Binding of Pb-Melatonin and Pb-(Melatonin-metabolites) complexes with DMT1 and ZIP8: implications for lead detoxification

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Abstract

We have applied the docking methodology to characterize the binding modes of the divalent metal transporter 1 (DMT1) and the zinc transporter 8 (ZIP8) protein channels with: melatonin, some melatonin metabolites, and a few lead complexes of melatonin and its metabolites, in three different coordination modes (mono-coordinated, bi-coordinated and tri-coordinated). Our results show that bi-coordinated and tri-coordinated lead complexes prefer to bind inside the central region of ZIP8. Moreover, the interaction strength is larger compared with that of the free melatonin and melatonin metabolites. On the other hand, the binding modes with DMT1 of such complexes display lower binding energies, compared with the free melatonin and melatonin metabolites. Our results suggest that ZIP8 plays a major role in the translocation of Pb, bi or tri coordinated, when melatonin metabolites are present. Finally, we have characterized the binding modes responsible for the ZIP8 large affinities, found in bi-coordinated and tri-coordinated lead complexes. Our results show that such interactions are greater, because of an increase of the number of hydrogen bonds, the number and intensity of electrostatic interactions, and the interaction overlay degree in each binding mode. Our results give insight into the importance of the ZIP8 channel on lead transport and a possible elimination mechanism in lead detoxification processes.

Keywords Lead poisoning · Melatonin · AFMK · AMK kynuramine cpd · CHMI · Molecular docking simulation · DMT-1 transporter · Zinc transporter ZIP8

Introduction

Environmental contamination with lead comes from industrial activities and from the use of some products such as:

leaded paint [1], lead-acid batteries [2], solder, stained glass, ceramic, jewelry, toys, cosmetics, ammunition, printing, batteries, glasses, pigments and fuel production processes and traditional medicines [3]. Due to lead use

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in industry, environmental lead concentration has been dramatically increased in the past decades. Regarding the hard numbers about deaths related with lead poisoning, the Institute of Health Metrics and Evaluation (IHME) reports 853,000 death cases in 2013 due to long-term lead exposure in low and middle-income countries. Effects of lead on health as: 9.3% of idiopathic intellectual disability, 4% of the ischemic heart disease and 6.6% of strokes caused by long-term lead intoxication was reported by IHME [3]. The major source of lead for humans is diet, lead intoxication generally results from gastrointestinal absorption [4].

Regarding lead toxicity mechanism, it interferes with several biochemical routes leading to the inhibition of the synthesis of several proteins and enzymes such as hemoglobin and additionally can cause damage of the central nervous system [5]. An explanation for this behavior has been proposed by Blair and co-workers [6], who found that lead interacts with phosphate ions on tissue, effectively removing up to 95% of lead. But some dietary components produce lead chelation yielding neutral species that are less probable to interact with intestinal tissue and thereby promoting their transport, probably regulated by the same physiological mechanism which controls the metabolism of calcium and phosphorus [7]. Continuous exposure to lead results in its gradual accumulation in the body, longer term causes accumulation in the skeleton with a half-life estimate of 17 years. Lead poisoning treatment is still an urgent health issue to be addressed. It is extremely harmful to young children, where the absorption is 4–5 times greater than in adults [8]. Lead accumulates over time in several biological compartments as kidney, bones, liver and brain. Moreover, lead is released from bones into blood during pregnancy, and then it becomes a source of intoxication to the fetus [3].

