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RATIONAL DESIGN OF NEW MEDICINES FOR NEURODEGENERATIVE DISEASES

RACIONALNI DIZAJN NOVIH LEKOVA ZA LEČENJE NEURODEGENERATIVNIH BOLESTI

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Ključne reči
Parkinsonova bolest, inhibitori, racionalni dizajn lekova, monoamino oksidaza, metabolizam.

Abstract

Over the last decade, as well as even before, new insights and steps have been made in understanding the mechanism of neurodegenerative diseases such as Parkinson's and Alzheimer's disease are. New findings have also been possible, because of the development of the so called computational part of the research, which is especially useful when it comes to explaining and understanding the mechanisms of action on an atomic or even an electronic scale, thus offering a hint for more successful planning and design of real experiments. Many new approaches for a more rational and effective design of new medicines for Parkinson's disease have been tried out, wherein a very important role have also molecular simulations, especially in elucidating the mechanism of action of the medicine. By using the principles of molecular simulations (molecular modelling) an insight on the metabolic pathway of the medicine once inside the human body could be given. „In the article it is based on the concrete examples presented how a new medicine can be designed for neurodegenerative disease such as Parkinson's disease is".

INTRODUCTION

Nowadays, thanking to the rapid development of different experimental methods, the amount of data concerning the neurodegenerative processes, and consequently neurodegenerative deviations, which result in the form of disease is growing. This rapid development is possible also due to the more and more acknowledging the usefulness of theoretical approaches, which are based on the quantum chemical principles, especially as they help one to get an impression and to understand what actually goes on at the level inaccessible to the naked eye. And the later most probably represents the biggest obstacle on the way to find a medicine to cure and to prevent further development of neurodegenrative diseases. Many diseases became managable and curable, since based on the combintaion of theoretical and experimental findings, researchers were able to design a successful system to prevent and to cure different diseases. Unfortunatelly when it comes to neurodegenerative disease that is still not the case. At the moment there are medicines with which relief of the symptoms can be achieved to a certain degree. However the proceeding of the disease is not stopped. With the help of the microscopical devices it can be seen what happens at the microscopic level, nevertheless there is a constant need for devices, which would be able to show the nano world, and would thus represent a tremendous breakthrough in understanding the processes that take place at the atomic scale. And computer simulations or more precisely molecular simulations have been offering great support in understanding various mechanisms of chemical or it is better to use expression biochemical reactions, which take place in different living organisms. Nevertheless without the experimental part the results of molecular simulations are meaningless. Probably the most representative examples of the later are for instance radical reactions, where by molecular simulations practically the whole pathway of a radical reaction can be explained, nevertheless they are often not confirmed or justified by real laboratory experiments, since the persistency of the radicals is very hard to be followed, thus very sensitive, accurate experimental methods and approaches have to be used. Such it was the case with the proposed radical mechanism by Silverman et at. for monoamine oxidase (MAO) [1-7], which has been for the last decade completely

left out of the discussion concerning the possible mechanisms in the case of MAO, since it could not be fully confirmed by the real experiments. Thus more or less the researchers have been discussing polar nucleophilic [8,9], and hydride mechanism [10-15]. Otherwise monoamine oxidase is a very important member of the flavoenzyme family, since its two isozymic forms MAO A and MAO B are involved in the methabolic pathway of primary and secondary amines, among which many neurotransmitters can be found. And the discountinued production of the later has one of the cruical roles in the appereance of the neurodegenerative diseases such as Parkinson's disease is. At the later dopaminergic neurons, which are basically somesort of production sites of the neurotransmitter dopamine, are diminishing, and consequently also the production of dopamine severely decreases. Dopamine is a common substrate of both isoforms (MAO A and MAO B) [16,17], and is an important neurotransmitter involved in the control of voluntary movement. It has been established, that insufficient dopaminergic stimulation of the basal ganglia, where more MAO B than MAO A is expressed, is characteristic for Parkinson's disease. Since MAO B metabolizes dopamine that decreases the concentration levels of dopamine even more. Hence one of the strategies is to inhibit MAO B in order to stop the degradation of dopamine. Currently the inhibitors used for the relieve of the symptoms of Parkinson's disease are all based on the irreversible inhibition, where a covalent bond between inhibitor and FAD co-factor in the active site of enzyme is formed, which completely inhibits the activity of MAO. There is a seek for reversible inhibitors, which would disable the activity of an enzyme only for a certain period of time, but according to the available data none has been yet officially used in the treatment of Parkinson's disease. However that does not stop the progress of Parkinson's disease, since dopaminergic neurons are still diminishing. Thus the researchers are being eager to find the cause of diminishing of the dopaminergic neurons, but until then the use of irreversible, and perhaps in the future also of reversible inhibitors of MAO B in the combination with levodopa, which is a precursor of dopamine, represents the first line in managing of Parkinson's disease. And here the molecular simulations take their place, since they help to distinguish between more and less plausible mechanisms of reactions, and thus they reduce the number of real experiments, which have to be conducted to confirm theoretical predictions. When making a plan for a theoretical study it is always beneficial to have as much data as possible from the already conducted laboratorial experiments. Thus the crystal structure of an enzyme with as better resolution as possible is definitely one of the key factors in a theoretical experiment, since on the basis of that researchers try to propose most plausible catalytic mechanism and to rationalize the substrate selectivity [16-21]. For instance MAO A is based on its crystal structure known to be a more abundant form of the two isozymes (MAO A and MAO B) in the human body due to the presence of only one cavity [22], and is therefore more susceptible for »bulkier« substrates such as serotonin and norepinephrine are, of which imbalance is known to be involved in the appereance of depression-like symptoms and mood disorders. On the contrary MAO B can metabolise smaller substrates such as benzylamine is, since it has two cavities of different size [23], which are »controlled« by Ile199 residue, the later acting as gator between the two cavities [23]. Moreover when assessing the catalytic mechanism (methabolic pathway of substrates) of MAO B three important structural features have to be considered: the hydrophobicity of its active site, the structure of aromatic cage surrounding the FAD co-factor flavin moiety, and the conformation of FAD co-factor. The hydrophobicity of MAO B active site is important for substrate access to MAO active site, since MAO substrates (amines) in cytoplasm are usually protonated, and therefore protonation states could be changed upon entering the active site or prior to rate-limiting step in a catalytic reaction. Beside the hydrophobicity of the active site, the aromatic cage surrounding the flavin co-factor also plays an important role in MAO. There are two tyrosyl residues (Tyr398, Tyr435), which form the aromatic cage [24-25], constraining the substrate access to the FAD co-factor. In order to investigate their function in the catalytic reaction along with the nearby Tyr188 residue their pK_a values were calculated [26]). It turned out that all three residues are not likely to change their protonation states in the presence of a protonated or non-protonated amine substrate, and could thus more likely be involved in the catalytic as well as inhibition reaction indirectly, possibly through some kind of π-π interactions [26].

