The coupling among electron transfer, deformation, screening and binding in electrochemically active macromolecules

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Experimental data are presented demonstrating that electrochemically active macromolecules show a coupling among electron transfer, deformation, screening and binding. The work includes dependence of the redox potential of synthetic and natural electrochemically active polymers on the electrolyte pH (electron transfer-binding coupling), the changes in volume during the redox switching of synthetic electrochemically active polymers (deformation-electron transfer coupling) and the changes in the macromolecular conformation during the acid–base titration of polyelectrolytes and proteins (deformation-binding coupling). A simple equilibrium statistical thermodynamic model is presented that allows explaining these couplings effects. The model is based on the assumption that a macromolecule is composed of segments of different length that may bind species present in the external solution and that also contain redox centers that may be oxidized and reduced. The partition function of the system is obtained, and from it the expressions for the redox potentials, the total length and the chemical potential of the bound species are obtained. Simple calculations shows that the model satisfactorily explains the qualitative behavior of the experimental results.

1. Introduction

Electrochemically active macromolecules are substances that can be oxidized and reduced in a reversible way. These macromolecules, both natural and synthetic, have received a great deal of attention: natural macromolecules, mostly metalloproteins, because of their obvious importance in biochemical reactions,[1] and synthetic macromolecules, mostly polymers, because of their potential applications in several fields.[2–5]

In this work we propose that, in electrochemically active macromolecules, there is a coupling among electron transfer, deformation or state of tension, binding and the ionic screening of the charged sites. By electron transfer we are referring to the ability of the macromolecule to transfer electrons to a suitable redox couple in the same solution or to an electrode submitted to a suitable potential. By deformation we are referring to the appearance of a state of tension in the macromolecule as a consequence of which the chemical bonds of different parts of the macromolecules become stretched. The deformation embodies several interaction forces in the macromolecule such as interaction with the solvent, conformations states, ionic screening, coulombic interaction between the charges sites in the macromolecule, hydrogen bonding, *etc*. The state of binding refers to the amount of specifically bound ionic or neutral species on the macromolecule, or parts of it. A typical example could be the binding of protons and hydroxyls on the amino acid residues present in different metalloproteins. The state of screening refers to the weakening of the coulombic interactions between the fixed charged sites of the macromolecule by the ionic atmosphere surrounding them. Obviously, this state of screening depends on the ionic strength of the external solution. These couplings manifest themselves in the sense that changing the state of one of them causes all the others to change. Thus, for instance the decrease of the pH of the external solution produces proton binding and this makes all the other mentioned states change. These couplings have a wide variety of consequences on the equilibrium and kinetic behavior of electrochemically active macromolecules. At this stage, it is worth noting that in macromolecular substances the characteristic magnitudes measuring the deformation, the redox potentials, the binding constants, *etc*., do not have a unique value, as happens with single site substrates, but a distribution of them.

The choice of magnitudes to describe the state of a macromolecule, whether in solution or as an amorphous solid or a gel, is not arbitrary; it is in agreement with the accepted thermodynamic and molecular descriptions of polyelectrolytes, both natural and synthetic.[6–8]

On the other hand, the change of these states can be effectively monitored by different experimental techniques.[7] Thus, the average state of deformation of a single macromolecule in solution can be monitored by measuring the viscosity or by light scattering, or by NMR shifts or, in the case of gels, by simply measuring the volume. The state of binding can be determined by some kind of titration. The state of screening is generally ascertained determining the dependence of some binding constant (acid–base or electron) on the ionic strength of the solution. The influence of the binding and screening on the redox potential can be determined by measuring it as a function of the activity of the binding species or the ionic strength, respectively.

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In the literature there are many antecedents of these type of coupling effects. Deformation/binding couplings are found in the *mechanochemistry* described by Katchalsky *et al.*,[9] to explain the folding of synthetic polyelectrolytes during acid–base titration. Also, deserving of mention is the Bovine serum albumin expansion to pH changes, that was described more than fifty years ago.[10]

With *electrostatic screening* something similar happens. During the acid–base titration of polyelectrolytes[11,12] and proteins[6,13,14] there is a coupling between binding and screening. Also, the folding of proteins produced by changes of ionic concentration in the external electrolyte is an example of deformation/screening coupling.[15,16] As will be discussed in the next paragraph, since the redox potential is dependent on the state of tension, it will also be influenced by the ionic strength of the solution.

