was deleted. The lowest energy conformer of VPA was optimally docked in the binding pocket by using the docking routine within HyperChem6. The hydrogen-bond donors and acceptors within the active core surrounding the ligand, the ligand itself, and the selected torsion bonds were defined. The docked HDAC-VPA complex was geometrically optimized using 1000 steps of steepest descent, followed by conjugate gradient minimization until an energy convergence of 0.01 Kcal/molÅ was reached (17). Solvation energies were not explicitly considered; however, calculations were performed with a distance-dependant dielectric constant of $\epsilon = 4$ and a non-bonded cut-off of 12Å to simulate the solvation effect of the ligand in the enzyme environment. The energy of interaction was calculated according to the formula: $E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{ligand}} + E_{\text{enzyme}})$ (18-20).

Results and discussion

VPA has long been known as an anticonvulsant agent, acting through the upregulation of the inhibitory GABA-system. Besides, VPA produced a number of slower, GABA-independent biological responses that appeared to be of genomic origin (10). These include potent teratogenic potential, a lithium-like capacity to treat bipolar disorders, and distinct cytodifferentiating actions on malignant cells (11, 12). Subsequent mechanistic studies revealed that these nuclear effects are produced by inhibition of the enzyme HDAC; a critical nuclear component that can alter gene expression and nucleosome conformation (16). HDAC catalyses the removal of acetyl group from the ϵ -amino group of a lysine residue clustered near the amino terminus of nucleosomal histones, a process that is associated with transcriptional repression (16). The deregulation of nuclear HDAC recruitment appears, therefore, to be one of the mechanisms that contributes to tumorigenesis. Moderate inhibition of HDAC, as seen with VPA, appears to selectively induce tumor cellular differentiation, while robust suppression of this enzyme, as seen with TSA causes suppression of vital host cells as well (12). The molecular mechanisms dictating VPA-triggered inhibition of HDAC, when characterized, can accordingly fuel a valuable strategy to develop superiorly effective/selective

anticancer VPA-homologs. This study was, hence, designed to delineate the mode of VPA binding to HDAC active site.

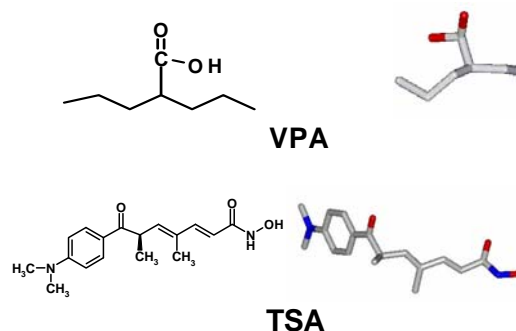


Figure 1. Chemical structures of valproic acid (VPA) and trichostatin-A (TSA) in solid bonds and their corresponding energy-minimized, three-dimensional structures.

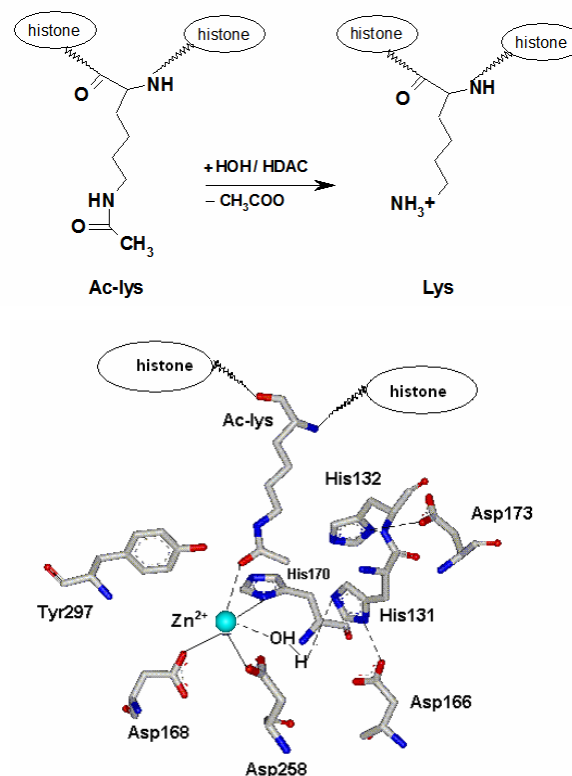


Figure 2. The proposed catalytic mechanism of deacetylation of acetylated-lysine, representing the key active site residues of HDAC with its acetylated-lysine substrate poised for hydrolysis.

