Atomistic modelling of the effects of protein adsorption on the (100) MgO surface

Gabriel Cava, José Dávila, Arturo Fiorentini, Jun Sotelo, Abel Gutarra
Laboratorio de Materiales Nanoestructurados - Universidad Nacional de Ingeniería
e-mail: ivancava@gmail.com

Mirko Zimic
Laboratorio de Bioinformática - Universidad Peruana Cayetano Heredia

Carlos Silva
Laboratorio de Microelectrónica - Pontificia Universidad Católica del Perú

In this work, a first atomistic approach to describe the processes taking place on an oxide-electrolyte interface due to the presence of a complex molecule such a protein is reported. A brief description of the classical approach is given. The spatial distributions of molecules and charges are reproduced using molecular dynamics techniques. Potential curves and Debye lengths determined in our simulations seem to agree with those calculated using classical models. Our results show that a small protein, the bovine pancreatic trypsin inhibitor (BPTI), can generate a *potential shift across the interface. Even more, the Debye length is modified by the presence of the protein.*

Keywords: oxide-electrolyte interface, protein adsorption, atomistic modelling.

En el presente trabajo se reporta un primer enfoque atomístico de los procesos que tienen lugar en una interfase oxido-electrolito debido a la presencia de una molécula compleja tal como una proteína. Las distribuciones espaciales moleculares y de carga son reproducidas utilizando técnicas de dinámica molecular. Las curvas de potencial y las longitudes de Debye determinadas en las simulaciones concuerdan con aquellas calculadas usando modelos clásicos. Nuestros resultados muestran que una pequeña proteína, el inhibidor de tripsina pancreática bovina, puede generar un cambio en el potencial en la interfase. Más aún, la longitud de Debye es modificada por la presencia de la proteína.

Palabras claves: interfase óxido-electrolito, adsorción de proteínas, modelamiento atomístico

1. Introduction

Currently, protein-surface interactions are being widely studied due to their important role in many biochemical techniques and biological processes, such as chromatographic separation of protein molecules, or the develpment of novel biosensors. Understanding the physico-chemistry of a protein adsorption process on an oxide surface in aqueous media is of extreme importance in medical, pharmaceutical and biotechnological research and development. Determination of proteins by measuring an intrinsic property such as the charge density of the protein itself is an attractive possibility for constructing an immunosensor. An oxide-water interface is a simple system which can be described using classical continuous models such as the well-known double layer and site-binding models[1], the comprehension of the effect of a biomolecule on the oxide surface in that system requires to be complemented by the use of atomistic models, widely used to describe complex systems such as proteins.

MgO is a basic oxide widely used as a support for catalytic processes and it is commonly used as a model system for understanding interfacial processes on oxide materials. Several experimental and theoretical studies have investigated the properties of the $MgO - water$ interface[2-3]. In this paper we investigate the effects of the adsorption of a small protein, the bovine pancreatic trypsin inhibitor (BPTI), on the (100) MgO surface in aqueous media using an atomistic approach.

2. Classical approach

The first step for studying the effect of a protein on an oxide surface is to understand the behaviour of the oxide in aqueous media. The mechanism responsible to modify the potential across the oxide-electrolyte interface can be described by the site-binding and double layer models, which describe the equilibrium between the so-called amphoteric surface sites and all the ions in the solution. According to these models, the double layer capacitance consists of a series network of a Helmholtz-layer capacitance (the Stern capacitance) and a diffuse layer capacitance. The Helmholtz layer models the effect that the ions in the solution have a finite size and the centres of these ions cannot approach the surface any closer than their ionic radiuses including a layer of water molecules[4], which means that there exists two planes of closest approach for the centres of the ions, the inner and outer Helmholtz planes, IHP and OHP, repectively. For reasons of simplicity, we will consider that only H^+-ions can reach the IHP and all other solvated ions can only reach the OHP, since this is enegetically favourable. The diffuse layer, starting from the OHP, contains a charge distribution formed of mobile ions. This is shown in figure 1. In thermodynamical equilibrium the surface reactions are:
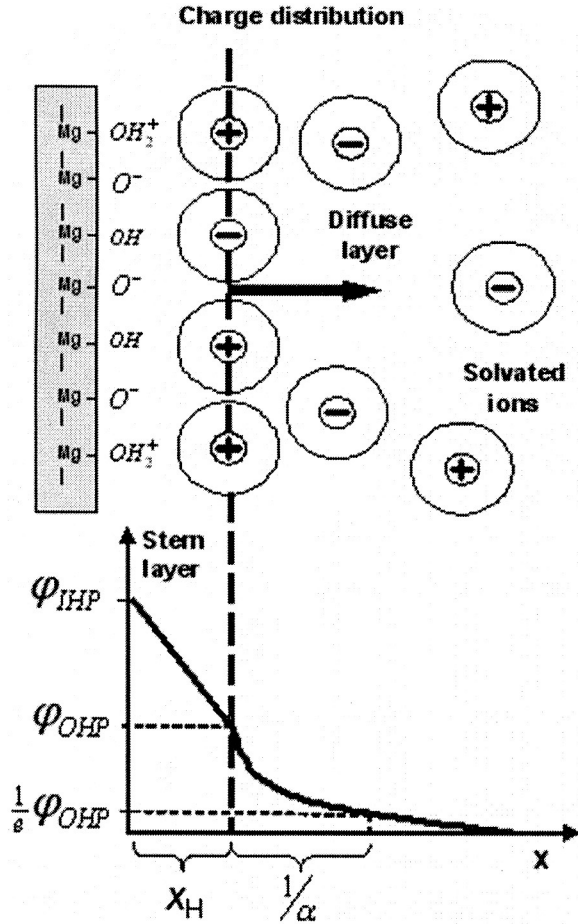
$$MgOH \rightleftharpoons MgO^- + H^+, \qquad (1)$$

