

Research Article

Quantitative *In silico* Analysis of Alcohol Dehydrogenase Reactivity

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Abstract

The enzyme activity of alcohol dehydrogenases 4W6Z and 1MG5 demonstrated good agreement with the calculated α -carbon atomic partial charges of the substrates. The correlation coefficients were found to be more than 0.8 ($n = 9$) for both the oxidation and reduction reactions. The stereo structure of cinnamylalcohol dehydrogenase (ELI3-2) was constructed modification in 1PIW (cinnamyl alcohol dehydrogenase) structure. The enzyme activity of ELI3-2 was related to the atomic partial charges on the aldehyde α -carbon atom ($r = 0.785$, $n = 6$). The oxidation process was an electron transfer-deprotonation reaction, whereas the reduction process involved direct protonation. A combination of the molecular mechanics and MOPAC-PM5 programs was used for the quantitative analysis of the enzyme reactivity.

ABBREVIATIONS AND NOTATIONS

APC- Atomic Partial Charge; MM- Molecular Mechanics; DAO- D-Amino Acid Oxidase; ADH-Alcohol Dehydrogenase; NAD- Nicotine Amide Adenine Dinucleotide; LADH-Liver Alcohol Dehydrogenase; CAD- Cinnamyl Alcohol Dehydrogenase; SCADH6p-Saccharomyces Cerevisiae; PDB- Protein Data Bank; TFE -Tri Fluoro Ethanol; FAD- Flabine Adenine Dinucleotide

INTRODUCTION

Quantitative analysis of an enzyme's reactivity allows for the design of mutants required for the development of practical immunoassay methods, enzymatic biosensors, engineered enzymes, and new drugs. The basic phenomenon of molecular recognition by proteins has been studied based on quantitative analysis of molecular interactions using chromatography. Columbic interactions have been studied by the analysis of ion-exchange liquid chromatographic retention, while steric effects have been investigated using quantitative analysis of enantiomer recognition in liquid chromatography. Hydrophobic interactions have been studied by the analysis of reversed-phase liquid chromatographic retention. Furthermore, quantitative analysis of the atomic partial charges (apc) on key atoms has been used to analyze the chemiluminescence intensities. In one study, the albumin-drug binding affinity was predicted using molecular mechanics (MM2) calculations based on liquid chromatographic analyses. These quantitative *in silico* analyses were summarized in the form of a book [1]. A variety of docking programs are available to study protein-drug interactions; however, the docking performance depends on a number of factors that are often difficult to equalize. Occasionally, in an effort to produce

unbiased results across programs, by attempting to equalize all parameters, one could accidentally forfeit the performance, which ultimately makes the docking comparisons very difficult. Most of the previous work in this regard has focused on assessing structure-based applications [2].

In one study, molecular mechanics calculations were first applied to study the enantiomer recognition of a D-amino acid oxidase (DAO) mutant, M213R, (1C0P in PDB) from the yeast *Rhodotorulagracilis*, which was developed to assess food product quality [3]. The enzyme activity of the M213R mutant was quantitatively analyzed using the MM2 and MOPAC-PM5 algorithms incorporated in the CAChe™ program. The selective enzymatic activity of DAO was related to the apc on the key atoms. Further, the results in this study demonstrated the feasibility of quantitative analysis of enzyme reactions using computational chemical methods. Further, the selectivity of the coenzyme was also analyzed using computational chemical calculations [4, 5].

Alcohol dehydrogenase (ADH) belongs to the oxido reductase family, a class of enzymes responsible for the catalysis of biological oxidation and reduction reactions. ADH catalyzes the inter-conversion between alcohols and the corresponding aldehydes or ketones using NADH as a coenzyme. The reaction mechanism for this enzyme is proposed as follows. A protein fluorescence quenching study ruled out the necessity of product dissociation [6]. The liberation of protons during the turnover of liver ADH was studied using transient and inhibition kinetics and by direct titrimetric determinations. The proton release preceded NAD⁺ reduction at neutral pH value. The liberation of protons occurred at the same rate from the substrates while the rates of bound NADH formation were very different; therefore,