All of the above mentioned structural features, which have to be taken in consideration, are not only important in terms of catalytic mechanism, but also for reaction of inhibition.

As it can be seen internal protein environment (different amino acid residues) has a very important role in the catalytic mechanism as well as in mechanism of inhhibiton of MAO B, and despite the extensive studies on mutant enzymes [27,28], at present there is still no consensus about the actual mechanism of the catalytic step. So far three mechanisms, by which MAO could oxidize (deaminate) amine susbstrates have been proposed:

1) hydride mechanism [10-15],
2) radical mechanism [1-7], and
3) polar nucleophilic mechanism [8,9].

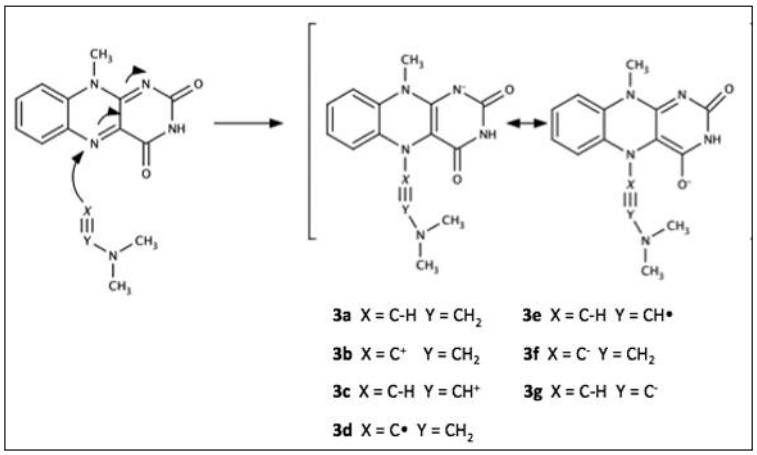
The rate-limiting step in all three mechanisms of MAO catalysis is the abstraction of the α-C hydride, hydrogen atom or proton, respectively, proximal to amino group, which is believed to be picked up by the flavin N5 atom. For hydride mechanism it was assumed unlikely to take place, due to a barrier, which is too high to be readily crossed [8, 27]. On the other side Edmondson and coworkers in their studies with benzylamine analogs showed that electron-withdrawing groups, which are attached to *para*-position of benzylamine increase the rate of the reaction in MAO A [8, 9]. On the basis of kinetics, structural data and Taft correlation for benzylamine series of substrates the authors proposed the polar nucleophyllic mechanism for MAO A [8, 9]. In this study it was also found that the effect on the reaction rate is much less noticeable in MAO B than MAO A, which could be the consequence of where these two isozymic forms are expressed in majority in comparison to each other [8,9]. The radical mechanism was proposed by Silverman and coworkers [1-7], but as it has been already mentioned previ-

ously in the last few years no experiments were conducted to provide more information on the plausibility of this mechanism. All of the above proposed mechanisms address the oxidation of biogenic amines. Practically no detailed mechanistic or computational studies have been performed on clinically used irreversible acetylenic inhibitors, such as selegiline and rasagiline, until recently, when a model system for the irreversible inhibition reaction with acetylenic inhibitors in the active site of MAO B was designed [29], based on seven different mechanism studied. The calculations suggested that the covalent bond is most likely formed between deprotonated terminal acetylenic carbon atom of acetylenic inhibitor and N5 atom of FAD co-factor [29], which was in agreement with the three dimensional structures of MAO B in complex with rasagiline and selegeline [30-35]. It was assumed that the most plausible mechanism among seven mechanisms studied, was the polar nucleophilic or the so called acetylenidic mechanism [29]. In Scheme 1 the model for seven studied mechanisms of irreversible inhibition in MAO B is presented. By further investigations it was shown why the polar environment has to be considered, and how it effects on certain parameters.