Concerning the factors that influence the redox potentials of electron transfer proteins a relatively large number of works have been published.[17–21] One of the most important conclusions of the work of Mauk and Moore[17] is that "the possibility of redox state-dependent conformational changes should be considered". Precisely, the idea of a redox potential being dependent on the state of tension (electron transfer/deformation coupling) was introduced by Evans *et al.*,[22–24] to explain the redox potential distribution of poly(vinylferrocene). The coupling of electron transfer, deformation, binding and electrostatic screening effects, was introduced to explain the redox potential distribution during the redox switching of arylamine substituted polymers.[25–27]

These couplings manifest themselves not only in systems at equilibrium but also on the kinetic of electron transfer reactions. Thus electron transfer triggers the folding of proteins[28] and the electrochemical induced ageing of conducting polymers.[29] Moreover, another example of kinetic effects of these couplings is the *entactic effect* by which "the chemistry and the energetics of protein folding generate a stereochemical and/or electronic state around a metallic ion that increase its potential catalytic functions".[30]

Previously,[25] we developed a very simplified model to explain the distribution of redox potentials as a coupling between the redox state and the deformation of a polymer. In the present work, we extend that model to include the possibility of binding some species to the polymer. In this way, we will be able to explore the coupling among the different processes that participate in the electron transfer of electrochemically active macromolecules. In this work, the model is employed in a qualitative way. From that point of view, it allows to demonstrate the basic relations between the different contributions to the Helmholtz free energy of the system. Then, the predictions of the model are compared with selected experimental results previously obtained by us and by other workers. Thus, the dependence of the redox potential on the concentration of the binding species in solution is compared with the experimental results obtained both for synthetic and natural electroactive macromolecules. Also, experimental results for the change of volume with the applied potential to synthetic electroactive polymers are compared with the model predictions. Finally, it is shown that the pH changes observed during the redox titration of electroactive macromolecules[31] may also be qualitatively explained with the present model.

This model is neither intended for a quantitative representation of such complicated systems as those used as examples nor to answer specific questions about a particular system, such as the mutagenesis of a particular protein site, but it provides a general framework of how the coupling of effects works out and how the couplings affect the properties of electrochemically active macromolecules.

2. Methods

Statement of the problem

Let us consider the system as a gel composed by intertwined polymer chains deposited on top of a suitable metallic base electrode, in contact with an electrolytic solution, in thermodynamic equilibrium, at constant temperature.

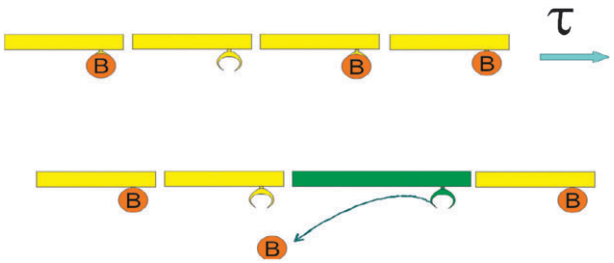
There are four external variables that determine the state of the system: the electrode potential, the concentration of binding species, the concentration of the electrolyte and the mechanical external force applied to the system. As one of these variables is changed, the change in the others can be measured. Thus, we may change the external concentration of the binding species and measure the changes in deformation, the electrode potential, and so on.

The model is based on the following considerations. First, we will consider that a polymer chain is composed of structureless segments: short segments and long ones. Short segments convert into long ones by stretching the chain.

Second, associated to each segment there is a binding site where a species present in the external solution can bind. In general, binding constants for short and long segments will be different. This binding process preferentially stabilizes one type of segment (according to the relative values of the binding constants) and the presence of a bound species modifies the tension necessary to convert one type of segment into the other.

Third, associated to a short segment there is a reduced redox center and associated to a long segment there is an oxidized redox center. As we will consider the polymer as a part of a galvanic cell (see below), stretching the chain will make some reduced redox centers become oxidized and some current will flow along the external circuit. So, short segments will be converted into long ones, by simply applying a suitable potential difference to the cell.

Fourth, we will not consider the structure of the solvent, disregarding the effect of its structure on the conformation of the macromolecular chains. Also we will assume that the concentration of ions in the external medium is high enough to efficiently screen the charges in the polymer chains. This is tantamount to disregarding the electrostatic interactions between the charges in the polymer chains. These charges may be due to fixed charges in the polymer chains or generated by the binding of charged species on the binding sites of the polymer chains. In the latter case, the bound ions will increase the charge of the polymer chains, and counterions from the external electrolyte, will enter into the polymer in order to maintain the electroneutrality (Donnan equilibrium).