$$MgOH + H^+ \rightleftharpoons MgOH_2^+. \qquad (2)$$
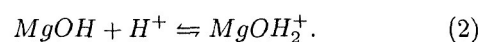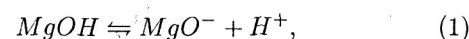
Figure 1. *Charge and potential distributions across the double layer.*

Considering NaCl as the salt in the electrolyte, the surface charge densities, are given by:

$$\sigma_{IHP} = q\left(\nu_{MgOH_2^+} - \nu_{MgO^-}\right),\qquad(3)$$

$$\sigma_{OHP} = q\left(\nu_{Na^+} - \nu_{Cl^-}\right),\qquad(4)$$

where ν is in moles per unit area, and q is the electron charge. Due to the geometry of the system, the potential across the Stern layer is given by,

$$\varphi_{OHP} - \varphi_{IHP} = -\frac{\sigma_{IHP} - \sigma_{OHP}}{2\varepsilon_s\varepsilon_0}\cdot x_H,\qquad(5)$$

where ε_s is the relative electrical permitivity in the Stern layer. By the other hand, the diffuse charge distribution is given by,

$$\rho(x) = q\Sigma_i\nu_i(x)z_i,\qquad(6)$$

where ν_i and z_i are the concentration and valence of each specie i. Those concentrations can be related by the Boltzman equation,

$$\nu_i(x) = \nu_i^0 exp\left(\frac{-qz_i\varphi(x)}{kT}\right),\qquad(7)$$

The potential across the diffuse layer is given by,

$$\frac{d^2\varphi}{dx^2} = -\frac{q}{\varepsilon\varepsilon_0}\Sigma_i\nu_i^0 z_i exp\left(\frac{-qz_i\varphi(x)}{kT}\right),\qquad(8)$$

which results in,

$$\varphi(x) = \varphi_{OHP} exp\left(-\alpha(x - x_H)\right),\qquad(9)$$

where $1/\alpha$ is the Debye length, and it is given by,

$$\frac{1}{\alpha} = \left(\frac{\varepsilon\varepsilon_0 kT}{2q^2 I}\right)^{\frac{1}{2}},\qquad(10)$$

where I is the ionic strength. Then, the Debye length is defined as the distance from the OHP where the electrical potential φ has dropped to $\frac{1}{e}\varphi_{OHP}$, and it depends on the ionic strength I.

Only charges located within the Debye length contribute with the potential across the double layer. This length should change if a protein is placed on the insulator because the overlapping of the double layers of the oxide-electrolyte interface and of the protein itself. Then, the sensitivity of φ due to a charge distribution change caused by a protein attached on the oxide surface will depend on the Debye length. However, the Debye length of that system is too difficult to calculate due to the complexity of a protein.

3. Atomistic approach

A complete simulation procedure contains three parts: derivation of interatomic potentials parameters, formation of simulated material structure, and evaluation of an effective simulation strategy for the investigation of the protein adsorption effects. The key of a computer simulation study is the availability of suitable interatomic potentials. For an oxide-electrolyte interface, we need interatomic potentials for the adequate description of the individual structures of which our system is composed: the oxide surface, the protein, and water molecules. There are many consistent interatomic potentials for proteins and water molecules implemented in many force fields. For this work, the consistent-valence force field, or CVFF, for the protein and water molecules was used. The interatomic short-ranged potential acting between the surface atoms Mg and O_S, and water atoms O_W and H, has the expression given in eq. (11), where the two first terms are called the Buckingham potential and the last term is the coulombic potential. The respective interatomic potential parameters for our system were taken from the work of McCarthy et al[5], derived by fitting to energy surfaces obtained from a series of Hartree Fock quantum mechanics calculations. These parameters are shown in table 1.