proton release appears to be uncoupled from the hydride transfer step. After ternary complex formation, the catalytic step results in the formation of the enzyme-NADH-aldehyde complex. The proton comes from the enzyme rather than from the hydroxyl group of the alcohol. The role of other functional groups and the zinc ions, considered to be involved in the catalysis, have not been elucidated; however, a likely role for the zinc ions would be to increase the acidity of the alcohol hydroxyl group, thus facilitating hydride transfer by a related mechanism [7-9]. The stoichiometry of the oxidation reaction is suggested the release of one proton from the alcohol. However, a key question is which of the following represent the correct mechanism for hydrogen transfer between the coenzyme and the substrate carbonyl group: a single-step hydride transfer (-H); a multi-step electron transfer-hydrogen atom substitution (e^- , H^+); or a three-step sequence, electron transfer, proton transfer, electron transfer (e^- , H^+ , e^-). Further no free radical reduction was observed involving the carbon-hydrogen bond cleavage, which was probed using halo ketones to test for the involvement of a radical intermediate in the NADH/liver alcohol dehydrogenase reduction reaction. A three steps reaction mechanism was considered rather than a direct release mechanism [10].

The alcohol molecule binds to the zinc ion through the oxygen atom, and this inner sphere binding induces the ionization of the alcohol to the alkoxide form. The formation of a zinc-bound alkoxide ion is necessary to form the closed conformation of the LADH ternary complex prior to the hydride transfer. The lower the pK_a value of an alcohol, the greater is the inductive effect on the hydride-donor carbon atom. For alcohols with electron-donating substituents at the C2 atom, the ionization would occur with a relatively higher pK_a value, and the hydride transfer would therefore occur more easily [11]. The catalytic efficiency of the amphibian alcohol dehydrogenase enzyme ADH8, which is the vertebrate ADH with coenzyme NADPH, is greater for the medium- or long-chained substrates such as hexanal, *trans*-2-hexanal, and several retinoid isomers, in corresponding alcohols or retinols [12].

In an early study, yeast ADH structure and catalysis were extensively studied by the comparison of their three dimensional (3D) structures with that of the other ADHs. The study suggested that a zinc ion may mediate the displacement of the zinc-bound water molecule with an alcohol or an aldehyde molecule, and the water was not assigned for any catalytic role [13].

The possible electron transfer can be determined by the electron localization of the targeted atoms and by the location of the alcohol hydroxyl group. The detailed crystal structure of the cinnamyl ADH from *Saccharomyces cerevisiae* has been determined [14]. Further, the crystal structure of the ternary complex of the slow-allele form of *Drosophila melanogaster* ADH with NADH and acetate has also been determined [15]. In this study, most of the biochemical and kinetic data were gathered for the allele forms and induced mutants of this species. The stereo structure of the *Drosophila melanogaster* ADH-S and a theoretically built homology model for the enzyme were compared. Moreover, the differences in the crystal structures of five available *Drosophila lebanonensis* ADHs in multiple complex forms were evaluated, residue conservation at the active site was

examined, and the metagenesis data available for the enzymes were interpreted [15].

CAD, aYMR318C gene product, is specific for NADPH and shows activity for a wide variety of substrates. In context of CAD, aldehydes are processed with a 50-fold higher catalytic efficiency than the corresponding alcohols [16]. The apo and holo structures of an NADPH-dependent cinnamyl alcohol dehydrogenase from *Saccharomyces cerevisiae* have also been reported. The *Saccharomyces cerevisiae* ScAdh6p is structurally a heterodimer composed of one subunit in the apo conformation and the other subunit in the holo conformation. The specificity of ScAdh6p towards NADPH is mainly due to the tripod-like interactions of the terminal phosphate group in NADPH with the S-210, R-211, and K-215 residues of the enzyme. The size and shape of the substrate-binding pocket correlated well with the substrate specificity of ScAdh6p towards cinnamaldehyde and other aromatic compounds. The structural relationships of ScAdh6p with the other medium-chain dehydrogenase-reductase enzyme super family were also analyzed. The function of the CAD enzymes found in bacteria and yeast need to be established clearly [14]. CAD enzymes were originally discovered in plants, where they catalyze the biosynthesis of lignin precursor. Transgenic trees that exhibit reduced CAD activity are easier to delignify; and hence, this family of enzymes is a favored target for biotechnological modifications [17]. However, one of the members of the CAD family, ELI3, shows different enzymatic activities that are not related to lignin synthesis. The enzyme mannitol dehydrogenase, found in celery, oxidizes mannitol to mannose. The ELI3 pathogenesis-related proteins from parsley and *Arabidopsis* aremannitol dehydrogenases [18]. The biochemical function of *Arabidopsis thaliana* defense-related protein ELI3-2, found in *Escherichia coli*, is an aromatic alcohol: NADP⁺ oxidoreductase enzyme. This enzyme demonstrates a strong preference for various aromatic aldehydes in comparison to the corresponding alcohols; however, no mannitol dehydrogenase activity was detected [19].

