

Electric Field Effect on Protein Dissociation: a Computational Evaluation of the Energy Barrier over Thiol-Disulfide Interchange Reaction in TRX-TXNIP Complex as an Interaction Model for Biological Antioxidant Response

D. Fracassi* ⁽¹⁾, V. D'Annibale ⁽¹⁾, M. D'Abramo ⁽¹⁾, G. d'Inzeo ⁽¹⁾

(1) La Sapienza University of Rome, Rome, Italy; e-mail: fracassi.1705177@studenti.uniroma1.it; valeria.dannibale@uniroma1.it; marco.dabramo@uniroma1.it; guglielmo.dinzeo@uniroma1.it

Abstract

Cells are susceptible of oxidative stress and have intracellular signaling pathways able to sense it and activate an appropriate response. In between these, we identified Thioredoxin (TRX), a major antioxidant protein, and its endogenous inhibitor TXNIP. TXNIP bonds TRX by means of a disulfide bridge that saturates its active site. Such an interaction is redox-dependent as ROS can dissociate it resulting in a Thiol-Disulfide Interchange Reaction. Following a growing interest in finding subcellular targets and mechanisms linked to a possible cytoprotective effect of electromagnetic (EM) fields, we studied the influence of electric field over this system through a hybrid QM/MM approach. Results show a strong catalytic effect of the static electric field over the TRX-TXNIP complex dissociation reaction and a consistent products' stabilization. A preliminary study on nanopulses also shows the effect to be significant in the transient period, making time a relevant variable only if the field amplitude is increased. These results are promising for a further characterization of this interaction mechanism, which could be the way (or one of many) in which EM fields might directly influence intracellular signaling pathways.

1 Introduction

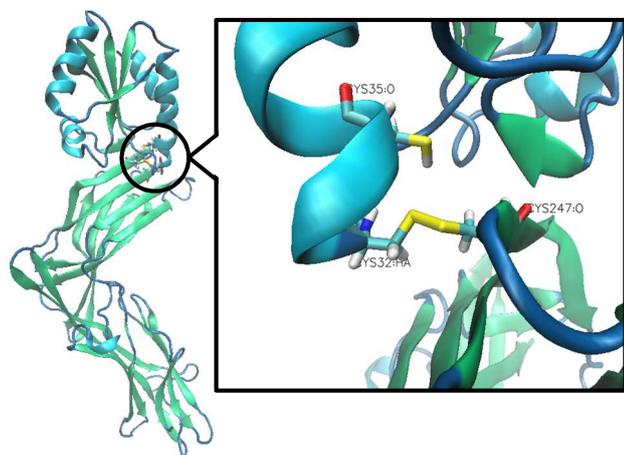
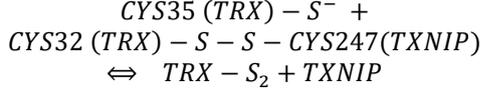


Figure 1. TRX-TXNIP complex: close up of the disulfide bridge between Cysteine 32 of TRX and Cysteine 247 of TXNIP with nearby thiol of Cysteine 35 of TRX.

The redox state of the intracellular environment plays a pivotal role between the chemical-physical properties that guarantee cellular homeostasis. In particular, an excess of Reactive Oxygen Species (ROS) can lead to oxidative stress, cell damage and is well known to be linked to aging [1]. Living organisms therefore need an intracellular signaling pathway able to sense such stress conditions and activate a defense mechanism. In between the different intracellular systems, we identified the action of the Thioredoxin protein (TRX) and its endogenous inhibitor Thioredoxin Interacting Protein (TXNIP) [1-3].

Thioredoxin is a small globular protein present in all types of cells and well known to have a major antioxidant role, as it is expressed as a defense to different kinds of stress [1,2]. Its active site can be found in the N-terminal part of its α -helix and contains a CGPC motif: Cysteine 32, Glycine 33, Proline 34 and Cysteine 35 [1, 4]. The two lateral cysteines carry out the antioxidant role of TRX, cyclically passing from their reduced form of thiol (R-SH) to their oxidated form of disulfide bridge (R-S-S-R') [1, 4]. TRX takes part in the Thioredoxin system together with Thioredoxin Reductase (TrxR) and a NADPH molecule; TRX reduces oxidated proteins by oxidating itself, while TrxR and the NADPH molecule later reduce TRX [2, 3].