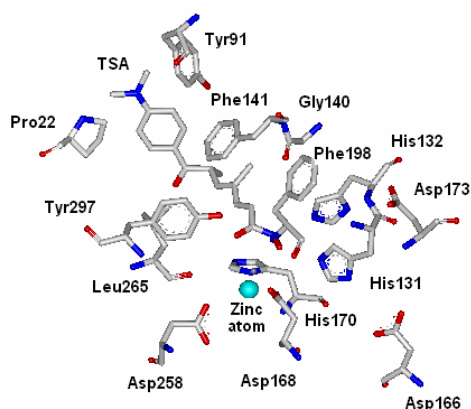


Figure 3. Schematic representation of TSA binding at the HDAC substrate-binding site.

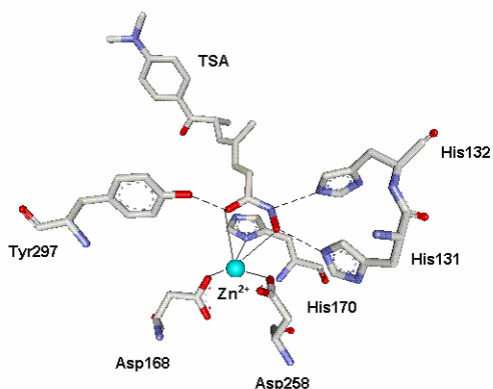


Figure 4. Binding mode of the energy-minimized and geometrically optimized TSA at the HDAC binding pocket showing residues involved in its recognition (dashed lines). Solid lines indicate chelation of Zn-atom with TSA and HDAC active site residues.

HDAC active site:

As depicted in (Figure 3), the active site of HDAC consists of a tubular pocket, a zinc-binding site and two Asp-His charge-relay systems (Asp166-His131 and Asp173-His132). The walls of the pocket are covered with some hydrophobic and aromatic residues (Pro22, Gly140, Phe141, Phe198, Leu265 and Tyr297). The zinc ion is positioned near the bottom of the pocket and coordinates with Asp168, His170 and Asp258. The Asp166-His131 charge-relay pair is positioned deeper inside the pocket and is more buried than the Asp173-His132 charge relay, which is partially solvent exposed (16).

The importance of the buried asp166-his131 charge-relay system relates to its negative charge, which enhances the nucleophilicity of this moiety to stimulate hydrogen bonding with a water molecule (16). Mutation studies of the histidine and aspartic acid residues (Asp166-His131) of the buried charge-relay system abolished the HDAC activity (16, 21). Conversely, mutation of histidine of the exposed charge relay (Asp173-His132) only-reduced the enzyme activity. An additional crucial HDAC-pocket residue is Tyr297, as evidenced also by mutation studies (21).

Mechanism and computations of TSA-induced HDAC inhibition:

TSA reversibly inhibits HDAC activity by displacing the zinc atom, thus rendering the charge-relay system dysfunctional. This process interferes with the removal of the acetyl groups from lysine residues in the amino-terminal tails of the nucleosomal core histones, and ultimately causes transcriptional repression (Figure 4) ⁽¹⁶⁾. TSA has been established as the most potent reversible HDACi (IC₅₀ in low nM range) (13). To trigger HDAC inhibition, TSA snugly fits its five-carbon long unsaturated branched aliphatic chain into the pocket tunnel making multiple *van der waals* contacts with all of the hydrophobic groups lining the pocket (Figures 3, 4) (16). The TSA's hydroxamic acid group at one end of the aliphatic chain reaches the polar bottom of the pocket to coordinate with zinc in a bidentate fashion. In this optimal orientation, the hydroxamic acid group forms three hydrogen bonds with both charge-relay histidines (His131, His132), and with the Tyr297's hydroxyl group (16). Further, the hydroxamic acid group replaces the zinc-bound water molecule *via* its hydroxyl group (Figure 4). At the other end of the TSA chain, the aromatic dimethylamino-phenyl capping group occupies the hydrophobic area near the tunnel entrance of the pocket. Lastly, the length of the aliphatic chain appears to be optimal for spanning the length of the pocket and allowing contacts both at the bottom and at the entrance of the pocket (Figures 3,4). Computational studies (Tables 1 and 2) indicate the stability of the TSA-HDAC complex, as evidenced by low conformational-energy change ($\Delta E = 0.5$ Kcal/mol) and RMS deviation value ($\approx 0.3\text{\AA}$). Likewise, only slight movement of the pocket residues (RMSd) was required to achieve binding and recognition of TSA.