$$U_{ij}(r_{ij}) = A_{ij}\cdot exp\left(\frac{-r_{ij}}{\rho_{ij}}\right) - \frac{C_{ij}}{r_{ij}^6} + \frac{q_iq_j}{r_{ij}}.\qquad(11)$$

Table 1. *Interatomic potential parameters used for the $MgO - H_2O$ system*

| i	j	A_{ij}	ρ_{ij}	C_{ij}
O_S	O_S	$5,24599x10^5$	0.149000	$6,42500x10^2$
O_S	Mg	$2,22839x10^4$	0,315000	$0,00000x10^0$
O_S	O_W	$1,78234x10^5$	0,229130	$1,96997x10^2$
O_S	H	$8,18944x10^3$	0,259700	$6,73483x10^1$
Mg	O_W	$8,66508x10^4$	0,233350	$4,34161x10^2$
Mg	H	$3,98139x10^3$	0,261534	$1,48432x10^2$

The underlying MgO crystalline unit cell structure was obtained from the cubic *Fm-3m* space group configuration. Using the Wyckoff notation, Mg atoms are in the 4a [0 0 0] position, and O atoms are in the 4b $[\frac{1}{2}\frac{1}{2}\frac{1}{2}]$ positions. The lattice parameter used was a=4.2112 $Å$. The MgO surface was built by repeating the unit cell along the three axes in a 16x16x4 proportion, resulting in a system of eight atomic layers with an area of 67.3792x67.3792 $Å^2$, and a depth of 16.8448 $Å$. In order to obtain the minimum energy structure, and with the interatomic potentials parameters and the system defined, a molecular dynamics (MD) process was performed with periodic boundary conditions (PBC) which permit us to consider it as an infinite surface, within the framework of the Born model of the solid wherein the Mg and O_S atoms were treated as non-polarizable point ions with integer atomic charges of +1.996 and -1.996 q. For this work *Insight II* from *Accelrys*, a commercially available software, was used. The resulting structure presented an area of 67.33795x67.33795 $Å^2$, and a depth of 16.83449 $Å$.

Water molecules were treated, according to CVFF, as semirigid molecules with an $H - O$ bond distance of 0.96 $Å$ and an $H - O - H$ angle of 104.5° , while the charges on the hydrogen and oxygen atoms were +0.41 and -0.82 q, respectively. On the MgO surface, a 15 $Å$ thick layer of water was placed. The bottom three layers of the MgO structure were fixed at their bulk position. A new MD process with PBC was performed. The resulting $MgO - H_2O$ system is shown in figure 2. As we can see, a first uniform continuous layer of water has formed on the surface, 3.7 $Å$ further up a second layer of water is observed. These layers correspond to the IHP and OHP, according to the classical approach (see Sec. 2).
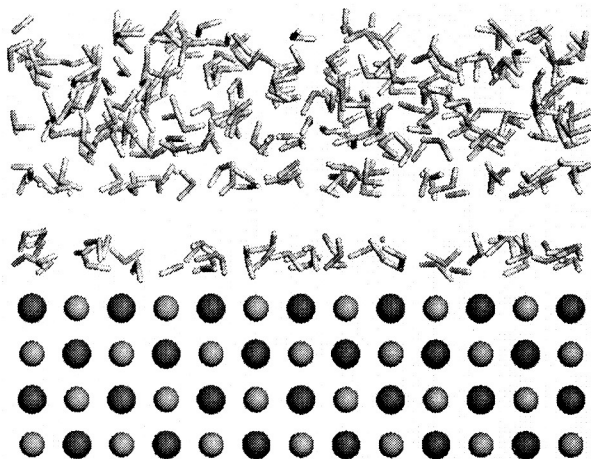

Figure 2. *$MgO - H_2O$ system after the MD process.*

In order to vary the ionic strength I, neutral salt molecules were added to the system. The salt chosen was $NaCl$ and the ionic radiuses taken were 0.95 and 1.81 $Å$ for the Na^+ and Cl^- atoms respectively. The potential across the system φ was calculated using a nonlinear Poisson-Boltzman method implemented in *Insight II*. Results are shown in figure 3 for different values of ionic strength.
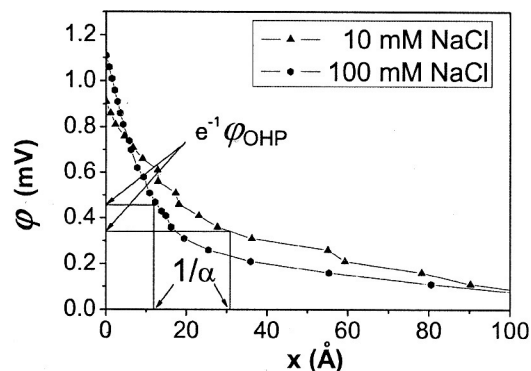
Figure 3. *Potentials across the $MgO - H_2O$ system for two different values of ionic strength at pH 7.0.*