Computational Methods

As a basis to calculate the activation free energy for the rate-limiting step of MAO B inhibition by irreversible inhibitors rasagiline and selegiline the model system **3f** from Scheme1 presented in Introduction part was used, where the only difference to the original Scheme 1 was that the two methyl groups were substituted with the appropriate substituents in accordance with the rasagiline (Figure 1) and selegiline (Figure 1) molecular structure. And thus the rate-limiting step was the formation of a covalent bond between terminal C atom of the acetylenic group in the irreversible inhibitor and N5 atom of flavin co-factor, since the latter was showing more of the electrophilic and the first more of the nucleophilic character. The calculations were performed in the following way: at each step the geometry was optimized, and the corresponding zero point energy was calculated. Then the optimized geometries were taken, which were corresponding to the reactant complex in order to perform new geometry optimization, but this time without any constraints and again the vibrational zero point energy in the harmonic approximation was calcuated. To investigate the transition state, the geometry with highest energy was taken, and the same as before a non-constrained optimization towards the transition state was performed, and the corresponding zero point energy and vibrational frequencies to detect and define the transition state geometry were calculated. The gas phase activation energies were all zero point energy corrected [59].

Ab initio and Density Functional Theory (DFT) calculations have been performed on the Hartree–Fock (HF) and B3LYP [37-39] level of theory in conjunction with the 3-21G, 6-31G(d), 6-31G(d,p) and 6-31+G(d,p) basis sets in terms of showing the energy differences in gas phase between these two theoretical methods, and the effect of polar environment on the barrier height. The applied basis sets were flexible enough to allow for reasonably accurate calculations. For the reactant minimum all frequencies were real, while for the transition state one imaginary frequency was found. Visualization of the eigenvector corresponding to the imaginary frequency reveals formation of the bond between N5 atom of flavin co-factor and terminal acetylene carbon atom of irreversible inhibitor. Effects of solvation (polar environment) were considered by Langevin dipoles implemented in the program Chemsol 2.1 of Florian and Warshel [39-41].



Scheme 1: *presentation of the model system for the irreversible inhibition reaction in MAO B for seven possible mechanisms (3a-3g).*

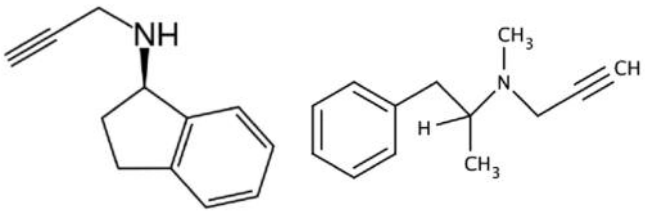
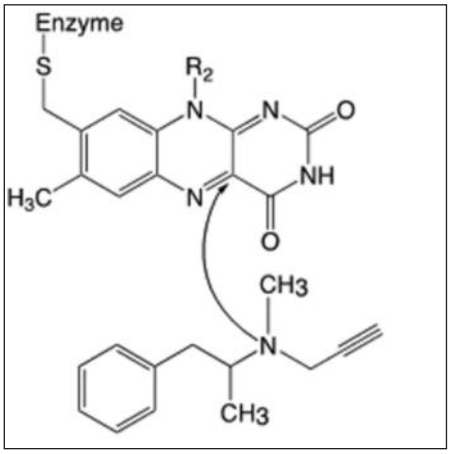


Figure 1: *clinically used irreversible inhibitors of hMAO B rasagiline (left) and selegiline (right).*



Scheme 2: *Assuming the polar nucleophyllic mechanism, it was first tried to calculate the barrier height for the attack of inhibitor N atom to flavin C4a atom. It was concluded that the associated barrier of 83.6 kcal mol-1 is too high for the reaction to take place in this way and proceeded with the mechanism shown in Scheme 1.*