The understanding of the mechanism of lead excretion is fundamental to define strategies to deal with lead intoxication. Nevertheless, such excretion mechanism is unrevealed so far; lots of efforts have been made to understand this process. In some recent reports, it has been probed the aid of some natural products in the excretion of lead. Hoseob and co-workers probed the modulation of lead excretion by Vitamin C, using rats as biological models [9]. Thereafter, in our group, Hernández-Plata and co-workers used rats as biological models to demonstrate the reduction of lead levels in blood, brain and bone mediated by melatonin [10] when rats are inoculated by lead acetate. As a matter of fact, lead acetate is one of the most used compounds in experiments with animals to get insight about lead intoxication, because of its relatively moderate toxicity and carcinogenicity compared with different lead compounds [11]. In addition, Limson and co-workers have shown experimentally that complexes of melatonin, its precursors and lead are formed [12]. Vitamin C and Melatonin have the main property of acting as hormones and

antioxidant agents. Nevertheless, their recently observed biological activity place them as feasible solutions to lead intoxication and to the understanding of the lead excretion mechanism. [9, 10].

Melatonin (*N*-Acetyl-5-methoxytryptamine) is a hormone secreted in the circadian cycle by the pineal gland. In addition, it is secreted by other organs and body-cells like skin, gut, lymphocytes, neutrophils, retina, pancreas, and bone marrow. Melatonin is metabolized by liver yielding active metabolites like *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK), *N*1-Acetyl-5-Methoxykynuramine (AMK) and cyclic hydroxymelatonin (OH-Met) [13, 14] whose structures are shown in Figs. 2, 3, 4, and 5. Melatonin is involved in a variety of biological processes such as inducing cell arrest and apoptosis in HepG2 cells [15], regulates inflammatory and immune responses [16], exerts a protective action against gentamicin toxic effects in rat kidneys [17], decreases the testicular toxicity of doxorubicin in rats [18], ameliorates the effects of toxic radiation in normal mouse splenocytes [19], inhibits toxic rotenone cell injury in HeLa cells [20], and decreases tetrachloride hepatotoxicity in rats [21]. This is a receptor dependent biological activity of Melatonin in which many secondary messengers and cellular functions are activated. There are several physicochemical studies that evince that melatonin protects against metalotoxicity via free radical scavenging [22]. There are different melatonin action mechanisms that are not receptor dependent, Gulcin and coworkers report a scavenging effect on metals and free radicals. Such action mechanism is structural dependent [23].

Tan and co-workers have reported that a major portion of melatonin is metabolized to AFMK and AMK [24], and that important functions of melatonin are amplified and mediated by AFMK and AMK. Nevertheless, OH-Met is found in considerable quantities under conditions of high oxidative stress. Galano and co-workers have reported a theoretical study where melatonin, AFMK, AMK and OH-Met together are the fundamental molecules to chelate copper species and to inhibit oxidative stress induced by Cu. [25] Moreover, Galano and Reiter report an exhaustive review regarding the oxidative stress inhibition of melatonin and its metabolites in which the molecular docking approach is not present [26]. In a recent study, we performed an exploration of the conformational space of 45 complexes of lead and some other important endogenous metals with melatonin, melatonin-metabolites and ethylenediaminetetraacetic acid (EDTA) [27]. Our findings were useful to establish the stability and the thermodynamic properties of such metal complexes. We found that melatonin-metabolites are very likely to form metal complexes. For this reason, it is crucial to include the main melatonin metabolites in the present study.

Among the proteins known to play a major role in metal transport are divalent metal transporter 1 (DMT1) and human zinc transporter SLC39A8 (ZIP8), Fig. 1a and 1b,

Fig. 1 Tertiary structure of **a)** DMT1 and **b)** ZIP8 channels obtained by homology modeling. As can be seen, both proteins have transmembrane structures, as is expected for transporting channels. Color code: Alpha helices on red, beta sheets on blue

respectively. DMT1 was identified for the first time on the gastrointestinal tract, it is highly expressed in the renal tissue, and it is clearly involved in the transport of trace elements. In addition, DMT1 was found to be involved also in the transport of highly toxic divalent cations like Pb^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} and Pt^{2+} [28]. However, DMT1 is not the unique renal transporter involved in the transport of toxic metals, there is evidence for Zn^{2+} transporters like ZIP8 which are involved in the transport of this metal at renal tubules [29]. In addition, ZIP8 studies with a murine model, expressed in *Xenopus oocytes*, showed a competitive inhibition of Pb and Zn uptake [30], another study showed that Pb is the second-best inhibitor after Cd for Zn uptake [31]. In addition, the involvement of DMT1 in the transport of lead has been observed in vitro and in vivo [32, 33].