Scheme 1 Effect of stretching a polymer chain. Pale grey blocks represent short segments and dark grey blocks long ones. Applying some tension to the chain, short segments are converted into long ones. Also, note that, as a consequence of this transformation, one bound species is desorbed from the segment ($K_R > K_{Ox}$).

As we have already stated, we assumed that the macromolecule is composed of intertwined chains; and that each chain is composed by two types of segments short, R, and long, Ox, respectively. The system is depicted in Scheme 1. If a tension τ is applied to the chain, short segments are converted into long ones. The length of the short segments is λ_R and that of the long ones, λ_{Ox}. The number of each type of segments is M_R and M_{Ox}, respectively, and the total number of segments is $M = M_R + M_{Ox}$. It is clear that the length of the chain is: $\lambda = \lambda_R M_R + \lambda_{Ox} M_{Ox}$. In order to stretch the chain by an amount $d\lambda$, it will be necessary to do mechanical work, $\delta w_{mec} = \tau d\lambda$.

We will consider there is no interaction neither between the segments nor between them and the solvent. This is we are disregarding some forces contributing to the stability of macromolecules, particularly in the case of biological macromolecules. These can be taken in part into account employing more refined models, as it has been done before.[26]

Each type of segments may bind a species that we will call B. In Scheme 1 a binding site is represented by a short stick perpendicular to the segment. The binding sites may be empty or occupied. We will assume that the binding species are present in the external solution with a chemical potential $\mu_B = \mu^0_B + kT \ln a_B$, where μ^0_B is the standard chemical potential, a_B its activity and k and T have the usual meaning. The number of species bound to the R and Ox segments is N_R and N_{Ox}, respectively, and the total number of bound species is $N = N_R + N_{Ox}$. For the sake of simplicity we will assume that the binding equilibrium for the R and Ox segments can be described by the Langmuir isotherm (no interaction between the bound species):

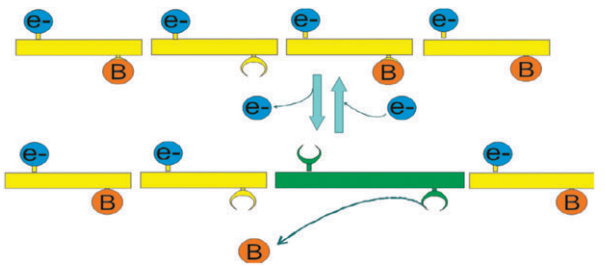
$$\theta_{N,R} = K_R a_B/(1 + K_R a_B) \quad (1)$$

and similarly for the oxidized ones,

$$\theta_{N,Ox} = K_{Ox} a_B/(1 + K_{Ox} a_B) \quad (2)$$

where, $\theta_{N,R} = N_R/M_R$, and $\theta_{N,Ox} = N_{Ox}/M_{Ox}$, are the fractions of occupied sites in each type of segment and K_R and K_{Ox} are the corresponding binding constants.

The absence of interactions between the bound sites is a simplifying assumption. One-dimensional systems such as linear polymers can be treated by the exact Ising model; for two and three dimensions, the Bragg–Williams approximation can be employed.[8]



Scheme 2 Effect of oxidizing and reducing one segment.

Since both types of segments are in binding equilibrium with the external solution, their respective chemical potentials, $\mu_{N,R}$ and $\mu_{N,Ox}$, must be equal, *i.e.*,

$$\mu_{N,R} = \mu_{N,Ox} = \mu_B \quad (3)$$

At this point, we will consider the conversion of short segments into long ones as the chain is stretched (see Scheme 1). It is interesting to note that, according to its relative binding constants (K_R and K_{Ox}), stretching the chain may also cause some B species to become bound or released (see Scheme 1).

The segments can be reversibly oxidized (or reduced) either electrochemically or by a chemical oxidation (or reduction) reaction, according to eqn (4) and Scheme 2.

Here, also for simplicity, we have assumed that the reduced center is uncharged and that the number of electrons transferred during the oxidation is one and, consequently, the Ox center has a positive charge.

The polymer is deposited on top of a base electrode, and as we stated, it is considered as a part of a suitable electrochemical cell such as:Base Electrode/Polymer/External Solution//Reference Electrode

Then, the potential can be changed simply by applying a different potential between the base and the reference electrodes.

The electrochemical reaction is:

$$R \leftrightarrow Ox^+ + e \quad (4)$$

As the short segments are also the reduced ones and the long segments are the oxidized ones, henceforth we will refer to them as reduced, R, and oxidized, Ox, segments.