The intracellular pathway able to activate the antioxidant action of TRX is linked to the regulatory activity of its endogenous inhibitor TXNIP [5, 6]. TXNIP is usually found in the cell nucleus but under stress conditions it transmigrates in the intracellular environment to interact with TRX [3]. This interaction saturates TRX's active site by means of a disulfide bridge between TRX's Cysteine 32 and TXNIP's Cysteine 247 [1, 3, 5], creating the TRX-TXNIP complex (Fig. 1). To trigger an antioxidant response such a covalent bond must be dissociated, and this is exactly what happens in the presence of oxidative stress. In fact, ROS can directly influence the interaction between TRX and TXNIP resulting, indeed, in a redox-dependent regulation [5]. In fact, the proximity of a free radical to the disulfide bridge can lead to the formation of a thiosulfinate (R-S(=O)-S-R), intermediate species that in the presence of a nearby thiol - like that of Cysteine 35 - can cause the formation of a new disulfide bridge [5, 7]. The overall reaction is labeled as a Thiol-Disulfide Interchange Reaction and leads to the dissociation of the TRX-TXNIP complex:



Having identified this system of intracellular signal pathway and related antioxidant defense mechanism, we now aim to study if an external electric or EM field could interact with it. In particular, we aim to find a catalytic effect of the external field that could alone compare to the action of ROS. This interest generates from the growing literature of *in vitro* cell experiments hinting at a cytoprotective effect of EM fields over ROS accumulation [8, 9]. To pursue this goal, we performed a computational study using a QM/MM hybrid approach to obtain an estimation of the Energy Barrier of the TRX-TXNIP complex's dissociation under different exposure conditions.

2 Methods

The computational method used to study the dissociation of the TRX-TXNIP complex is a hybrid QM/MM approach, necessary due to the system's dimension (10^4 atoms) and the subatomic precision requirement. In particular, the quantum center (QC) is identified with the lateral chains of Cysteines 32 and 35 of TRX and Cysteine 247 of TXNIP, while the rest of the system is treated with classical physics. The hybrid approach chosen is the Perturbed Matrix Method (PMM) [10] in which the perturbed Hamiltonian of the QC, \hat{H} , is obtained perturbing the unperturbed one, \hat{H}^0 , as in:

$$\hat{H} = \hat{H}^0 + \hat{V} \quad (1)$$

Where \hat{V} is the perturbation operator obtained by a multipolar expansion of the electrical potential V centered in the QC center of mass, r_0 , as in:

$$\hat{V} = \sum_i V(r_i) q_i \cong \sum_i [V(r_0) - E(r_0) \cdot (r_i - r_0) + \dots] q_i \quad (2)$$

The i -index refers to the QC nuclei and electrons, as q_i is particle charge and r_i the corresponding distance from the center of mass. $V(r_0)$ is the electrostatic potential and $E(r_0)$ is the electric field both exerted by the perturbing environment on the QC center of mass. The quantum properties of the perturbed QC are evaluated by diagonalization of the Hamiltonian matrix; this way we obtain the QC perturbed energy ΔA at each time step. The convenience of this hybrid approach dwells in the fact that quantum calculations are run only once to obtain \hat{H}^0 , while the perturbative term is evaluated for each DM simulation. To estimate the reaction's energy barrier, we can consider it as a sum of steps between ensembles along the reaction coordinate, typically described by a reactant state (R), a transition state (TR) and the products state (P). Therefore, the perturbed energy change, ΔA , can be considered a

function of the reaction coordinate ξ transitioning from R to TR to P. For a generic transition between a point i and $i+1$ along the reaction coordinate, the energy difference could be designed as follows [12]:

$$\Delta A_{i \rightarrow i+1}(\xi) = -k_B T \ln \langle e^{-\beta \Delta H} \rangle_{i \rightarrow i+1} \approx -k_B T \ln \langle e^{-\beta \Delta U} \rangle_{i \rightarrow i+1} \quad (3)$$

Where the QC environment whole ground energy difference, ΔH , is approximated with the QC perturbed electronic ground state energy difference, ΔU . The sum of each free energy variation constitutes the overall free energy profile along the reaction coordinate:

$$\Delta A_{tot}(\xi) = \sum_{i=1}^n \Delta A_{i \rightarrow i+1} \quad (4)$$

Where n is the total number of points selected along the reaction coordinate, in our case 11.