RESULTS AND DISCUSSION

For the catalytic mechanism in MAO it was proposed that an adduct between C4a atom of flavin co-factor and amine moiety of amine substrate is formed. Therefore in the first instance it was tested if the same mechanism can be applied also for the irreversible inhibition in MAO B. Thus the idea of the polar nucleophylic mechanism was followed, where an inhibitor was used instead of an usual substrate (Scheme 2), which was attacking C4a atom of flavin co-factor with its amine moiety (for atom labeling in fully reduced flavin co-factor see Figure 2). The barrier height of 83.6 kcal mol^{-1} was calculated, which is much too high to be easily crossed, and also the process was endothermic, being another evidence that irreversible inhibition does not follow the same mechanism as it was proposed for the amine substrates in MAO B. This was in agreement with the results of previous studies where firstly the Lewis/Brønsted acid–base properties of the isolated flavin fragment, the inhibitor molecules and the model system were investigated in order to obtain preliminary information on their reactivity in the enzyme active site, and to justify why the truncated system is a sufficient and good model for representing and evaluating the properties and reactivities of the two full-sized inhibitors rasagiline and selegiline in the first stage of mechanistic studies. The affinities of N5 and O4 atom of flavin co-factor towards H- (hydride), H• (hydrogen radical) and H+ (proton) species were also considered. The Lewis/Brønsted acid–base properties were implying that N5 atom of flavin co-factor was more favoured for the attack of the irreversible inhibitor than carbonyl C4a atom. Therefore it can be understood why it resulted in a barrier height of 83.6 kcal mol^{-1} for reaction between C4a atom of flavin co-factor and amine moiety of irreversible inhibitor. One of the reasons why the barrier is so high are also the steric effects, which are for the reaction just mentioned very unfavourable, since there is very little space available to irreversible inhibitor amine moiety to approach C4a atom of favin co-factor. Even if one included the effects of polar environment, it would not lower the barrier height up to the point that it could be readily crossed. All of this led back to the mechanism, where a covalent bond is formed between terminal C atom of acetylene group of irreversible inhibitor and N5 atom of flavin co-factor. And N5 atom shows more of electrophilic character, whereas C atom of acetylene group in irreversible inhibitor shows more of the nucleophylic character. The later is consistent with experiments performed by Miller and Edmondson [8,9], who showed that attaching electron-withdrawing groups to the *para*-position of the benzylamine substrate increases the rate of the reaction in MAO A. However the purpose of the study presented herein was also to critically evaluate the contributions of polar environment to the free energies of activations and hydrations for polar anionic mechanism of irreversible inhibition in MAO B. The selection of flexible basis set is of crucial importance, especially the inclusion of diffusion functions due to the presence of ionic species. In conjunction with suitable solvation models reliable results were obtained.

The results for polar anionic mechanism of MAO B irreversible inhibition in the gas phase are presented in Table 1. It should be immediately emphasized that these results were

Table 1: *Activation energy (E□) for the reaction of selegiline and rasagiline with flavin moiety of MAO-B (second column) and the corresponding reaction energies (third column). Please note that the zero point energies are included. The experimental value of the activation free energy corresponding to the transition state barrier height was calculated from the experimentally determined rate constant by equation*

$$k_{inact} = \frac{k_b T}{h} e^{-\Delta G / k_b T N_A}$$

SELEGILINE		
	E□ [kcal mol^{-1}]	Reaction energy [kcal mol-1]
HF/3-21G	14.00	-52.01
HF/6-31G(d)	20.19	-37.72
HF/6-31G(d,p)	25.10	-37.65
B3LYP/6-31G(d)	14.64	-37.65
B3LYP/6-31+G(d,p)	8.85	-37.65
Experimental value[56]	21.30	/
RASAGILINE		
	E□ [kcal mol-1]	Reaction energy [kcal mol-1]
HF/3-21G	19.86	-47.55
HF/6-31G(d)	19.70	-40.82
HF/6-31G(d,p)	21.19	-39.29
B3LYP/6-31G(d)	8.32	-39.82
B3LYP/6-31+G(d,p)	16.81	-28.90
Experimental value[43]	20.83	/

Table 2 *Free energies of activation (ΔG□) for chemical reaction between flavin and inhibitors selegiline and rasagiline in aqueous solution. Please note that for calculations of $\Delta G_{\ddagger}^{\ddagger}$ the in vacuo classical barrier height was corrected for zero point energy contribution and free energy of hydration for transition state minus the corresponding value for reactants. The experimental value corresponds to the reaction in enzyme.*

SELEGILINE	
	ΔG□[kcal mol^{-1}]
HF/3-21G	12.63
HF/6-31G(d)	26.40
HF/6-31G(d,p)	31.17
B3LYP/6-31G(d)	15.62
B3LYP/6-31+G(d,p)	18.15
Experimental value[56]	21.30
RASAGILINE	
	ΔG□[kcal mol^{-1}]
HF/3-21G	24.76
HF/6-31G(d)	28.80
HF/6-31G(d,p)	28.99
B3LYP/6-31G(d)	10.22
B3LYP/6-31+G(d,p)	18.81
Experimental value[43]	20.83