The results of lead excretion mediated by melatonin [10] present the challenge to get insight about the mechanism of lead excretion mediated by melatonin, some of the questions to be solved are: what kind of melatonin-Pb

complexes are involved in lead excretion? Are the melatonin metabolites important in the excretion process? Are DMT1 and ZIP8 involved in such lead excretion? The present work contributes to the understanding, from the theoretical and computational chemistry perspective (using the docking methodology), of the possible role of melatonin and its metabolites in the excretion of lead, analyzing the factibility of melatonin or melatonin metabolites adduct formation as a plausible mechanism to lead elimination through lead quelant formation. Due to the absence of reports on molecular docking calculations, our main goal is to characterize the plausible binding modes of Pb^{2+} complexes with melatonin and its metabolites within protein channels of DMT1 and ZIP8, known to play an important role in the transport of heavy metals, to better understand the observed Pb elimination mediated by melatonin. In addition, we believe that our results give insight regarding the Pb elimination mechanism.

Computational methodology

The Molecular docking methodology is a useful tool for understanding important phenomena such as enzymatic inhibition, biological activity and biological mechanism of natural substrates and drugs. Nowadays, it is one of the most used methodologies for computer aided drug design [34]. Protein-ligand docking is a geometric optimization search problem. The protein and ligand conformations, as well as their relative orientations, are the relevant degrees of freedom of this methodology. Whereas a given protein structure is reasonably well known, the protein-ligand conformation is usually unknown. Therefore, most docking approaches assume ligand flexibility and keep the protein rigid, although there are examples of methods that allow protein flexibility. To perform protein-ligand docking computational experiments, a 3D structure of the target protein at atomic resolution must be available. The most reliable sources are crystal and solution structures provided by the Protein Data Bank (PDB) [35]. Homology models [36, 37] are an alternative when 3D experimental protein structures are unknown. Energetic differences between alternative ligand conformations are often small, compared to the total binding affinity between a ligand and a target protein. In general, flexible ligands display different bioactive conformations, energy minima, in solutions and in gas phase [38]. Small drug-like molecules are typically flexible; 70% of drug-like molecules contain between two and eight rotatable bonds [39]. Therefore, ligand flexibility is typically handled in docking approaches by combinatorial optimization protocols such as fragmentation, ensembles, genetic algorithms, or simulations techniques.

There are no crystallographic structures reported in the Protein Data Bank (PDB) for the modeled channels in this project. Therefore, we constructed tertiary structures for the DMT1 and ZIP8 channels, to use them in our docking calculations. For this purpose, we introduced the amino acid sequences for each protein in the I-TASSER software [40–42] to perform homology modelling, obtaining tertiary structures for these channels. The so constructed structures were exported into the PDB format and studied with the molecular docking methodology that we describe below.

The Molegro Virtual Docker (MVD) program suite [43] was employed to visualize, manipulate and render the channels structures and thereafter perform the docking calculations, during which we kept each protein as rigid biological targets, keeping fixed the positions of all their residues, to reduce the computational effort, although the ligands can freely vary their conformations and positions along the search to minimize the total binding energy. Such procedure ensures the exploration of all possible conformers and allows getting the set of poses with the best interaction energies (see SI). Blind docking calculations for the DMT1 and ZIP8 channels were performed employing the MolDock Score function [44, 45] with a grid resolution of 0.4 Å. The search site was established specifying the center of each protein as the origin and defining a grid sphere of radii 42 Å

and 43 Å, for DMT1 and ZIP8, respectively, to cover the whole protein surface. For all calculations, we have used the MolDock SE search function [46] with 10 runs, 1500 maximum number of iterations, a maximum population size of 10 individuals, an energy threshold for pose generation of 100 kcal/mol, and the simplex evolution parameters with 300 as maximum number of steps and a neighbor distance factor of 1.00 Å. For each calculation, the five poses with the lowest energies were selected (all the poses obtained can be found in the SI).