Above mentioned reports do not provide a clear answer for the ADH reaction mechanism. The role of a cofactor is to activate the protein by either changing its 3D structure, or by actually participating in the overall reaction mechanism. All reports related to zinc-mediated isopropanol oxidation are based on indirect evidences. The contribution of zinc in the ADH mechanism have been studied using a model compound in non-aqueous condition, however, the effects of zinc catalysis in terms of rate enhancements or product yield were small, and did not give a proper understanding of the important enzymatic process [20, 21]. Therefore, in this study, the crystal structures of a zinc-dependent *Saccharomyces cerevisiae* ADH (4W6Z in PDB) and a zinc-independent *Drosophila melanogaster* ADH (1MG5 in PDB) were used as model enzymes, and their binding affinities were evaluated after replacing the substrate with alcohols and aldehydes to study the ADH reaction mechanism. Further study was carried out for the quantitative analysis for the enzyme reactivity of CAD ELI3.

EXPERIMENTAL

The stereo structures of 4W6Z (previous 2HCY), 1MG5, CAD-2CF5, and 1PIW were downloaded from the PDB file [22]. The

previously downloaded 2HCY structure was used in this study. 2CF5 does not include the coenzyme NADP; therefore, 1PIW was used to build the stereo structure of ELI3. The sequence datum of ELI3 was obtained from Uniprot (Q02972) [23]. All water molecules and ligands were removed, and the structure was optimized. The original aldehyde was replaced with other alcohols and/or aldehydes. The ionization form of the coenzyme was fixed, then the new complexes were optimized using the molecular mechanics force-field parameters of version 7.5 CAChe MM2 (Version 7.2 from Fujitsu, Japan), and the apc were calculated using CAChe MOPAC-PM5. Differences in the atomic distances and apc of key atoms and their relationships to the enzyme activities were examined. Calculations were performed using a Dell Optiplex GX270 with 2.4 MHz CPU (Dell, Japan). The minimum energy level was 10^{-7} kcal mol⁻¹.

RESULTS AND DISCUSSION

The two structures (4W6Za and 4W6Zb) for ADH were obtained from the downloaded 4W6Z file; however, the two structures were not identical due to a few differences in their secondary structure. The atomic distances of the key atoms between the TFE-oxygen and the C5 proton of NAD⁺ were found to be 2.981 Å in 4W6Za and 3.283 Å in 4W6Zb, respectively. After optimization using the MM2 program, the atomic distances were 3.290 Å in 4W6Za and 3.366 Å in 4W6Zb, respectively. The 4W6Za conformation was selected for further studies.

The amino acid residues within 3 Å radius of the ethyl alcohol molecule in the 4W6Za-NAD⁺ complex were T-45, H-66, W-92, Y-294, and V-295; and NAD⁺. The nearest atom from the ethylalcohol hydroxyl hydrogen was the C5 atom of the pyridine ring, with a distance of 3.173 Å. The conformation of the complex of 4W6Za-NADH with acetaldehyde was found to be very similar to the conformation of the complex of 4W6Za-NAD⁺ with ethyl alcohol. The nearest atom from the aldehyde α -carbon atom was found to the proton on the C4 atom of the pyridine ring, with a distance of 2.671 Å. 4W6Za contains 14 tyrosyl residues; out of which, only Y-294 was close to the reaction center with its the hydroxyl group facing away from the reaction center. Thus, the hydroxyl group does not seem to be acting as a hydrogen acceptor or a donor. The zinc ion was found to be placed very near ethyl alcohol and acetaldehyde molecules. The distances between the zinc ion and the hydroxyl hydrogen of ethyl alcohol and the oxygen in the aldehyde were 3.838 Å and 2.923 Å, respectively.