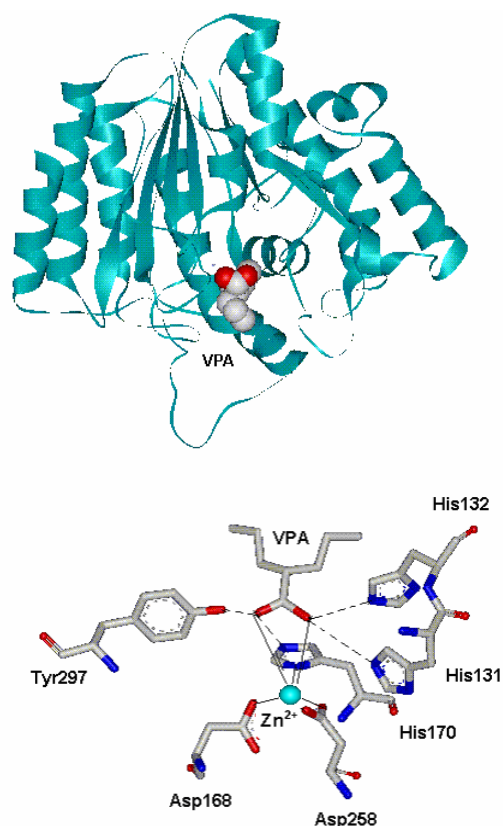


Figure 5. Upper panel: Flat-ribbon presentation of HDAC in complex with the lowest energy conformer of VPA (space-filling). Lower panel: Binding mode of energy- minimized VPA in the HDAC binding pocket, showing residues involved in its recognition (dashed lines). Solid lines indicate chelation of Zn-atom with VPA and HDAC active site residues.

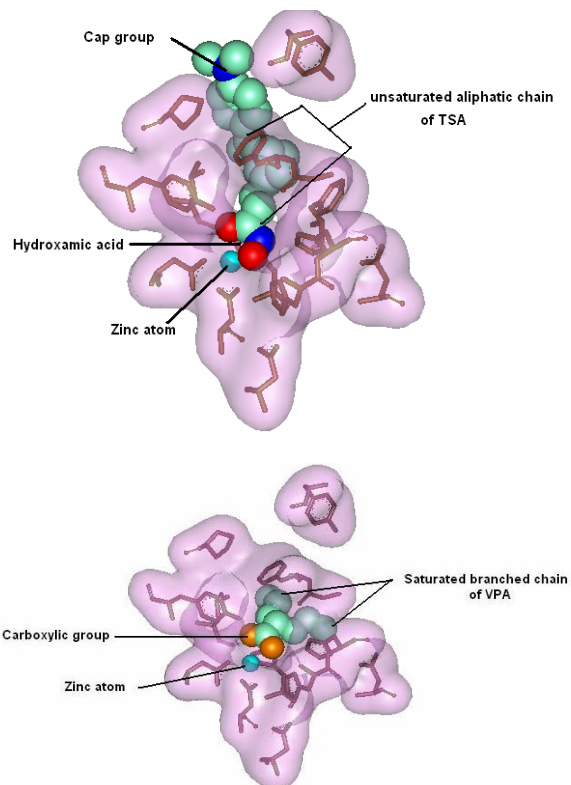


Figure 6. Upper panel: space-filling representation of TSA in the HDAC pocket's active site, showing packed binding of TSA within the HDAC tunnel. Lower panel: space-filling representation of VPA in the HDAC pocket's active site, showing loose binding of VPA within the HDAC tunnel.