Pb species were built in silico, based on the metal-ligand binding sites described by Galano and co-workers [25] and in a recent research performed by our group. [27] The modeled species were subjected to geometry optimization and calculation of electrostatic atomic charges (employing the CHELPG charge scheme) using the QUILD software as implemented in the ADF package [47–49]. To get insight from computational results of our previous experimental work [10], we decided to use lead acetate complexes as the ligands for the present in silico experiments. The selected level of theory was the density functional theory (DFT) Perdew-Burke-Erzenhohf (PBE) functional [50] and the chosen basis set was the optimized Slater-type Triple Zeta with two polarization functions (TZ2P), [51] the solvent effect was included using the

Fig. 2 Structures of **a**) melatonin, **b**) Pb coordinated with melatonin, **c**) Pb coordinated with melatonin and an acetyl group, and **d**) Pb coordinated with melatonin and two acetyl groups. Color code: carbons in light grey, Pb in dark grey, oxygens in red and nitrogen atoms in blue

COSMO solvation model [52] with water as solvent and adding relativistic corrections to the Pb atom using the ZORA approach [53–55]. The optimized structures were exported into the Sybyl Mol2 format, keeping the computed atomic charges, so we could thereafter manipulate them with the MVD software.

A limitation of the present methodology lies in the fact that chemical interactions are not characterized from a high-level quantum chemical calculation. However, using the molecular docking methodology, we can get accurate enough binding energies and protein-ligand poses to get information on the plausible molecular mechanism. Besides, it is possible to get an overview of the relevant interactions; i.e. hydrogen bonds, electrostatic and steric interactions. Nevertheless, a high chemical accuracy of such interactions is not possible using this methodology. To deal with this issue, our alternative that is a compromise between computational cost and obtained accuracy, is to use the best molecular docking poses obtained herein to perform a quantum chemical study, such as DFT, to get accurate information about the chemical interactions responsible of such binding.

Results and discussion

One of the principal observations of our reported biological tests [10], is that the excretion of Pb species was apparently propelled by the presence of melatonin, which was administrated to the biological models. A question to be solved emerges from such observation: in what manner melatonin aids in the excretion of heavy metals? In this study, we perform docking calculations intended to shed light into that question, between protein channels such as DMT1 and ZIP8 and Pb-containing ligands, constructed by the union of Pb to melatonin and its metabolites. We study how the sole melatonin molecule and its metabolites binding to each protein channel, and how this binding is affected by the presence of Pb in each of these molecules. In Fig. 1, it is shown the structure of DMT1 and ZIP8 obtained by homology modeling. To evaluate the quality of our DMT1 and ZIP8 homology model, we use the ProsaII [56] software to test the overall model quality. The Z-Scores of our homology models are −5.71 and −4.43 for DMT1 and ZIP8, respectively. PROSAIL compares these values with the ones displayed by the

Fig. 3 Structures of **a**) AFMK, **b**) Pb coordinated with AFMK, **c**) Pb coordinated with AFMK and an acetyl group, and **d**) Pb coordinated with AFMK and two acetyl groups. Color code: carbons in light grey, Pb in dark grey, oxygens in red and nitrogen in blue

Fig. 4 Structures of **a**) AMK, **b**) Pb coordinated with AMK, **c**) Pb coordinated with AMK and an acetyl group, and **d**) Pb coordinated with AMK and two acetyl groups. Color code: carbons in light grey, Pb in dark grey, oxygens in red and nitrogen in blue

Fig. 5 Structures of **a**) OH-Met, **b**) Pb coordinated with OH-Met, **c**) Pb coordinated with OH-Met and an acetyl group, and **d**) Pb coordinated with OH-Met and two acetyl groups. Color code: carbons in light grey, Pb in dark grey, oxygens in red and nitrogen in blue

structures of the PDB proteins, obtained by crystallography or NMR, we observe that the quality of the DMT1 and ZIP8 models fall in the range of the structures of proteins obtained only by reliable experiments. (See SI).