The amino acid residues within 3 Å radius of the ethyl alcohol molecule in the 1MG5-NAD⁺ complex were L-96, T-141, Y-152, I-184, L-189, and V-190. The nearest atom from ethyl alcohol hydroxyl hydrogen was the Y-152 hydroxyl oxygen, with a distance of 2.453 Å. The hydrogen transfer may involve the Y-152 residue in its ionized form, as it is reported that the tyrosine residue of *Drosophila melanogaster* ADH plays a catalytically crucial role [24]. The atomic distances between the ethylalcohol hydroxyl hydrogen atom and the C4 and C5 atoms of the pyridine ring in NAD⁺ were found to be 3.497 Å and 3.636 Å, respectively. Further, the amino acid residues within 3 Å radius of the aldehyde molecule in the 1MG5-NADH complex were L-96, S-139, V-140, T-141, Y-152, I-184, and V190. The nearest atom from the aldehyde oxygen was the Y-152 hydroxyl hydrogen, with a distance of 2.201 Å. The distance between the aldehyde

oxygen and the hydrogen on the C5 atom in the pyridine ring was 2.520 Å. Thus, these results suggest that both the C4 and C5 atoms may possibly work as hydrogen donors.

These conformations were further studied using the MOPAC-PM5 program to calculate the apc on the key atoms, and to analyze the possibility of electron transfer. The apc values on the selected atoms correlated with the enzyme activity. The enzyme activity required for the oxidation of alcohols V_{\max}/K_m (min⁻¹mg⁻¹) reported in the literature [25] correlated well with the apc on the α -carbon atom of alcohols in the 4W6Za enzyme. The correlation coefficient was found to be 0.861 (n = 9). The enzyme activity and apc values are summarized in (Table 1).

The enzyme activity for the reduction of aldehydes V_{\max}/K_m (min⁻¹mg⁻¹) reported in the literature [24] also correlates well with the apc values on the corresponding pyridine ring C5 atom, and the α -carbon in the aldehydes. The correlation coefficients were 0.875 and 0.812 (n = 9), respectively. Further, the enzyme activity for the oxidation of alcohols V_{\max}/K_m (min⁻¹mg⁻¹) reported in the literature [24] also correlates well with the apc on the α -carbon atom of alcohols in the 1MG5 enzyme. In this case, the correlation coefficient was found to be 0.838 (n = 9). The enzyme activity for the reduction of aldehydes V_{\max}/K_m (min⁻¹mg⁻¹) reported in the literature [25] also correlates well with the apc on the corresponding pyridine ring C4 atom and the α -carbon atom of the aldehyde; and the correlation coefficients were 0.900 and 0.915 (n = 9), respectively.

Moreover the contribution of zinc ion in the enzymatic mechanism was studied by examining the 4W6Za structure after the removal of zinc ions. The crystal structure of 4W6Za includes the zinc ions, and the original substrate TFE bound to the zinc ion, which in turn interacts with two cysteine residues (C-43 and C-153) of the enzyme. The following studies were performed, after removing the zinc ion and balancing the charge on the TFE oxygen atom from the above-mentioned structure of the enzyme. The original atomic distances between the TFE hydroxyl hydrogen atom and NAD⁺ pyridine ring C5 atom were 3.398 Å and 4.373 Å, respectively. These distances depend on the enzyme structure selected from the 4W6Z crystal structure file. The above mentioned distances between the TFE hydroxyl hydrogen atom and NAD⁺ pyridine ring C5 atom were 2.981 Å and 3.283 Å, respectively, after optimization of the structures using the MM2 program. The distance between the TFE hydroxyl hydrogen atom and zinc ion was 4.565 Å, resulting in the TFE molecule being unable to bind to the zinc ion. The presence of zinc ion is reported to increase the acidity of the alcohol hydroxyl group [8]; however, the atomic distance mentioned above does not support these speculations, even if a water molecule was present. The amino acid residues within 3.5 Å radius of the TFE molecule were selected and were unlocked except for the C and N atoms of the peptide bond; the conformation was further optimized using the PM5 program. This resulted in an increase in the atomic distance between the TFE hydroxyl hydrogen and the zinc ion to 4.798 Å. To determine whether alcohols were ionized under physiologic conditions, the same conformational analysis was performed after ionizing the alcohol molecules. The ionization of TFE oxygen atom resulted in an orientation in which it moved away from the zinc ion.