observed only in the gas phase, where no solvent was present, and therefore no effects of polar environment were considered. Hence one can not make any conclusions or even assumptions regarding the free energy of activation based on that. As it can be seen from Table 1 in the gas phase the best results are given by the HF/6-31G(d) level of theory for selegiline, and HF/6-31G(d) or HF/6-31G(d,p) in the case of rasagiline, if one compares them directly with the experimentally observed results (21.3 kcal mol^{-1} for selegiline [42], and 20.8 kcal mol^{-1} for rasagiline [43]. Results obtained from the gas phase calculations can not be compared with the experimental results due to the absence of polar environment as it has been already emphasized above. The later can be further on supported by the results obtained with the DFT methods. The DFT calculations performed on B3LYP/6-31G(d) level of theory gave an activation energy of 14.84 kcal mol^{-1} for selegiline and 8.3 kcal mol^{-1} for rasagiline, which is definitely too low for comparison with the experimentally observed values. It is worth stating that DFT calculations often give lower barriers than the corresponding Hartree-Fock (HF) values, which can be explained with overestimation of the correlation energy, especially in the gas phase. Therefore aqueous environment must be considered to obtain relevant results, and that can be compared with the experimental ones, since in an experiment usually a solvent (aqueous solution) is used.The effects of polar environment must be included in order to compare theoretical results with the experimental values, since solvent interacts with solute and in this way contributes to the overall energy of the system, especially through the free energies of hydration. For this purpose the model of Langevin dipoles by Florian and Warshel was used [39-41], since it was shown to be well parametrized for different biological systems by giving reliable and meaningful results [41,44,45]. Although the substrate cavity of MAO B is believed to be hydrophobic, the free energies of solvation in aqueous solution for the reactant complex and the transition state were calculated, since elucidating the effects of aqueous environment on this reaction can serve as a reference reaction in the Empirical Valence Bond (EVB) calculation [46].

Table 3 *Free energies of hydration calculated on different levels of theory with Langevin dipoles method (39-41,45). values are given for the reactant minimum (R), transition state (TS) and the products (R).*

SELEGILINE	Δ_{Ghydr} [kcal mol^{-1}]		
	R	TS	P
HF/3-21G	-77.58	-78.95	-67.58
HF/6-31G(d)	-75.55	-69.34	-68.19
HF/6-31G(d,p)	-75.41	-69.34	-68.26
B3LYP/6-31G(d)	-69.85	-68.87	-62.74
B3LYP/6-31+G(d,p)	-77.52	-68.22	-65.42
RASAGILINE	Δ_{Ghydr} [kcal mol^{-1}]		
	R	TS	P
HF/3-21G	-82.9	-78.0	-75.3
HF/6-31G(d)	-84.5	-75.4	-75.7
HF/6-31G(d,p)	-83.3	-75.5	-75,7
B3LYP/6-31G(d)	-76.9	-75,0	-69,9
B3LYP/6-31+G(d,p)	-75.9	-73.9	-71.6

Table 4 *Calculated values for the terminal C atom of irreversible inhibitor (CI) and flavin N5 distance that is a measure for the course of inhibition reaction with selegiline and rasagiline. The values are given for the reactant minimum (R), transition state (TS) and the products (R).*

SELEGILINE			
	Distance[Å]		
	R	TS	P
HF/3-21G	4.67	2.50	1.33
HF/6-31G(d)	4.76	2.15	1.34
HF/6-31G(d,p)	4.74	2.15	1.34
B3LYP/6-31G(d)	4.70	2.72	1.34
B3LYP/6-31+G(d,p)	4.88	2.28	1.34
RASAGILINE			
	Distance[Å]		
	R	TS	P
HF/3-21G	4.63	2.29	1.33
HF/6-31G(d)	4.73	2.16	1.34
HF/6-31G(d,p)	4.73	2.16	1.34
B3LYP/6-31G(d)	4.64	2.74	1.34
B3LYP/6-31+G(d,p)	4.74	2.23	1.34

It can be clearly seen that inclusion of polar environment increases the hydration free energy typically by 6-10 kcal mol^{-1} on the Hartree-Fock and B3LYP level of theory (Table 3 and Table 4). This results confirmed the assumptions that the effects of polar environment must be included when one wants to study chemical reactions, which are planned to be used as the reference reactions for the future calculations in the active centers of enzymes. Furthermore it is also shown that after including contributions of hydration free energies of solvent, the B3LYP method in conjunction with an appropriate basis set gives much better results than Hartree-Fock method, which is expected and understandable, since one would expect that Hartree-Fock method would overestimate the energy. Table 3 presents how free energies of hydration change from the reaction state, through the transition state to the product state. It is clear that free energies decrease in the same order. Geometric parameters associated with the inhibition reaction are collected in Table 4. They are comparable with the geometrical parameters from previous studies of irreversible inhibition of MAO B with rasagiline and selegiline, which were obtained by (CPCM)/BMK/6-311++G(2d,2p)//B3LYP/6-31+G(d) approach. There the distance between the interacting C terminal atom of acetylene group in irreversible inhibitor and N5 atom of flavin cofactor in the transition state was 2.28 Å for rasagiline and 2.27 Å for selegiline, respectively, while herein the distances fluctuate from 2.15 to 2.7 Å, since different levels of theory were used. The best match with the previous studies for both rasagiline (Figure 3) and selegiline (Figure 4) can be here found at the B3LYP/6-31+G(d,p) level of theory, which also points out the importance of using diffusion functions (+) in