Figure 2 shows the equilibrium geometry structures of melatonin and the possible complexes of this compound with Pb. Meanwhile, Fig. 3 shows the equilibrium geometry structures of the melatonin metabolite AFMK and the possible complexes of this compound with Pb. In addition, Fig. 4 shows the equilibrium geometry structures of the melatonin metabolite AMK and its possible complexes with Pb. Finally, Fig. 5 shows the equilibrium geometry structures of the melatonin metabolite OH-Met and its possible complexes with Pb. All the structures shown were obtained in our previous work [22].

Regarding the docking results, as can be seen from Fig. 6a, melatonin and its metabolites interact in different places over the periphery of the DMT1 channel. It is evident that these molecules tend to interact over the outer surface of the channel. From Table 1, it is seen that melatonin exhibits the best ligand efficiency (LE, Eq. 1), so this is the molecule predicted to have the best interaction

towards the DMT1 channel. Meanwhile, melatonin and its metabolites tend to interact with ZIP8 channel in just one specific place on the periphery of this protein, except for the AFMK molecule, which is the only molecule interacting in the central region of ZIP8 (Fig. 7a).

$$LE = E_{total}/No.heavy.atoms \quad (1)$$

where the heavy atoms are any atoms different to hydrogen.

From data on Table 1, we can observe that melatonin, once again, shows the best interaction towards ZIP8 channel as can be seen from its major ligand efficiency. In summary, melatonin and its metabolites tend to bind towards DMT1 in the periphery of this protein, each one in distinct places, whilst these same molecules have their preferred binding site in the same place on the outer surface of ZIP8 channel, except for the AFMK molecule which is the only one interacting inside this protein in its central region. Therefore, we can establish that none of these molecules (except AFMK) can travel on its own through these channels.

Let us now analyze how this behavior is affected by the presence of a Pb atom linked to the structure of melatonin and

Fig. 6 Best poses obtained from blind docking calculations between DMT1 and: **a)** Melatonin (green), AFMK (yellow), AMK (blue) and OH-Met (pink) as flexible ligands. **b)** Melat-Pb (green), AFMK-Pb (yellow), AMK-Pb (blue) and OH-Met-Pb (pink) as flexible ligands. **c)** Melat-Pb-AcO (green), AFMK-Pb-AcO (yellow), AMK-Pb-AcO (blue)

and OH-Met-Pb-AcO (pink) as flexible ligands. **d)** Melat-Pb-AcO₂ (green), AFMK-Pb-AcO₂ (yellow), AMK-Pb-AcO₂ (blue) and OH-Met-Pb-AcO₂ (pink), as flexible ligands. Color code: alpha helices on red, beta sheets on blue

its metabolites. We observe from Fig. 6b that when melatonin and its metabolites are mono-coordinated with a Pb atom, the binding sites of these molecules are located in the periphery of the DMT1 channel, almost in the same way shown for melatonin and its metabolites without Pb, Fig. 6a. However, the presence of a mono-coordinated Pb atom in these molecules does affect their affinities towards the DMT1 protein, as can be seen from the values of LE reported in Table 1. We note that the affinities of melatonin metabolites towards the DMT1 protein decrease by the presence of Pb, as can be corroborated by the lower LE values. Only melatonin itself attains better affinity for the DMT1 protein when it is mono-coordinated with Pb, as can be observed from its larger LE value reported. Therefore, a co-adjuvant effect from the complexed molecules (melatonin and its metabolites) in lead transport is plausible, promoting affinity between these complexes and DMT1, due to the number of interacting moieties in the formed mono-coordinated complexes.