3 Results

3.1 Static Electric field

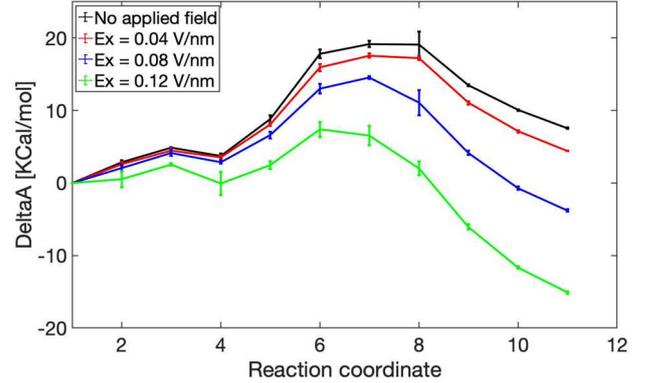


Figure 2. Energy barrier evaluation for electric field values of 0.04, 0.08 and 0.12 V/nm on 100 ns simulations transient excluded

We simulate the action of a static electric field by considering a simulation time of 100 ns, having first deleted 20 ns of transient. The results are shown in Fig. 2 where the black curve represents the control condition and the other three are obtained for exposures of 0.04, 0.08 and 0.12 V/nm electric fields. A strong field effect is noticeable as the energy profile undergoes a consistent change when the system is exposed. In particular, the profile tends to lower its peak values as the intensity of the field increases, implying that the reaction tends to occur more easily as the field amplitude rises. There is also a significant effect in the stabilization of products (last three points), as their energy gradually lowers with the field intensity. This same effect isn't found in the reactants area (first three points), where the energy values stay similar in the four different conditions. This can be explained minding the fact that the

stabilization of a chemical specie is mainly related to the solution. As previous studies show [12], our QC in the reactants phase is found to be interdicted to the solvent and therefore has less possibility of being stabilized by the nearby solution.

Table 1. Maximum amplitude of the evaluated energy barrier for the different exposure conditions.

Electric Field [V/nm]	ΔA_{\max} [Kcal/mol]
0.00	20.91
0.04	18.28
0.08	15.30
0.12	9.03

From the estimated energy barrier curves we extrapolated the maximum ΔA as shown in Tab. 1. Such a value represents the activation energy E_a of a reaction, which is the amount of energy needed to initiate it. The ΔA_{\max} values give a further quantitative confirmation of the detected effect.

3.2 Nanopulses

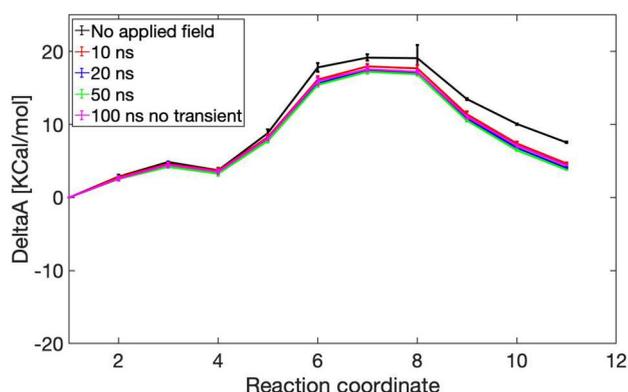


Figure 3. Energy barrier evaluation for 0.04 V/nm, comparison of 10, 20, 50 and 100 ns exposure.

