John von Neumann Institute for Computing

NIC

Molecular Dynamics in Excited States: Landau-Zener Model of Nitric Oxide Geminate Recombination to Nitrile Hydratase

K. Kubiak, W. Nowak

http://www.fz-juelich.de/nic-series/volume36

Molecular Dynamics in Excited States: Landau-Zener Model of Nitric Oxide Geminate Recombination to Nitrile Hydratase

Karina Kubiak and Wieslaw Nowak[*]

Institute of Physics, N. Copernicus University,
ul. Grudziadzka 5, 87-100 Torun, Poland
[*]*E-mail: wiesiek@fizyka.umk.pl*

Molecular dynamics (MD) simulations on two coupled electronic surfaces are employed to investigate the geminate recombination of nitric oxide to photoactive biotechnological enzyme nitrile hydratase (NHase). NHase enzymatic activity is triggered by photodissociation of NO molecule[1,2]. The crossing between the ground and the excited state surfaces is treated using the Landau-Zener model[3,4]. The NO geminate recombination curve and recombination rate were calculated. Results suggest that the NO recombination is a picosecond time-scale process.

1 Introduction

The microbial enzyme nitrile hydratase (NHase, EC. 4.2.1.84, CAS registration no. 82391-37-5) is widely used in biotechnological production of amides[5,6] from nitriles. Out of two types of NHases (Fe and Co-type) only the Fe-type NHase is photosensitive. This enzyme loses activity in the dark conditions when the endogenous nitric oxide molecule (NO) blocks the active centre. This process is totally reversible and the enzyme recovers catalytic ability upon light irradiation[1,2]. The electronic mechanism of NO controlled photoactivity remains unknown, despite several model systems of NHase have been investigated[7,8] and theoretical models have been calculated[9–14]. In the earlier study of the photosensitivity phenomenon Nowak *et al.*, described structural changes upon NO ligand binding to the iron center ("inverted doming")[9]. The MD calculations of NHase gave an insight into the channel dynamics[15], however the kinetics of recombination is still mysterious.

In this paper the NO recombination kinetics rate is theoretically calculated for the protonated enzyme model. Similarly to Li *et al.*[16] classical Landau-Zener (LZ) MD model of recombination is employed.

2 Computational Methods

In the LZ method the system may evolve on two alternative, ground and excited state crossing hypersurfaces. At the crossing point, the LZ probability P is calculated (1) and the decision whether to switch the energy surface is made[3,4]

$$P = e^{-\frac{4\pi\varepsilon_{12}^2}{h\nu|s_1 - s_2|}},\tag{1}$$

where V is a velocity, ε_{12}^2 is energy difference between two energy states and $|s_1 - s_2|$ is a difference in the energy curves slopes.

All computations were performed for Fe-type NHase (2AHJ) in the MOIL package[16,17]. The parametrization of the NHase active site is described elsewhere[15]. Only crystallographic water molecules were present. Initially minimized system (the enzyme with NO molecule bounded via Morse potential, 3000 steps) was heated up to 300 K (50 ps) and equilibrated for 50 ps. From short 100 ps run 6 random structures were chosen as starts for production simulations of the NO recombination. For each start a 10 ps trajectory at 250, 275, 300, 325 and 350 K was generated. In each of 30 trajectories NO molecule was photodissociated (by excitation) at the very beginning. The SHAKEB protocol was used, data were collected in each 1 fs step. The temperature was held constant by velocity scaling, cutoffs for electrostatic and van der Waals interactions were 12 Å and 9 Å, respectively. The nonbonding interactions were recalculated in each step.

The excited state curve $U(r)$ was modeled by (2) and the binding potential $B(r)$ was approximated by the Morse function (3) (see Fig. 1):

$$U(r) = A_{rep}e^{-\beta r} - B_{rep}, \tag{2}$$

and

$$B(r) = D_{mor}e^{-2\alpha(r-r_{eq})} - 2e^{-\alpha(r-r_{eq})}. \tag{3}$$

Parameter r_{eq} is the equilibrium Fe–N(NO) bond length of 1.65 Å, parameters D_{mor}, α, A_{rep}, β and B_{rep} were 30 kcal/mol, 2 Å^{-1}, 80 kcal/mol, 1 Å^{-1} and 4 kcal/mol, respectively. Other parameters required for excited state MD were adopted from[16].
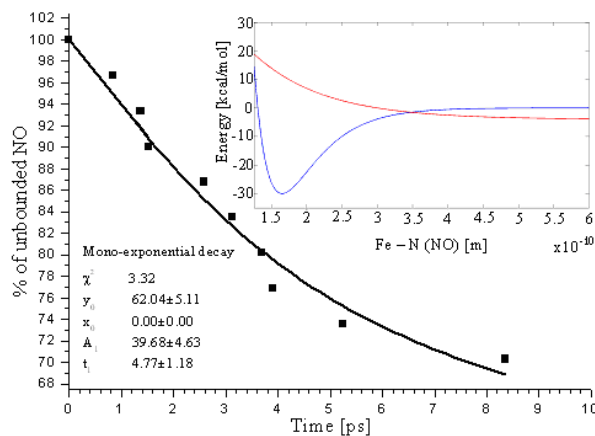
3   Results and Discussion

Figure 1. Kinetics of NO recombination to NHase active site. Points indicate a percent of recombined ligands. A mono-exponential decay $y = y_0 + A_1 \exp\left(-\frac{x-x_0}{t_1}\right)$ is fitted. **Inset**: Potential curves used in simulations of NO recombination.