Meanwhile, the mono-coordinated complexes of melatonin and its metabolites with Pb exhibit a similar behavior towards the ZIP8 protein when these molecules are not

forming complexes with Pb. Figure 7b clearly shows that these complexes bind in the same region in the outer surface of the ZIP8 channel, hence the behavior of these molecules towards the ZIP8 channel does not differ significantly in terms of the binding site. Once again, the principal difference when Pb is mono-coordinated to melatonin and its metabolites is the binding energies and ligand efficiencies of these complexes towards ZIP8 protein. From data reported in Table 1, one clearly observes an increase of these values when Pb is mono-coordinated with melatonin and its metabolites, indicating the corresponding increase in the affinity of these molecules towards the ZIP8 protein in comparison with the affinity shown by these molecules in the absence of a Pb atom in their structures.

Let us now consider how the binding modes of melatonin and its metabolites are modified by a distinct coordination form of Pb, namely, those shown in the structures of the c) sub-sections of Figs. 2, 3, 4, and 5, where the Pb atoms are bi-coordinated. Figure 6c shows the best poses obtained from blind docking calculations of each of the ligands with bi-coordinated Pb, towards the DMT1 channel. We observe in this case, that all complexes tend to bind in the same region of the DMT1 protein except for the complex AFMK-Pb-AcO. In fact, as can be seen from Table 1, this compound has the second-best affinity towards the protein, as can be seen from its LE value. Nevertheless, all of them bind on the periphery of the protein.

When we dock these same ligands to the ZIP8 protein we observe an interesting behavior, two of the metabolites bi-coordinating the Pb atom (namely AMK-Pb-AcO and OH-Met-Pb-AcO, Fig. 7c) bind inside the ZIP8 protein at its central region. This behavior evinces the importance of these two bi-coordinated Pb species as carriers of Pb to be transported by this channel, in accordance with our initial hypothesis for melatonin and its metabolites, although, the binding properties of the bi-coordinated Pb species with ZIP8 can favor the metal transport by the ZIP8 and DMT1. The decrease of LE when going from mono to bi-coordinated species can be related with the steric impediment in the protein cavities due to a larger size of the complexes.

Finally, we analyze the binding behavior of the tri-coordinated Pb species towards the channels we have studied. Figure 6d shows the best poses obtained from blind docking calculations for each of these ligands towards the DMT1 channel. As we can see, the molecules have increased their volumes to the extent that none of them can bind inside the protein. Again, it is observed that their affinities have decreased in comparison with the other Pb-Melatonin species discussed above, as can be seen from their LE values shown in Table 1.

Conversely, the blind docking of these last group of Pb complexes towards the ZIP8 channel show that, while their affinities remain almost unaltered, as can be seen

Table 1 Binding energies in kcal/mol, (E) and ligand efficiency (LE), in kcal/mol.atom for the best poses obtained from blind docking calculations between melatonin and its metabolites mono, bi and tri-coordinating a Pb atom, towards DMT1 and ZIP8 channels

	DMT1		ZIP8	
	E*	LE†	E	LE
Melat‡	−115.98	−6.82	−108.87	−6.4
AFMK	−116.22	−6.12	−102.80	−5.41
AMK	−108.25	−6.37	−98.78	−5.81
OH-Met	−103.39	−5.74	−99.78	−5.54
Melat-Pb§	−131.78	−7.32	−134.63	−7.48
AFMK-Pb	−116.87	−5.84	−128.40	−6.42
AMK-Pb	−122.38	−6.80	−128.32	−7.13
OH-Met-Pb	−106.69	−5.61	−99.83	−5.25
Melat-Pb-AcO**	−125.88	−5.04	−130.25	−5.92
AFMK-Pb-AcO	−142.29	−5.93	−131.55	−5.48
AMK-Pb-AcO	−137.83	−6.26	−125.22	−5.69
OH-Met-Pb-AcO	−123.84	−5.38	−94.45	−4.11
Melat-Pb-AcO2††	−149.55	−5.75	−132.06	−5.08
AFMK-Pb-AcO2	−143.96	−5.54	−128.63	−4.59
AMK-Pb-AcO2	−139.71	−5.37	−146.46	−5.63
OH-Met-Pb-AcO2	−130.54	−4.83	−105.37	−3.90