As an example, we may consider the redox switching of polyaniline (Pani). For this polymer, the reduced form (leucoemeraldine) is oxidized to a partially oxidized form (emeraldine). This reaction can be roughly represented as: [32–35]

$$(B\text{–}NH\text{–}B\text{–}NH)_{2\nu} \rightleftharpoons (B\text{–}NH\text{–}B\text{–}NH\text{–}B\text{–}N{=}Q{=}N)_\nu + 2\nu H^+ + 2\nu e \quad (5)$$

Here ν is the number of segments in a chain. It is generally accepted that in the leucoemeraldine form the reacting segment is composed by four amine units (here represented as –NH) and that the emeraldine form is composed by two amine units and two imine groups (here represented as –N=).[32–35] Then, it is clear that, in this example, the reduced segment have two electrochemically active centers.

In writing eqn (5) we have assumed that both leucoemeraldine and emeraldine forms are in their base forms. According to the pH of the external medium both will be protonated to a certain

degree that will be different for each type of segments. The pK_a of the amine groups in the leucoemeraldine has been determined to be around 2.5 and that of the imine groups in the emeraldine form to be about 5.5.[36]

Then, we will consider that associated to each type of segment there are n_{Ox} and n_R redox centers, the total number of them, $n = n_{Ox} + n_R$ is constant. The conversion of R to Ox occurs through reaction (4). Moreover, we will define the fraction of oxidized centers as $\theta = n_{Ox}/n$.

According to the above definitions the following relations can be established:

$$M_{Ox} = \gamma n_{Ox} \quad (6)$$

$$M_R = \gamma n_R \quad (7)$$

In eqn (6) and (7) we have assumed that the number of redox centers on each segment is one. The total number of segments, M, results:

$$M = M_{Ox} + M_R = \gamma n = \gamma(n_{Ox} + n_R) \quad (8)$$

The total length of the polymer chain, λ, is given by:

$$\lambda = M_{Ox}\lambda_{Ox} + M_R\lambda_R = \gamma(n_{Ox}\lambda_{Ox} + n_R\lambda_R) \quad (9)$$

In the absence of mechanical effects, in order to oxidize a differential amount of R to Ox, an amount of charge, dq, has to be transferred along the external circuit of the galvanic cell. Therefore, one has to perform electrochemical work, $\delta w_{electrochen} = E\,dq$, where E is the emf of the galvanic cell. However, since to convert a short segment into a long one, it is also necessary to expend mechanical work, it is clear that, in the presence of mechanical effects, the total necessary work to be expended is the sum of the two contributions.

The basic thermodynamic equation

At constant temperature, the basic thermodynamic equation can be written as:

$$dA = \tau\,d\lambda + \mu'\,dM + \mu^*_{Ox}\,dn_{Ox} + \mu_R\,dn_R + \mu^*_{el}\,dn_{el} + \mu_{N,Ox}\,dN_{Ox} + \mu_{N,R}\,dN_R \quad (10)$$

Here A is the Helmholtz free energy. The first term refers to the work necessary to expand the segments in which τ is the tension and λ is the deformation. The second term is the contribution of the segments, μ' being its chemical potential. The following two terms are due to the redox centers, μ^*_{Ox} and μ_R are its electrochemical and chemical potentials, respectively, μ^*_{el} is the electrochemical potential of the electrons. $\mu_{N,Ox}$ and $\mu_{N,R}$ are the chemical potentials of the species bound to an oxidized and to a reduced segment, respectively.

Not all the variables are independent, so we will proceed to eliminate the dependent variables. From the stoichiometry of the electrochemical reaction (4), the following relations can be established: $dn_{Ox} = -dn_R = dn_{el}$. As a result:

$$\mu^*_{Ox}\,dn_{Ox} + \mu_R\,dn_{red} + \mu_{el}^*\,dn_{el} = (\mu^*_{Ox} - \mu_R + \mu^*_{el})\,dn_{Ox} = eE'\,dn_{Ox} \quad (11)$$

where we have defined $eE' = \mu^*_{Ox} - \mu_R + \mu^*_{el}$. Here, E' is the redox potential in the absence of mechanical effects and e is the charge of the electron.