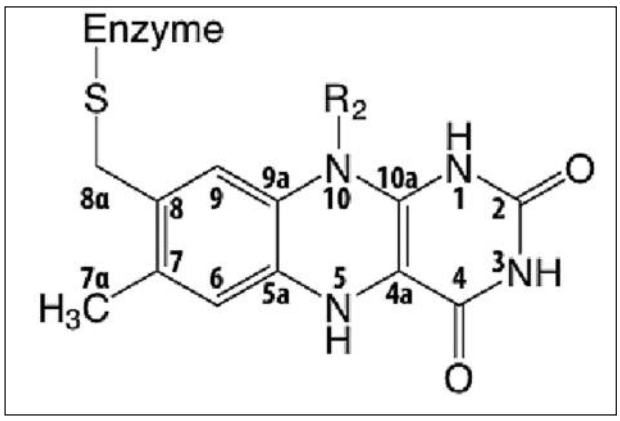


***Figure 2**:Atom numbering of flavin co-factor moiety - fully reduced form (left), and fully oxidized form (right).*

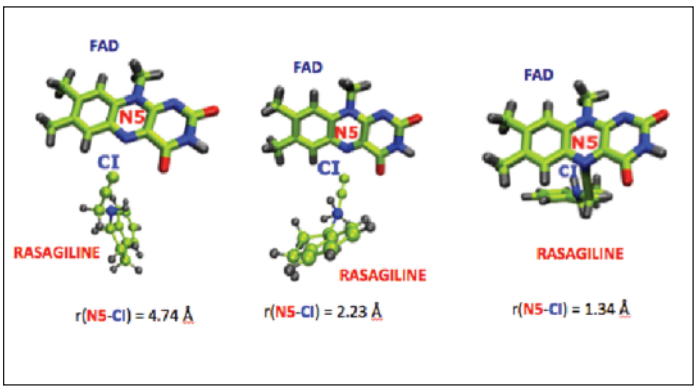


***Figure 3:** Structures of reactants (left), transition state (middle) and products (right) for rasagiline reacting with flavin co-factor (FAD). Structures were optimized at the B3LYP/6-31+G(d,p) level of theory.*

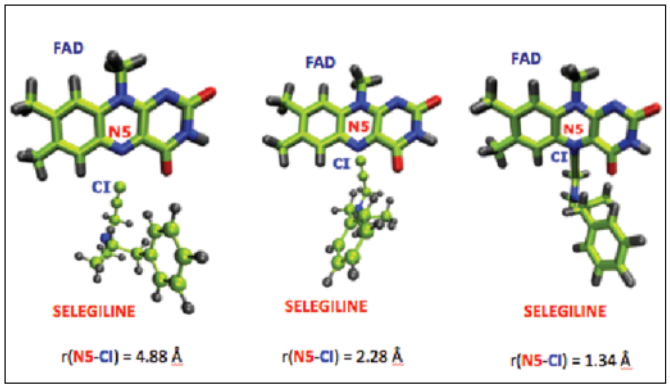


***Figure 4:** Structures of reactants (left), transition state (middle) and products (right) for selegiline reacting with flavin co-factor (FAD). Structures were optimized at the B3LYP/6-31+G(d,p) level of theory.*

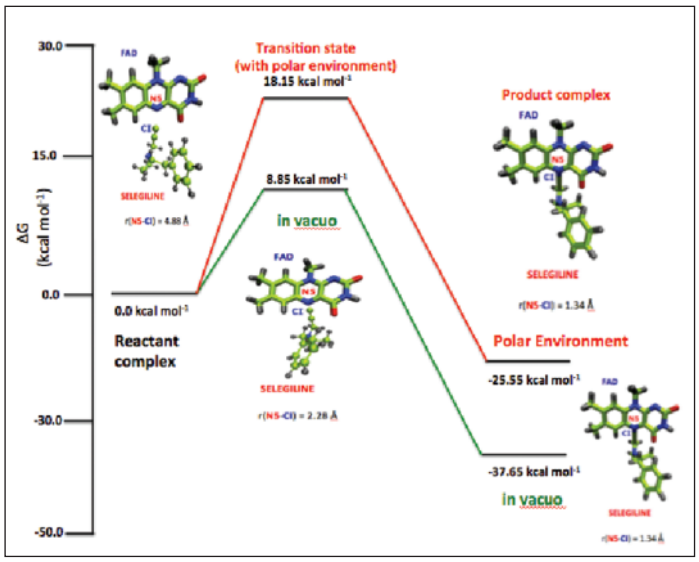


***Figure 5:** Free energy profile for selegiline reacting with flavin co-factor (FAD) in vacuo (green lines) and after considering effects of polar environment (red lines). Structures were optimized at the B3LYP/6-31+G(d,p) level of theory.*

the case of ionic species. For product state more or less all distances are around 1.34 Å for both types of irreversible inhibitors, which is practically the same as in the previous studies [48], which justifies the decision to critically evaluate the effects of polar environment at different level of theories in conjunction with Langevin dipoles model, since only in this way it was able to shed a light on the importance of this topic.