Table 1: Atomic partial charge (au) of key atoms.

Chemicals	V_{\max}/K_M	$\Delta\alpha C$	Chemicals	V_{\max}/K_M	$\Delta\alpha C$	C5
4W6Za						
Ethylalcohol	0.062	-0.007	Formaldehyde	0.426	0.011	-0.192
Propylalcohol	0.021	-0.008	Acetaldehyde	1.971	0.030	-0.244
Butylalcohol	0.016	-0.009	Propanal	1.567	0.045	-0.244
Pentylalcohol	0.009	-0.008	Butanal	0.338	0.000	-0.217
IsoPropylalcohol	0.002	-0.006	Pentanal	0.271	0.001	-0.216
IsoButylalcohol	0.004	-0.016	Benzaldehyde	0.009	0.019	-0.215
Furfural	0.022	-0.013	Furfurol	0.212	0.010	-0.205
Allylalcohol	0.073	-0.003	Acrolein	1.976	0.050	-0.270
Cinnamyl alcohol	0.124	0.012	Cinnamaldehyde	0.788	0.035	-0.222
Chemicals	V_{\max}/K_M	$\Delta\alpha C$	Chemicals	V_{\max}/K_M	$\Delta\alpha C$	C4
1MG5						
Ethylalcohol	0.062	-0.029	Formaldehyde	0.426	0.030	-0.007
Propylalcohol	0.021	-0.026	Acetaldehyde	1.971	-0.005	-0.038
Butylalcohol	0.016	-0.026	Propanal	1.567	0.000	-0.035
Pentylalcohol	0.009	-0.027	Butanal	0.338	0.019	-0.013
IsoPropylalcohol	0.002	-0.018	Pentanal	0.271	0.033	-0.012
IsoButylalcohol	0.004	-0.020	Benzaldehyde	0.009	0.049	-0.011
Furfural	0.022	-0.016	Furfurol	0.212	0.040	-0.011
Allylalcohol	0.073	0.011	Acrolein	1.976	0.007	-0.034
Cinnamyl alcohol	0.124	0.035	Cinnamaldehyde	0.788	0.026	-0.033

V_{\max}/K_M (unit : $\text{min}^{-1}\text{mg}^{-1}$) from reference 25

In the original 4W6Za structure optimized using the MM2 program, the atomic distance between the TFE oxygen and the NAD^+ pyridine ring C5 atom was 3.630 Å. The apc on the oxygen and α -carbon atoms of TFA and the C5 atom of pyridine ring of NAD^+ were -0.679, -0.039, and -0.205 au, respectively. The zinc ion was removed from the 4W6Za, and the structure was optimized using the MM2 program. The conformation with the amino acid residues selected within 3 Å of the TFE molecule was further optimized using the PM5 program in the unlocked state. The atomic distance between the TFE oxygen and the NAD^+ pyridine ring C5 atom showed an increase to 4.157 Å. After optimizing with the PM5 program, the apc on the oxygen, α -carbon atoms of TFE, and the C5 atom of pyridine ring of NAD^+ were -0.439, -0.177, and -0.180 au, respectively. The TFE hydroxyl group was displaced towards the H-66 residue, (located behind the zinc ion) and showed interactions with the imidazole ring, with an atomic distance of 2.057 Å. The apc on the TFE oxygen atom also supported this observation.