For each of 10 ps non-equilibrium trajectories on excited state energy surface the Fe–N(NO) distance, the repulsion energy value (E_{rep}), the switching moment from repulsion

to Morse potential and a value of the Morse potential (E_{morse}) were monitored. If the distance Fe–N(NO) was smaller than 2.5 Å or if the E_{morse} was smaller than -10 kcal/mol, we assumed that the recombination happened. A percent of recombinated ligands vs time gave data for estimation of recombination rate (see Fig. 1).

We assume that these data points are the best described by a mono-exponential decay since that assumption gives the smallest value of the χ^2 ($\chi^2 = 3.3$). Two and three exponential decays fitted to our MD data gave χ^2 of 4.7 and 8.7, respectively and times t_2 and t_3 were extremely long (data not shown). Fitted parameters (Fig. 1) indicate that the average lifetime of the free NO is 4.8 ps (the decay rate constant is 0.21 ps^{-1}). NO collisions with βArg56 or (less frequently) with αGln90 induced the recombination. Other collisions with the active site-solvent channel walls didn't result in the rebinding.

4   Conclusions

For the first time a geminate recombination of the NO molecule to Fe-type NHase active site was studied using the Landau-Zener approach and MD in excited state. We found that there is perhaps one energy barrier (4 kcal/mol) and the recombination rate is characterized by 4.8 ps lifetime of free NO. Residues βArg56 and αGln90 are critical for NO recombination. Better statistics is required for more quantitative estimates of NHase photophysics.

Acknowledgments

References

1. T. Noguchi, J. Honda, T. Nagamune, H. Sasabe, Y. Inoue, I. Endo, *Photosensitive nitrile hydratase intrinsically possesses nitric oxide bound to the non-heme iron center: evidence by Fourier transform infrared spectroscopy*, FEBS Letters **358**, 9 (1995).
2. M. Odaka, K. Fuji, M. Hoshino, T. Noguchi, M. Tsujimura, S. Nagashima, M. Yohda, T. Nagamune, Y. Inoue, I. Endo, *Activity regulation of photoreactive nitrile hydratase by nitric oxide*, J. Am. Chem. Soc., **119**, 3785 (1997).
3. L. Landau, Z. Phys. Sov., **2**, 46, (1932).
4. C. Zener, Proc. Roy. Soc. London, Sect. A (Math. Phys. Sci.), **137**, 696, (1932).
5. M. Kobayashi, T. Nagasawa, H. Yamada, *Enzymatic synthesis of acrylamide: a success story not yet over*, Tibtech, **10**, 402 (1991).
6. H. Yamada, S. Shimizu, M. Kobayashi, *Hydratases involved in nitrile conversion: screening, characterization and application*, Chemical Record **1**, 152 (2001).
7. H. L. Jackson, S. C. Shoner, D. Rittenberg, J. A. Cowen, S. Lovell, D. Barnhart, J. A. Kovacs, *Probing the influence of local coordination environment on the properties of Fe-type nitrile hydratase model complexes*, Inorg. Chem. **40**, 1646 (2001).
8. J. Shearer, H. L. Jackson, D. Schweitzer, D. K. Rittenberg, T. M. Leavy, W. Kaminsky, R. C. Scarrow, J. A. Kovacs, *The first example of a nitrile hydratase model complex that reversibly binds nitriles*, J. Am. Chem. Soc. **124**, 11417 (2002).

9. W. Nowak, Y. Ohtsuka, J. Hasegawa, H. Nakatsuji, *Density Functional Study on Geometry and Electronic Structure of Nitrile Hydratase Active Site Model*, Int. J. Quantum Chem. **90**, 1174 (2002).

10. A. J. Boone, Ch. H. Chang, S. N. Greene, T. Herz, N. G. J. Richards, *Modelling the spin-dependent properties of open-shell Fe(III)-containing systems: towards a computational description of nitrile hydratase*, Coord. Chem. Rev. **238–239**, 291 (2003).

11. T. C. Harrop, P. K. Mascharak, *Fe(III) and Co(III) Centers with Carboxamido Nitrogen and Modified Sulfur Coordination: Lessons Learned from Nitrile Hydratase*, Acc. Chem. Res. **34**, 253 (2004).

12. Ch. Chang, A. J. Boone, R. J. Bartlett, N. G. J. Richards, *Toward a Computational Description of Nitrile Hydratase: Studies of the Ground State Bounding and Spin-Dependent Energetics of Mononuclear, Non-Heme Fe(III) Complexes*, Inor. Chem. **43**, 458 (2004).

13. L. Peplowski, K. Kubiak, W. Nowak, *Insights into catalytic activity of industrial enzyme Co-nitrile hydratase. Docking studies of nitriles and amides*, J. Mol. Model. **13**, 725 (2007).

14. L. Peplowski, K. Kubiak, S. Zelek, W. Nowak, *A Comparative DFT Study of Substrates and Products of Industrial Enzyme Nitrile Hydratase* Int. J. Quant. Chem., (2007), *in print*.

15. K. Kubiak, W. Nowak, *Molecular dynamics of photoactive protein nitrile hydratase*, Biophys. J., *in preparation*.

16. H. Li, R. Elber, J. E. Straub, *Molecular dynamics simulation of No recombination to myoglobin*, J. Biol. Chem. **268**, 17908 (1993).

17. R. Elber, C. Simmerling, R. Goldstein, H. Li, G. Verkhiverer, C. Keasar, J. Zhang, A. Ulitsky, *MOIL: A program for simulations of macromolecules*, Comput. Phys. Commun. **91**, 159 (1995).