Figure 5 and Figure 6 offer a graphical representation of how the inclusion of polar environment contributions effect on the barrier height. As it has been already mentioned the height of the barrier increased when the solvent effects were considered. The solvent tries to surrund the solute and one of the very good sides of Langevin dipoles model is that the solvent is treated as dipoles on a cubic grid. In this way the calculation of free energies of hydration based on the interactions between dipoles of solvent and solute can be described more accurately. Therefore the differences between environment in an enzyme and polar environment (solvent) should always be very carefully considered and acknowledged, since the dielectric constant in an enzyme changes respectively to the one in solvent. This is specially important when calculating pKa values of the respective residues in the active site of MAO B [26], since it had to be considered the fact that due to the presence of many residues in the enzyme ,the prelectrostatic organization is different than the one in an aqueous solution. In the same way as the solvent here increases the barrier height for 6-10 kcal mol^{-1}, the residues in an enzyme can also contribute to free energies of activation or reaction for about 10 kcal mol-1, which was shown with QM/MM studies [48,49]. Therefore knowing the protonation states of residues is crucial, since in an enzyme electrostatic preorganization basically governs the mechanism of catalytic and inhibitory reactions. That also serves as a proof why calculating free energies of activation in the gas phase can not and must not be compared directly with the experimental values, and why it is so important to consider effects of polar environment. The later helps one to estimate free energies of reference reaction in aqueous solution. The properties in an enzyme environment can be very different, and mostly they are, compared to those in aqueous solution, because the prelectrostatic organization is probably one of the key components in studies of enzymatic reactions. Thus it is easier to understand why enzymes can catalyze many chemical reactions much better than they can be catalyzed in an aqueous solution in laboratory. Now if one takes a closer look at the possible tautomeric structures of the truncated FAD co-factor in Figure 7 it can be seen that atoms N1, O2, N3 and O4 have the ability to form

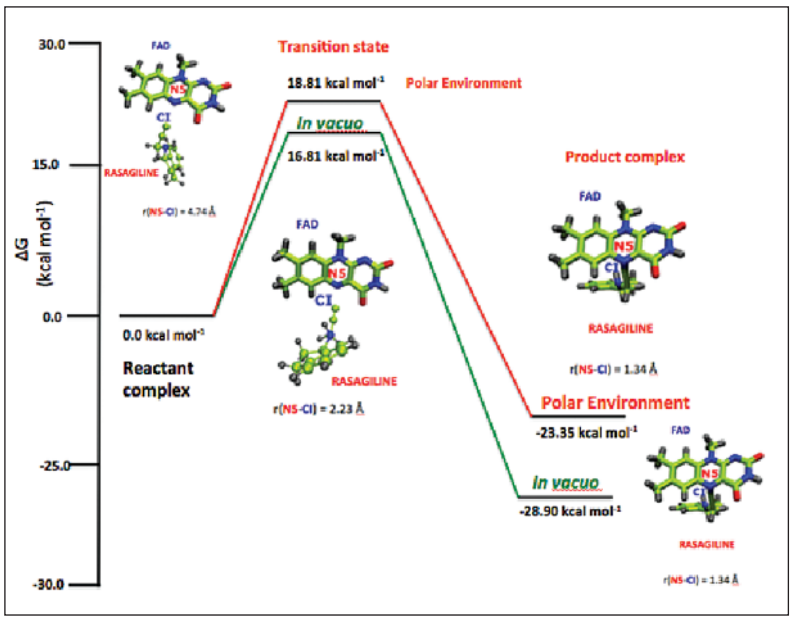


Figure 6: *Free energy profile for rasagiline reacting with flavin co-factor (FAD) in vacuo (green lines) and after considering effects of polar environment (red lines). Structures were optimized at the B3LYP/6-31+G(d,p) level of theory.*

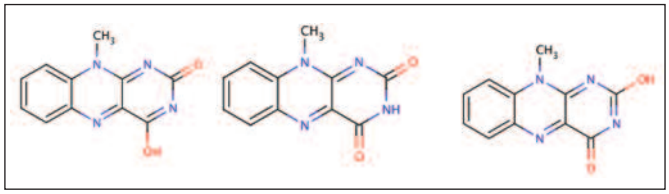


Figure 7: *Three possible tautomers of flavin co-factor (FAD).*

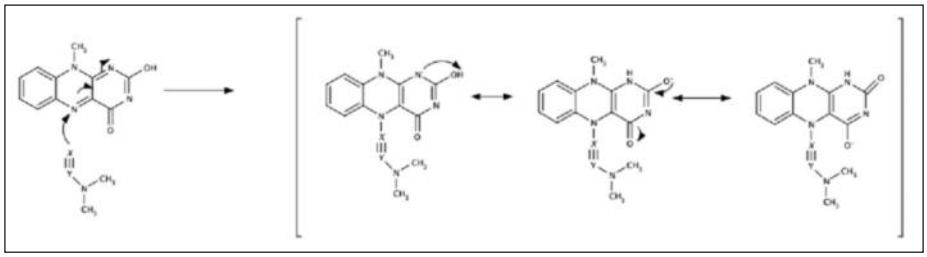


Figure 8: *Presentation of the irreversible inhibition reaction and possible tautomer and proton transfer structures in MAO B starting from the dominant tautomer structure presented on the right hand side of Figure 7.*