* Binding energy

† Ligand efficiency

‡ Melatonin

§ Lead-melatonin complex

** Lead acetate-melatonin complex

†† Lead diacetate-melatonin complex

Fig. 7 Best poses obtained from blind docking calculations between ZIP8 and **a**) Melatonin (green), AFMK (yellow), AMK (blue) and OH-Met (pink) as flexible ligands. **b**) Melat-Pb (green), AFMK-Pb (yellow), AMK-Pb (blue) and OH-Met-Pb (pink) as flexible ligands. **c**) Melat-Pb-

AcO (green), AFMK-Pb-AcO (yellow), AMK-Pb-AcO (blue) and OH-Met-Pb-AcO (pink), as flexible ligands. **d**) Melat-Pb-AcO₂ (green), AFMK-Pb-AcO₂ (yellow), AMK-Pb-AcO₂ (blue) and OH-Met-Pb-AcO₂ (pink), as flexible ligand. Color code: alpha helices on red

from their ligand affinities in Table 1, their binding site inside the protein does change, in a way that each molecule interacts inside the protein, near to its central region. In fact, two of these complexes, e.g. AFMK-Pb-AcO₂ and OH-Met-Pb-AcO₂ are seen to mainly interact with the protein in its central region (Fig. 7d). When we compare this behavior with the one shown by the previous Pb complexes, we can underscore the importance of Pb

binding in its tri-coordinated form towards melatonin metabolites to translocate Pb through the ZIP8 channel. Clearly, a tri-coordinated Pb complex displays the best interaction inside the ZIP8 channel. Therefore, apparently ZIP8 plays a major role in the translocation of Pb when melatonin metabolites are present.

Let us now try to identify the underlying reason for such behavior. For this purpose, we analyzed in depth the binding modes inside the ZIP8 channel of the three most important metabolites, AFMK, AMK and OH-Met in their bi-coordinated and tri-coordinated binding modes to the Pb atom. As we showed in Fig. 7a, the AMK molecule tends to bind to ZIP8 in the outer region of this protein. Table 2 shows the non-steric contributions to the binding energy of this pose and Fig. 8 shows a graphical representation of such different contributions to the binding energy. We can observe that the principal contribution is due to the hydrogen bond established within the AMK molecule and the Glu184 residue with an energy of -2.50 kcal/mol. The electrostatic contributions are weak and not greater than 1 kcal/mol. The interaction overlay of the AMK molecule shows that the principal contributors to the binding energy are the steric interactions established by the phenyl system and its lateral string.

Table 2 Principal non-steric contributions to the binding energy of the AMK molecule binding mode towards the ZIP8 channel

Type	Energy(kcal/mol)	Length (angstroms)
(Electrostatic)	−1.05	4.46
(Electrostatic)	−0.76	3.83
(Electrostatic)	−0.73	4.46
(Electrostatic)	0.93	2.86
(Electrostatic)	0.93	3.83
(Electrostatic)	0.97	3.81
(Electrostatic)	1.46	4.43
H.Bond*	−2.50	3.10

* Hydrogen bond

Fig. 8 Non-steric contributions to the binding energy of the AMK molecule binding mode. In **a**) we can see the electrostatic interactions, where green lines represent attractive interactions and red lines represent repulsive interactions. In **b**) are shown the hydrogen bond interactions.