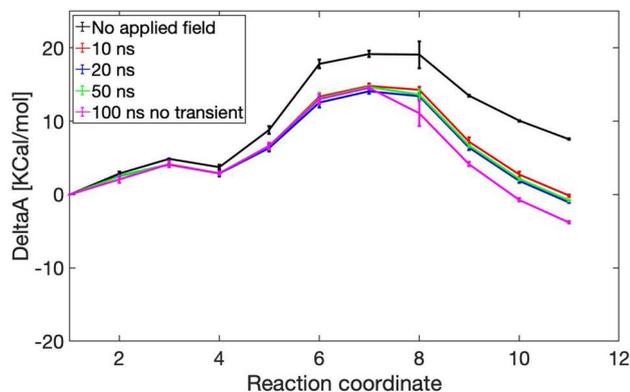


Figure 4. Energy barrier evaluation for 0.08 V/nm, comparison of 10, 20, 50 and 100 ns exposure.

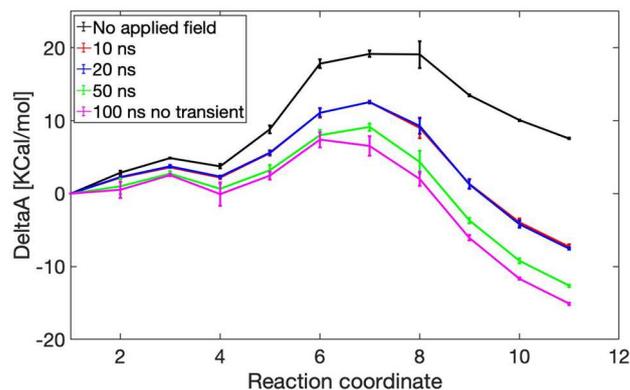


Figure 5. Energy barrier evaluation for 0.12 V/nm, comparison of 10, 20, 50 and 100 ns exposure.

In Fig. 3, Fig. 4 and Fig. 5 we compare the energy barrier estimated for static exposure with the ones obtained considering 10, 20 and 50 ns exposures respectively for 0.04, 0.08 and 0.12 V/nm field amplitudes. What we observe is that for an electric field of 0.04 V/nm there's not a significant difference in the obtained results for every exposure condition. Almost the same happens for 0.08 V/nm, as only the static exposure tends to diverge after the pick showing a slighter increased product stability. Only at 0.12 V/nm the obtained curves tend to diversify, showing an influence of the exposure time over the detected effect. These results state that the exposure time and the field amplitude aren't independent variables. In particular, the exposure time becomes significant only if the field amplitude is high enough.

4 Conclusion

In this study we aimed to find a possible effect of the electric field over the dissociation reaction of the TRX-TXNIP complex. This protein-inhibitor system takes part in the cell's intracellular signalling pathway for oxidative stress detection. In fact, their covalent bond is redox-dependent and enables TRX's antioxidant action once dissociated [5]. The groundwork of this research can be found in a rising interest in finding targets and mechanisms of a possible cytoprotective effect of the EM field [8, 9]. The study has been conducted computationally with a hybrid QM/MM approach by estimating the energy barrier of the dissociation reaction. The system has been exposed to electric fields of 0.04, 0.08 and 0.12 V/nm, comparing the effect of a different exposure time. Static exposure results show a significant change in the energy profile, identifying a catalytic effect of the electric field over the reaction. In fact, the activation energy halves - passing from 20.91 Kcal/mol of the control condition to 9.03 Kcal/mol of the 0.12 V/nm exposure - and a greater products stability is also shown. Nanopulses results show the effect to be significant also in the transient period. In particular, exposure time tends to be a significant variable only if the field intensity is high enough. Therefore, we

conclude to have identified a specific electric field effect on the dissociation of TRX-TXNIP complex in which the exposure time and the field amplitude aren't independent variables. These results are promising for a further characterization of the effect, having as next step the use of oscillating fields. The goal is to find a possible agreement of the computational side with the experimental one in which radiofrequencies are used. In this way we hope to find out the (or one of the) subcellular targets and related mechanisms on which such cell-level cytoprotective effect is based. Fully characterizing the detected interaction mechanism can give birth to a Biological Antioxidant Response Model which could be of clinical use.

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