Similarly, in view of eqn (8) not all the values of M are independent. We will choose M and M_{Ox} as the independent variables. Differentiating eqn (9):

$$d\lambda = \lambda_{Ox}\,dM_{Ox} + \lambda_R\,dM_R \quad (12)$$

So that the first two terms of eqn (10) can be written as:

$$\tau\,d\lambda + \mu'\,dM = \tau(\lambda_{Ox} - \lambda_R)\,dM_{Ox} + (\mu' + \tau\lambda_R)\,dM \quad (13)$$

Henceforth, we will consider M is constant and consequently we will drop the term $(\mu' + \tau\lambda_R)\,dM$. Similarly, we will consider N and N_{Ox} as the independent variables. Then the last two terms in eqn (10) can be written as:

$$\mu_{N,Ox}\,dN_{Ox} + \mu_{N,R}\,dN_R = (\mu_{N,Ox} - \mu_{N,R})\,dN_{Ox} + \mu_{N,R}\,dN \quad (14)$$

Since both types of segments are in binding equilibrium with the external solution, the respective chemical potentials, $\mu_{N,Ox}$ and $\mu_{N,R}$, must be equal (see eqn (3)). So, this term reduces to $\mu_{N,R}\,dN$.

Then, eqn (11) can be written as:

$$dA = \tau(\lambda_{Ox} - \lambda_R)\,dM_{Ox} + eE'\,dn_{ox} + \mu_{N,R}\,dN \quad (15)$$

In view of eqn (6), $dM_{Ox} = \gamma\,dn_{Ox}$, so that eqn (15) may be written as:

$$dA = [\gamma\tau(\lambda_{Ox} - \lambda_R) + eE']\,dn_{Ox} + \mu_{N,R}\,dN = eE\,dn_{Ox} + \mu_{N,R}\,dN \quad (16)$$

where we have defined $eE' = [\gamma\tau(\lambda_{Ox} - \lambda_R) + eE']$. According to eqn (16) E is given by:

$$(dA/dn_{Ox})_N = eE \quad (17)$$

Note that the quantity E is the redox potential *including* mechanical effects, as is clear from the definition of E.

The chemical potential of the species bound to the R segments results, from eqn (16), as:

$$(dA/dN)_{n_{Ox}} = \mu_{N,R} \quad (18)$$

Note that the procedure to obtain the redox potential is similar to those presented in most of the standard Physical Chemistry books.[37] Note also that, in the absence of external forces, the quantities E and $\mu_{N,R}$ are the experimentally and theoretically accessible quantities.

The statistical thermodynamic solution

In a previous work we have employed the formalism of the canonical partition function, $Q(M, M_{Ox}, N, N_{Ox}, n, n_{Ox}, T)$;[25] in this case we will proceed with the same formalism. However, it is interesting to note that the problem can be also solved within the formalism of the grand canonical partition function, $\Xi(M, M_{Ox}, \mu_B, N_{Ox}, n_{Ox}, E, T)$.[8]

The canonical partition function can be conveniently separated into three contributions:

(i) The partition function of the segments, Q_M:

$$Q_M(M_{Ox}, M, T) = M!{j_{Ox}}^{M_{Ox}}{j_R}^{(M-M_{Ox})}/(M - M_{Ox})!M_{Ox}! \quad (19)$$

where j_{Ox} and j_R are the internal partition functions of the segments associated to the Ox and R centers, respectively.

(ii) The partition function of the bound species, Q_N:

$$Q_N(M_{Ox}, M, N, N_{Ox}, T) = (M - M_{Ox})!M_{Ox}!{q_{Ox}}^{N_{Ox}}{q_R}^{(N-N_{Ox})}/(M_{Ox} - N_{Ox})!N_{Ox}![M - M_{Ox} - (N - N_{Ox})]!(N - N_{Ox})! \quad (20)$$

where, q_{Ox} and q_R are the internal partition function of the species bound to the segments, associated to the Ox and R centers, respectively. We have assumed that the partition function of an unoccupied site is unity.

(iii) The partition function of the redox centers, Q_n. This, as in the previous work,[25] is given by:

$$Q_n(n_{Ox}, n, T) = {p_R}^{n-n_{Ox}}{p_{Ox}}^{n_{Ox}}n!/n_{Ox}!(n - n_{Ox})! \quad (21)$$

where, p_{Ox} and p_R are the individual partition functions for the redox centers. The total partition function, Q, results:

$$Q = Q_M Q_N Q_n = [M!{j_{Ox}}^{M_{Ox}}{q_{Ox}}^{N_{Ox}}{j_R}^{(M-M_{Ox})}{q_R}^{(N-N_{Ox})}/(M_{Ox} - N_{Ox})!N_{Ox}![M - M_{Ox} - (N - N_{Ox})]!(N - N_{Ox})![{p_R}^{n-n_{Ox}}{p_{Ox}}^{n_{Ox}}n!/n_{ox}!(n - n_{Ox})!] \quad (22)$$