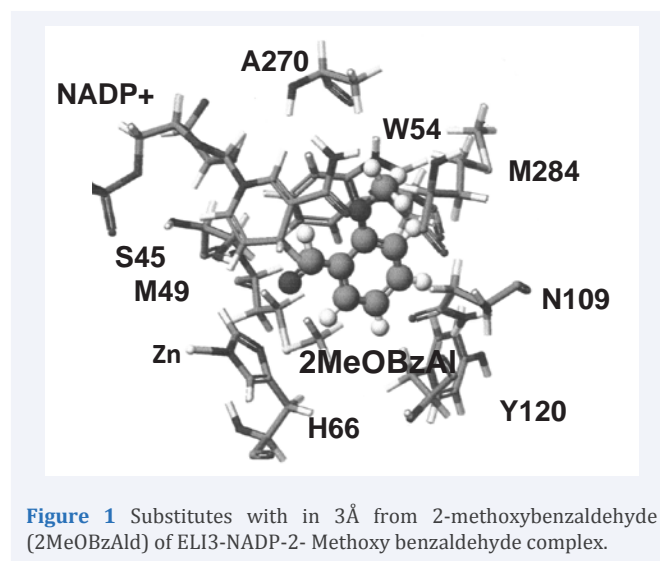
In the 4W6Z crystal structure, the residue R-340 interacts with the NAD^+ phosphate, and seven other arginyls, R-196, R-211, R-257, R-260, R-298, R-302, and R-310; however, these arginines are located at a distance from NAD^+ . The high pK_a value observed for this enzyme may be due to these arginyl residues, but only the R-340 residue is directly involved in the interactions with NAD^+ phosphate. In the case of 1MG5 crystal structure, five arginyl residues are involved in the interaction with NAD^+ phosphate atom; however, only the Y-152 residue is nearest. Further, no arginyl and lysinyl residues are located near the NAD^+

phosphate. In earlier studies, hydrogen bonding of dipole form pyridoxal and side chain was proposed [26]; however, this was not observed in this analysis. In the literatures, several enzyme reaction mechanisms have been compared and the proposed enzyme catalysis pathway seems to function as a chain reaction [27]. These results support an electron transfer-deprotonation system. The enzyme reactivity was supported by the apc on the key atoms, although the atomic distance did not support direct contact in some of the mutants. Thus, these results indicate that electron transfer from the alcohol hydroxyl group is the primary step for the metal ion-dependent enzyme reaction. Moreover, some of the water molecules may be involved in the dissociation and binding between the alcohol and the zinc ion [28]. The new results indicate that reduction the alcohol molecule was difficult in the absence of the zinc ion in 4W6Za. The zinc ion acts as a cofactor and may be necessary for the required 3D structure of the enzyme (4W6Z). However, the zinc ion may not directly participate in the overall reaction of the proton transfer from the alcohol molecule.

The semi empirical (AM1) molecular orbital theory used to investigate the oxidation of alcohols at the active site of liver ADH (LADH) revealed the following two possible mechanisms for the deprotonation of ethanol molecule: (1) by zinc-bound OH^- and (2) by the direct deprotonation of zinc-bound ethanol by imidazole group via serine hydroxyacetate. The model active site for the ADH enzyme consists of a zinc ion coordinated to two methylmercaptans (C-46, C-176), an imidazole group (H-67), and a water molecule. An imidazole group (H-51) hydrogen bonded