Table 1: The calculated ligand-binding interaction energy at the HDAC binding cavity (Kcal/mol)

	$E_{\text{interaction}}^a$	ΔE^b	RMS ^c	Relative potency ^d
TSA	-66.30	0.5	0.3	25
VPA	-53.80	2	0.7	1

Energy of ligand-free enzyme = -172.70 Kcal/mol.

^a $E_{\text{interaction}} = E_{\text{complex}} - (E_{\text{ligand}} + E_{\text{enzyme}})$.

^b ΔE : energy difference between energy of docked conformation and energy of the lowest energy conformer (kcal/mol).

^cRMS: root-mean-square deviation of active site residues (all atoms), as compared to the refined crystal structure.

^dRelative potency for HDAC (11).

Table 2: Hydrogen-bonding interaction of TSA and VPA with the conserved amino acid residues in the active site of HDAC

AA	TSA	VPA
His131	2.8*	3.2
His132	2.8	2.2
His170	-	3.1
Tyr297	2.4	3.0

* Values define hydrogen-bond length in Å.

Mechanism and computations of VPA recognition by the HDAC enzyme:

Studies showed that VPA relieves repression of transcription factors by inhibiting histone deacetylases. At the molecular level, VPA putatively inhibits HDAC by binding to and blockade of substrate access to the HDAC pocket (12). In this context, VPA is thought to mimic the interaction of TSA with HDAC; despite of the yet unexplained differences observed in catalytic and biological effects of these two inhibitors (12). It is generally agreed that these differences are partly entailed to some pharmacokinetic/ pharmacodynamic reasons, like altered cellular penetrability, metabolism, or presence of secondary targets/ HDAC-isoform specificity (12). However, the disparity in “*in vitro*” potency of inhibition of HDAC is most indicative also of altered mode and/or extent of binding to the HDAC active site. This preposition prompted us to attempt delineating the binding mode of both ligands to this enzyme pocket. The active site used is a HDAC-homolog that retains the active site core residues necessary for ligand binding and recognition, thus well represents various HDAC-isozymes (12). We presently report both qualitative and quantitative differences in the recognition of VPA and TSA by HDAC. Notably, the VPA-HDAC complex produced “four” H-bonds in total; in which the carbonyl oxygen formed two bifurcated H-bonds with His170 and Tyr297 (Table 1) and the carboxylic OH-group contributed two bifurcated H-bonds with His131 and His132 (Figure 5). Unlike TSA, absence of the N-hydroxamic group appears to weaken the nucleophilic environment and lessen the extent of enzyme catalysis (deacetylation). Energy calculations indicate that VPA had lower interaction with the enzyme core than TSA ($E = -53.8$ Vs. -66.3 Kcal/mol; Table 1). This binding affinity for VPA is also inferred from the necessarily higher movement (stretching) of key pocket residues to achieve the binding (RMS deviation values are higher than those of TSA by (Table 1). Moreover, a four-fold higher conforma-

tional change (ΔE) in the structure of VPA, relative to that of TSA was inevitable for VPA to accommodate itself at the HDAC binding site (Table 1). Figure 6 depicts the molecular under-pinnings of VPA/TSA binding to the HDAC enzyme. Notably, the VPA’s saturated aliphatic chain is shorter than that of TSA, and is packed less snugly in the pocket tunnel, with fewer *van der waals* contacts. Also, the orientation and length of VPA’s branched saturated chain (shorter than that of TSA) blunt the chances of interaction with the pocket aromatic residues. Unlike TSA, the lack of unsaturated double bonds leads to increased flexibility of the branched chain of VPA. Besides, the absence of a terminal aromatic group (cap moiety) obviously limits the packing of VPA at the cap region of the enzyme. Based on these observations, we propose that these major computational; conformational and binding differences would, at least in part, underlie the disparity in potency of VPA and TSA against HDAC; and, subsequently, the higher selectivity found for VPA against cancer cells. Lastly, the characteristics of VPA binding to HDAC can propose for a new model of rational drug design of superior, VPA-like, anti-cancer agents.

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