Once the partition function is known, the Helmholtz free energy can be obtained from:

$$A = -kT\ln Q \quad (23)$$

This allows obtaining the chemical potential of the bound species on the reduced segments. According to eqn (18), this is:

$$\mu_{N,R}/kT = -(\partial \ln Q/\partial N)_{M,M_{Ox},N_{Ox},T} = \ln[\theta_{N,R}/(1 - \theta_{N,R})q_R] \quad (24)$$

Had we chosen N_R instead of N_{Ox} in the partition function Q_N (eqn (20)) we would have obtained for the chemical potential of the oxidized segments, $\mu_{N,Ox}$:

$$\mu_{N,Ox}/kT = \ln[\theta_{N,Ox}/(1 - \theta_{N,Ox})q_{Ox}] \quad (25)$$

Then, considering that both bound species are in equilibrium with the external solution in which the chemical potential of this species is μ_B, we may write eqn (24) and (25) in a more familiar form as those given by eqn (1) and (2).

It is clear that if θ is changed, keeping constant the concentration of species in the external solution, the fractional amount of occupied Ox sites, $\theta_{N,Ox} = N_{Ox}/M_{Ox}$, and of occupied R sites, $\theta_{N,R} = N_R/M_R$, must also remain constant. However, as θ is changed, M_{Ox} and M_R change and, therefore, N_{Ox} and N_R must also change.

Taking the logarithm of eqn (22), multiplying by kT and deriving respect to n_{Ox}, taking into account eqn (6) and (7), we obtain the potential,

$$E = (kT/e)\{\ln[p_R\theta/p_{Ox}(1 - \theta)] + \gamma\ln[(j_R(1 - \theta_{N,Ox})\delta/j_{Ox}(1 - \theta_{N,R})(1 - \delta)]\} \quad (26)$$

In eqn (26) we have introduced the definition $\delta = M_{Ox}/M$. The first term is the contribution of the redox centers and the second one the contribution of the expansion of the polymer. The presence of the latter is a consequence of the restriction that relates the number of redox centers to the number of segments (eqn (6) and (7)). Note that the first term of the rhs of eqn (26) is the difference of the chemical potentials of the redox centers, $\mu_{Ox} - \mu_R$, with $\mu_{Ox} = \mu_{Ox} + kT\ln\theta$ and $\mu_R = \mu_R + kT\ln(1 - \theta)$. It is important to remark that the definition of the chemical potential of the redox centers R and Ox implies ideality, that is the absence of interactions between them. Departures from ideality can be easily taken into account by applying the Bragg–Williams approximation.[25] Moreover, it is clear that the potential depends on the amount of bound species and on the internal partition functions of the segments. In turn, the fraction of bound species depends on its internal partition functions, as well as on its concentration in solution. That is, the potential depends on the concentration of the binding species in solution through the mechanical term.

The potential at which the oxidized fraction is $\theta = 0.5$ can be considered as the apparent formal potential, therefore ${E^{0\prime}}_{ap}$ results:

$${E^{0\prime}}_{ap} = (kT/e)\{\ln[p_R/p_{Ox}] + \gamma\ln[(j_R(1 - \theta_{N,Ox})/j_{Ox}(1 - \theta_{N,R})]\} \quad (27)$$

The equilibrium constant for electron binding in the absence of other effects is $P = p_R/p_{Ox}$. Similarly, the equilibrium constant for the segments in the absence of other effects is $J = j_R/j_{Ox}$. Also the equilibrium constant for the bound species on the R and O segments in the absence of other effects is $K = K_R/K_O$. Taking into account these definitions, eqn (27) can be written as:

$${E^{0\prime}}_{ap} = (kT/e)\{\ln P + \gamma\ln[JK\theta_{N,Ox}/\theta_{N,R}]\} \quad (28)$$

The influence of P, J and K on ${E^{0\prime}}_{ap}$ will be discussed in the next section.

3. Results and discussion

Henceforth, we will analyze the predicted theoretical dependences of the calculated magnitudes. Then we will make a qualitative comparison of the theoretical predictions with selected experimental results.

As a_B may change over several orders of magnitude we will employ, instead of this variable, minus the logarithm of this quantity which we will call pB. Therefore $pB = -\ln a_B$.

Instead of selecting the q values it is convenient to introduce the binding constants values, Ks in eqn (1) and (2).