to hydroxy-acetate (S-48) acts as the remote base. The new calculations demonstrated that LADH does not require a water molecule at the active site, as in the case of a number of other zinc-coordinated enzymes. Moreover, the results also indicate that the substrates were bound to an inner-sphere-penta coordinated complex with the zinc ion at the active site. The direct coordination of the substrate to the zinc ion leads to the small activation of proton transfer to the alkoxide of S-48. The hydride transfer step is the rate-limiting step, as determined experimentally for the oxidation of secondary alcohols by LADH enzyme. The zinc ions provide electrostatic stabilization and localization of the alcohol and alkoxide at the active site of the enzyme, as the formation of an alkoxide ion is known to facilitate hydride transfer for the alcohol substrate [29]. However, the conformation of 4W6Za is different; as the stereo structure of 4W6Za, C-43, H-66, and C-176 residues may be present as C-46, C-176, and H-66 residues, but the amino acid residues corresponding to H-51 and S-48 are absent. The PM5 calculations demonstrated that the alcohol molecule did not orient closer to the zinc ion; on the other hand, it had moved away. Further detailed studies are necessary to establish the ADH reaction mechanism. The stereo structure of 4W6Za is similar to that of DAO [4], and when NAD⁺ was replaced with FAD⁺, the reaction site of 4W6Za was found to be similar to that of DAO. Thus, if the zinc ion in 4W6Za could be replaced with the DAO arginyl residues, then 4W6Za may also be able to oxidize D-amino acids. However, such speculations need to be studied experimentally. Both zinc-dependent and zinc-independent alcohol dehydrogenases showed a similar relation between enzyme activity and the apc on the key atoms. In the oxidation process (from alcohol to aldehyde), the apc on the α -carbon of alcohol molecule demonstrated the best agreement with the enzyme activity, i.e. V_{max}/K_m ($\text{min}^{-1}\text{mg}^{-1}$). On the other hand, in the reduction process (from aldehyde to alcohol), the apc on the C5 and α -carbon atoms of the pyridine ring demonstrated a good agreement with the enzyme activity. The zinc ion, in a metal-dependent enzyme reaction, seems to provide the space required by a substitute of NAD⁺. The oxidation process was an electron-transfer-deprotonation reaction, while the reduction process was a direct proton transfer involving the C4 atom of the pyridine ring. Further, a tyrosine residue also may be involved in the metal-independent enzyme reaction.

The amino acid sequences of ELI3 and 1PIW are different. However, if the enzyme reaction mechanisms were to be same for both the enzymes, then they should have similar stereo conformations. Hence, the amino acid residues of 1PIW were replaced by those of ELI3, and the conformation was optimized using MM2 calculations. The conformation of ELI3-NADP⁺ with 2-methoxybenzaldehyde is shown in (Figure 1), where the amino acids located within 3Å radius of the aldehyde oxygen or zinc ion are indicated. The apc on the key atoms and the atomic distances for the same are summarized in (Table 2). The nearest hydrogen atom to the aldehyde oxygen was found to be the hydroxyl hydrogen of threonine, and the hydrogen on the C4 atom of pyridine ring of the coenzyme, located within a distance of 2.505Å. The apc on the C4 hydrogen atom was higher (0.279 au) than that of the threonine hydroxyl hydrogen atom (0.271 au); while the apc on the alcohol oxygen and C4 atom of pyridine ring were - 0.449 and - 0.203 au, respectively. These apc values



indicate that proton transfer could occur between the aldehyde oxygen and the C4 of the pyridine ring. The atomic distance between the zinc ion and C5 atom of pyridine ring was 3.606 Å, indicating that the zinc ion seems to be supporting the proton transfer from the coenzyme. The conformations of enzyme with 3-methoxybenzaldehyde, salicylaldehyde, and benzaldehyde are similar; on the other hand, those of cinnamaldehyde and 4-coumaraldehyde are different. These aromatic aldehydes moved toward the zinc ion. The apc on the threonine hydroxyl hydrogen was higher than that on the C4 hydrogen of pyridine ring, suggesting that the threonine hydroxyl hydrogen may contribute to the proton transfer.

V_{max}/K_m (unit: $\text{nkat}\cdot\text{mg}^{-1}\cdot\mu\text{M}^{-1}$) from reference 19, atomic partial charge (unit: au).

AC1: analyte carbon connected with aldehyde, AC2: analyte aldehyde carbon, ACO: analyte aldehyde carbon, C5: coenzyme pyridine ring meta-position carbon. C5H: C5- hydrogen, C4: the para-position carbon, C4Hf and C4Hb: para-position front and back side hydrogens, O-H: atomic distance between aldehyde oxygen and C4Hb hydrogen (A).