The interaction overlay per atom is represented by a green sphere over each atom. The larger the sphere of one atom the larger is the interaction overlay of such atom

As we discussed from Fig. 7c, where the AMK molecule forms a bi-coordinated complex with Pb, its binding site changes at the ZIP8 protein. Let us see what is the principal difference between this binding mode and the binding mode we just described above. There are three principal differences between them, specifically the number of hydrogen bonds, the number and intensity of electrostatic interactions, and the interaction overlay degree in each binding mode. As we can see from Fig. 9, AMK-Pb-AcO molecule establishes two hydrogen bonds with residues Asn 176, one with His 84 and one with Cys 74, in comparison with the only one that establishes AMK molecule by itself. Such behavior is due to the presence of additional hydrogen bond acceptors in the additional acetate

group, contained by the AMK-Pb-AcO complex. Even more important is the fact that, due to the presence of the Pb atom, there is a strong attractive electrostatic interaction of -3.29 kcal/mol (Table 3) with the Glu 197 residue (Fig. 9a) and a strong repulsive electrostatic interaction of 2.79 kcal/mol (Table 3) with Asn 176 residue (Fig. 9b). The presence of chelator residues such as Glu and polar residues such as His and Asn in the core of the Zip8 protein clearly shows its character as a metal translocator or transporter. Therefore, when melatonin or its metabolites bind with the Pb atom and its additional polar groups such as acetates, which are widely present in the physiological medium, we can expect, as we have shown by our computational analysis, that the complexes will

Fig. 9 Non-steric contributions to the binding energy of AMK-Pb-AcO molecule binding mode. In **a**) we can see the electrostatic interactions, where green lines represent attractive interactions and red lines represent repulsive interactions. In **b**) are shown the hydrogen bond interactions.

The interaction overlay per atom is represented by a green sphere over each atom. The larger the sphere of one atom the larger is the interaction overlay of such atom

Table 3 Principal non-steric contributions to the bind energy of the AMK-Pb-AcO molecule binding mode

Type	Energy (kcal/mol)	Length (angstroms)
(Electrostatic)	−3.29	3.57
(Electrostatic)	2.79	3.85
H. Bond	−2.50	2.77
H. Bond	−2.50	3.08
H. Bond	−2.50	2.60
H. Bond	−1.23	3.09

tend to locate at the core of channels such as ZIP8 or DMT1 and to translocate Pb by action of these type of channels.

Conclusions

The detailed characterization of the binding modes in the framework of the molecular docking simulation of DMT1 and ZIP8 with Melatonin-Pb Complexes is reported for the first time. From our preceding molecular docking analysis evidences, we can establish that the coordination of Pb towards melatonin and its metabolites plays an important role in the interaction of this metal with two of the most common translocator proteins able to exert a physiological metal detoxification effect, the so-called divalent metal transporter (DMT1) and the human zinc transporter SLC39A8 (ZIP8). We find that the species with the best ligand efficiencies and with the stronger interactions towards these channels are those that form tri-coordinated species with Pb, specifically AFMK-Pb-AcO₂ and OH-Met-Pb-AcO₂, followed by bi-coordinated species and in the last place, are those with mono-coordinated species. As we know, elemental Pb strongly interacts with the phosphate groups, this fact complicates the Pb transportation and elimination from physiological medium. In addition, the coordination with melatonin and its metabolites yields neutral Pb species that are more suitable to be transported through the channels considered in this work. This is possible due to the presence of H-bond acceptor groups, as well as the electrostatic interactions of Pb with the partial negative residues located along the inner surface of the channels. Such interactions are weaker, compared with electrostatic and coordination interactions that elemental Pb exerts with various physiological compounds, like phosphate groups ubiquitous in the physiological medium. This effect of melatonin and its metabolites can be compared with the effect of other compounds used as metal chelators in the metal detoxification therapies like EDTA and provide a useful alternative to those therapies, considering that actual chelation therapy has many toxic effects. The present study explores the feasibility of melatonin and melatonin's metabolites as molecules to be

used in alternative treatments of lead poisoning as chelators with less toxic effect.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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