Dependence of ${E^{0\prime}}_{ap}$ on the partition functions and pB

The first term in the right side of eqn (28) depends on the ratio of the partition functions of the redox centers $P = p_R/p_{Ox}$. This is a measure of the relative stability of the Ox and R states in the absence of other effects. If $P > 1$, it will be more difficult to oxidize the polymer.

The second term in eqn (28) depends on the ratio of the partition functions of the segments $J = j_R/j_{Ox}$ and on $(1 - \theta_{N,Ox})/(1 - \theta_{N,R})$. For θ constant, $\delta/(1 - \delta)$ is also constant (eqn (6) and (8)). The ratio $J = j_R/j_{Ox}$ reflects the intrinsic relative mechanical stability of the segments in the absence of tension. $J > 1$ implies that the reduced segments are

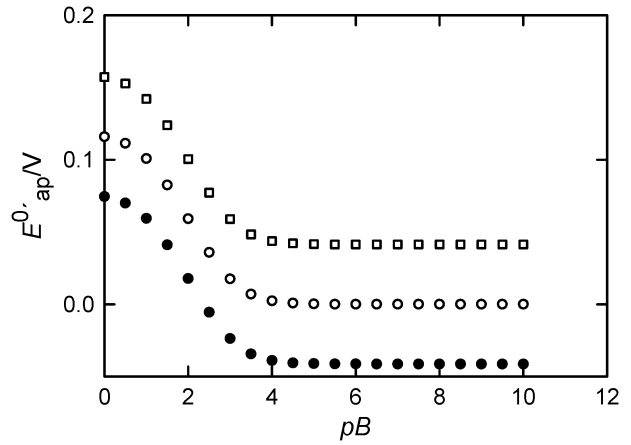


Fig. 1 Influence of the partition functions of the segments on the dependence of $E^{0\prime}{}_{ap}$ on the pB of the external solution for $\theta = 0.5$, $\gamma = 1$, $P = 1$, $K_R = 10^3$, $K_{Ox} = 10^1$: (●) $J = 0.2$, (○) $J = 1$, (□) $J = 5$.

mechanically more stable and consequently it is more difficult to oxidize the polymer. Thus, as J increases, $E^{0\prime}{}_{ap}$ becomes more positive (see Fig. 1)

The ratio $(1 - \theta_{N,Ox})/(1 - \theta_{N,R})$ is related to the relative stability of the bound species on the Ox and R segments. The bigger is $\theta_{N,Ox}$ the more negative $E^{0\prime}{}_{ap}$ and then it is easier to oxidize the polymer. This ratio depends, according to eqn (1) and (2), on the ratio of the binding constants K_{Ox} and K_R. Thus if $K_{Ox}/K_R > 1$, it would be easier to oxidize the macromolecule (Fig. 2).

It is also seen in Fig. 2 that, for $K_{Ox} > K_R$, $E^{0\prime}{}_{ap}$ increases as pB increases. The converse is true, for $K_{Ox} < K_R$, $E^{0\prime}{}_{ap}$ decreases as the pB increases. On the other hand, for the same K_{Ox} value, a decrease in K_R leads to an increase in the range of variation of $E^{0\prime}{}_{ap}$. When a_B is very small (high pB values), the effect of binding on $E^{0\prime}{}_{ap}$ disappears.

Comparison of Fig. 1 and 2 shows that changes in K_R and K_{Ox} do affect the pB value at which the inflection points appears. On the other hand, changes in j_R and j_{Ox} do not affect this pB value.

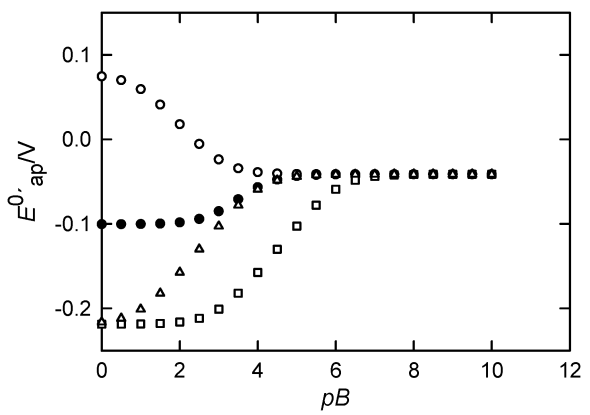


Fig. 2 Influence of the binding constants on the dependence of $E^{0\prime}{}_{ap}$ on the pB of the external solution for $\theta = 0.5$, $\gamma = 1$, $P = 1$, $J = 0.2$: (○) $K_R = 10^3$, $K_{Ox} = 10^1$; (●) $K_R = 10^3$, $K_{Ox} = 10^4$; (△) $K_R = 10^1$, $K_{Ox} = 10^4$; (□) $K_R = 10^3$, $K_{Ox} = 10^6$.