In this study, the enzyme reactivity of the aromatic aldehydes, V_{max}/K_m , correlated well with the apc on the analyte aldehyde carbon, the C4, and the H4 atoms of the pyridine ring (C4Hb). The correlation coefficients were found to be 0.785, 0.730, and 0.754 ($n = 6$), respectively. The highest correlation coefficient of 0.899 ($n = 6$) was obtained for the atomic distance between the aldehyde oxygen and C4Hb hydrogen (O-C4Hb). The apc on the aldehyde oxygen, para-position carbon and hydrogen atoms of the coenzyme pyridine ring, and zinc, demonstrate reasonable correlation. Out of the above-mentioned atoms, the atomic distance between the aldehyde carbon and hydrogen show the best correlation. Therefore, these atoms seem to be involved in the reduction reaction. The apc on the key atoms and the atomic distances are related to the enzyme reactivity. This *in silico* analysis using present computational chemistry programs does not clearly indicate the overall contribution of the zinc ion to the enzymatic reaction; however, it suggests the role of zinc in altering the 3D structure of the enzyme.

Table 2: Affinity of ELI3-2 for aromatic aldehydes and the properties.

Substrate	V_{max}/K_m	Atomic partial charge of key atoms				
		AC1	AC2	ACO	C5	C5H
2-Methoxybenzaldehyde	19.1	-0.302	0.315	-0.508	-0.046	0.248
3-Methoxybenzaldehyde	2.9	-0.223	0.319	-0.516	-0.047	0.248
Salicylaldehyde	1.1	-0.370	0.330	-0.496	-0.290	0.253
Benzaldehyde	1.1	-0.279	0.327	-0.507	-0.056	0.247
Cinnamaldehyde	0.7	-0.409	0.301	-0.476	-0.214	0.191
4-Coumaraldehyde	0.05	-0.402	0.302	-0.471	-0.207	0.190
		C4	C4Hf	C4Hb	Zn	O-C4Hb
2-Methoxybenzaldehyde	19.1	-0.203	0.255	0.279	0.486	2.505
3-Methoxybenzaldehyde	2.9	-0.199	0.254	0.274	0.477	2.537
Salicylaldehyde	1.1	-0.217	0.268	0.282	0.537	2.588
Benzaldehyde	1.1	-0.196	0.252	0.270	0.486	2.552
Cinnamaldehyde	0.7	-0.097	0.178	0.152	1.088	2.668
4-Coumaraldehyde	0.05	-0.087	0.172	0.141	1.090	2.703

CONCLUSION

Both the zinc-dependent and zinc-independent ADHs showed similar relationships between the binding affinity and the apc on the key atoms. In the oxidation process (from alcohol to aldehyde), the apc on the α -carbon of the alcohol demonstrated best agreement with the enzyme activity, V_{max}/K_m ($\text{min}^{-1}\text{mg}^{-1}$), where the correlation coefficients were found to be 0.861 and 0.838 ($n = 9$) for 4W6Z and 1MG5, respectively. Further, in the reduction process (from aldehyde to alcohol), the apc on the C5 and α -carbon atoms of the pyridine ring demonstrated a good agreement with the enzyme activity. The correlation coefficients for this process were 0.875 and 0.915 ($n = 9$) for 4W6Z and 1MG5, respectively. In a metal-dependent enzyme reaction, zinc ion seems to alter the 3D structure of the enzyme resulting in the easy access of a substitute for NAD^+ ; however, zinc does not directly participate in the overall reaction mechanism. The oxidation process was an electron transfer-deprotonation system, and the reduction process was a direct proton transfer involving the C4 atom of the pyridine ring of the coenzyme. Further, tyrosine residue may be involved in the reaction mechanism of the metal-independent enzyme. The aromatic aldehyde dehydrogenase (ELI3-2) enzyme activity was correlated with the atomic distance between the aldehyde oxygen and C4Hb hydrogen and a correlation coefficient of 0.899 ($n = 6$) was obtained. The correlation coefficient between the apc on the analyte aldehyde carbon and the reactivity was 0.785 ($n = 6$). This study suggests that a combination of the MM2 and MOPAC-PM5 programs could be used for the quantitative analysis of enzyme reactivity. Moreover, these results demonstrate that enzyme reactions can be quantitatively analyzed using computational chemistry methods, which could help in the design of inhibitors (drugs) and enzyme mutants for the purpose of conducting immunoassays. Further detailed computational chemistry studies could help in establishing a clear mechanism for ADH enzymatic reactions.

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