Debye lengths were extracted from five different curves of φ at different values of ionic strength and compared with those calculated using eq (10). Results are shown in figure 4.
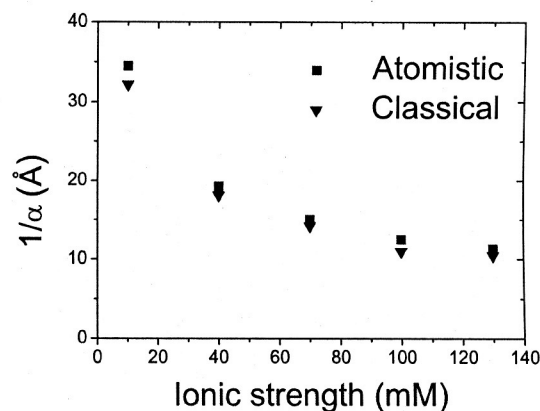
Figure 4. *Debye length values calculated using classical and atomistic models.*

To simulate the effect of a protein on the MgO surface, a new system was built. A small protein, the bovine pancreatic trypsin inhibitor (BPTI), was chosen. The structure of the BPTI was obtained from the Protein Data Bank (PDB). With this structure, a refinement process was performed in order to obtain the minimun energy structure and to eliminate possible bad bond lengths and angles. The dimensions of the protein in the .pdb file were 31.62x30.17x30.90 $Å^3$, and a 204.8 $Å$ -edge cube of water was used for the refinement. For this process, the available open-source software GROMACS[6] was used.

After the refinement process, neither bad bonds nor angles were present. Then, all water molecules less those on the first layer were removed from the $MgO - H_2O$ system and the refined structure of the BPTI was placed on it. Water molecules were added to complete the new system. The minimum energy configuration of the $MgO - BPTI - H_2O$ system, and its respective potential curve were obtained. The potential curve is shown in figure 5. Due to the lack of interatomic potential parameters for the protein, the neutral salt $NaCl$, and the MgO surface atoms, a first approximation was to consider those interactions as purely coulombic.
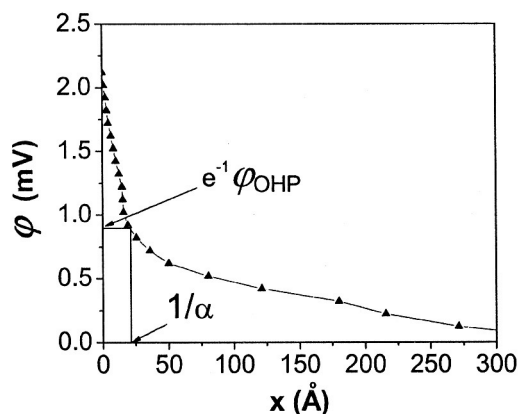
Figure 5. *Potential across the $MgO - BPTI - H_2O$ system for a ionic strength of 10mM at pH 7.0.*

It is shown how the adsorbed protein on the surface remarkably increases the potential φ at the interface compared with its value without the protein (see fig. 3). By the other hand, the Debye length is reduced.

4. Discussion

The atomistic treatment presented in this work gives a new approach to elucidate the mechanisms taking place in an oxide-electrolyte interface not only when that simple system is studied, but when the system becomes more complex due to the presence of a protein. A classical approach reveals its limitations when such system is studied. The examples given here are meant to be illustrative

rather than comprehensive. In that sense, the aim of this work is to use atomistic models and computer simulations to give a better approach for the possibilities and limitations about detecting proteins by measuring potential shifts across an oxide-electrolyte interfase.

Although φ_{IHP} depends on the surface properties, the Debye length is not related to the material of which the surface is made. Debye length only depends on the ionic strength (see eq. (10)), when no other than nonbonding interactions are considered. The shapes of the potential curves showed in figure 3 and their respective Debye lengths agree with those calculated using eq. (10). This means that the system behavior is in accordance with classical models. Water molecules move by the action of the interatomic potentials between them and the surface atoms so the key of a successful MD process is how accurate the parameters are in our potential model. If suitable parameters are used for the interaction between all the system atoms, the behavior of that system should be reproduced. A first approximation of these parameters in our simulation revealed that a protein attached to the surface can modify not only the potential φ_{IHP} but the Debye length too.

5. Conclusions

A first approach to reproduce the mechanisms which take place on an oxide-electrolyte interface is given using atomistic models. Simulations were performed in order to obtain potential curves across the double layer at different ionic strengths. Potential behavior seems to agree with those calculated using classical models. The presence of a small protein attached to the surface modifies the potential and Debye length across the double layer in our simulations. The work presented here is clearly of a preliminary nature, and results must be validated experimentally to consider this atomistic treatment accurately enough.

Acknowledges

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