new tautomers. In the literature it is still unknown from where the N1 atom receives the hydrogen when an inhibitor or a hydrogen atom binds to N5 atom, and so enables the FAD co-factor to transform into its complete reduced form (Figure 2), although few spectroscopic studies were made in order to investigate the nature and oxidation states of flavin co-factor [50,51]. When the dominant tautomer structure for the truncated FAD co-factor was investigated by Marvin Sketch calculation tool for tautomeric structures the results suggested the structure shown on the very right hand side of Figure 7 was a dominant tautomer structure (58). Two other tautomer structures were much less plausible according to MarvinSketch calculator. This is very interesting, since usually the FAD co-factor is represented by the tautomer structure shown in the middle of Figure 7. The idea in the case of irreversible inhibition and also the catalytic reaction of MAO B would then be that one should actually start from the tautomer structure of FAD co-factor shown in Figure 7 (right hand side), because it would be easier for N1 atom as a proton acceptor to receive hydrogen from O2 atom through some sort of tautomerization or proton transfer process (Figure 8). In this way O2 becomes O-, and the question is, whether it accepts the proton or the process goes on all the way to O4 atom, which can eventually become O- (Figure 8). This could be also one of the explanations why FAD co-factor can get into fully reduced form without any additional reagents. Of course that is only one of many possibilities, and many more experimental and computational studies must be done before the catalytic and inhibition reaction of MAO and MAO B will be completely understood. Moreover it offers a complete new perspective to which up to now not much attention has been paid, especially not from the theoretical part of view, and could give more information regarding the electronic structure and properties of FAD co-factor. The next step to make would be to see, whether O2 or O4 atom of FAD co-factor get protonated again by the one of the water molecules in vicinity or by the methionine residue Met436 of which N atom of peptide bond is according to 2XFN crystal structure of MAO B, taken from the PDB base [57], 2.73 Å away from the O2 atom FAD co-factor, and could form some kind of temporary hydrogen bond. In this way that atom looses the ability of transforming to proton acceptor again, while the activity of the whole MAO B decreases.

For now all it can be suggested and implied based on the results of the calculations presented in this manuscript is that for irreversible inhibition of MAO B the polar anionic mechanism turned out to be the most plausible one, and that effects of polar environment must be included in order to be able to compare calculated free energies of activation with the experimental results, and to see later, after performing the calculations with QM/MM methods, where the whole enzyme environment can be considered, how good the reference reaction in aqueous solution is.

As one can see a lot of effort has been put up to now in trying to explain the mechanism of inhibitory reaction in MAO B as well as in MAO in general, and molecular simulations have been really helpful in sheding the light on that. However despite all the effort, it has to be kept in mind. that such studies are target specific studies, meaning they are focused on one part of the whole system, where the later is human body. Therefore it is often the case that even if a mechanism of catalytic reaction of an enzyme is known and understood it still does not prevent the side effects, which a medicine can cause. And the reason for that is exactly the target specific approach. Thus in reality the whole route through which a medicine reaches its target should be considered. For instance it is known that rasagiline is included

in an extensive hepatic metabolism by cytochrome P450 type 1A2 (CYP1A2) [53], and the CYP system is believed to be reposponsible for production of a great number of selegiline metabolites too [54, 55]. This means there are all of a sudden, especially if the metabolism is very extensive, new molecules present, which have not been predicted or studied, and they might follow a completely different mechanistc pathway than the original medicine. Hence the future perspectives of molecular simulations are directed and lean towards to be able to model and follow the lifetime route of a medicine, which is probably as chalenging as it was chalenging to design the human genome.

CONCLUSIONS

In the article a concrete approach based on the use of molecular simulations is presented, which shows the steps that need to be taken in order to be able to design a new medicine. It is important to know that up to now more or less theoretical procedures have been all based on the target specific approach, where the interactions between a specific target and a active molecule were studied in terms of shedding the light on the mechanistic pathway by which specific target operates. Nevertheless that represents only a part of a very complex system, which is actually a route that has to be travelled by a active molecule (medicine) to reach its specific target. And in most cases the methabolic pathway in theoretical studies is neglected, thus the unwanted and unpredicted side effects are still present. In order to reduce even more the side effects of a medicine the whole route of administration should be considered by molecular simulations and subsequently supported by the results of real experiments. That represents a great challenge for the future and can be somehow compared with the design of human genome.

Sažetak

Racionalni dizajn novih lekova za lečenje neurodegenerativnih bolesti.

U toku poslednje dekade, a i ranije, dobijena su nova saznanja i napravljeni su novi koraci u razumevanju mehanizama neurodegenerativnih bolesti, kao što su Parkinsonova i Alchajmerova bolest. Nova otkrića su moguća zahvaljujući razvoju tzv. kompjuterskog dela istraživanja, koje je posebno korisno kada je potrebno objasniti mehanizam delovanja na atomskom ili čak elektronskom nivou, što omogućava bolje planiranje i dizajn novih eksperimenata. Napravljena su mnogobrojna istraživanja u oblasti racionalnijeg i efektivnijeg dizajna novih lekova za lečenje Parkinsonove bolesti, posebno u cilju boljeg objašnjenja mehanizma delovanja leka. Korišćenjem principa molekularne stimulacije (molekularno modelovanje) može se dobiti uvid u metabolički put leka u ljudskom organizmu. Ovaj rad je zasnovan na konkretnim primerima koji pokazuju kako novi lek može biti dizajniran za lečenje neurodegenerativnne bolesti kao što je Parkinsonova bolest.

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