It is observed that the model predicts a symmetric sigmoid dependence of $E^{0\prime}{}_{ap}$ on pB (Fig. 1 and 2). The value of $E^{0\prime}{}_{ap}$ at the inflexion point being its most probable value (mpv). The mpv of $E^{0\prime}{}_{ap}$ in Fig. 1, decreases as J decreases.

The value of $E^{0\prime}{}_{ap}$ at high pB is the value in the absence of binding effects but in the presence of mechanical effects. If the value of the redox potential in the absence of mechanical effects were available (the redox center alone in solution), it would be possible to obtain the contribution of the mechanical effect from the shift of $E^{0\prime}{}_{ap}$ in going from the redox couple alone in solution to the redox couple in the macromolecule. Separation of the redox and mechanical effects for Pani type polymers has been done both, from the analysis of the voltammetric behaviour[25,27] and from the volume changes during the redox switching.[38]

Note, also in Fig. 2, that the change in $E^{0\prime}{}_{ap}$ in the whole pB range depends on the ratio K_R/K_{Ox}.

Change in N with θ

Eqn (1) and (2) imply that N_{Ox} and N_R change as the fraction of oxidized polymer changes. If $K_{Ox} > K_R$, there will be an increase in the amount of bound species as θ increases. Consequently there will be a decrease in the amount of binding species in the solution. Employing suitable values of the internal partition functions and M, it is possible to calculate the sum $N_{Ox} + N_R$, as a function of the degree of oxidation, θ. From the sum we calculate the change in the amount of total bound species as the change $\Delta N = N_\theta - N_{\theta=0}$. The model predicts a linear change of ΔN with θ. This is shown in Fig. 3 for $M = 10^5$, $P = 1$, $J_R = 0.2$ and a selected range of K_R and K_{Ox} values.

Change of length with potential

With the help of eqn (6) and (9) it is possible to calculate λ for different values of θ_n and a given set of the partition functions. With eqn (26) it is possible to calculate E for these values of θ and the same parameters. These calculated values are shown in Fig. 4, where the relative length increase, $(\lambda - \lambda_0)/\lambda_0$ is shown as a function of the potential.

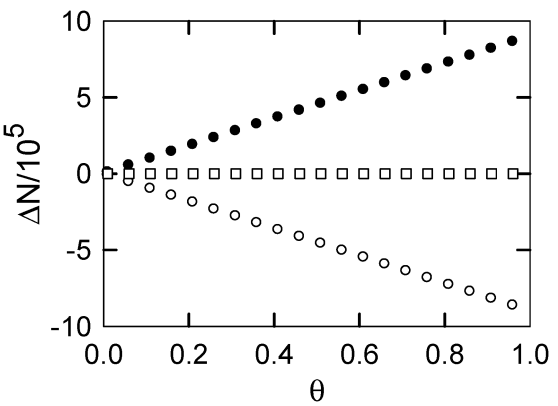


Fig. 3 ΔN as a function of the oxidation fraction, θ; $M = 10^5$, $P = 1$, $J = 0.2$, $\gamma = 1$, pB = 3: (●) $K_R = 10^1$, $K_{Ox} = 10^4$; (○) $K_R = 10^4$, $K_{Ox} = 10^1$; (□) $K_R = 10^1$, $K_{Ox} = 10^1$.

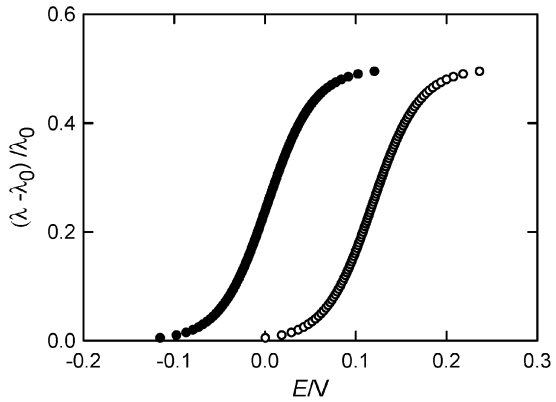


Fig. 4 Calculated relative increment of length, $(\lambda - \lambda_0)/\lambda_0$, as a function of the potential: $\gamma = 1$, $M = 10^5$, $P = 1$, $J = 0.2$, pB = 3, $\lambda_R = 1.0 \times 10^{-7}$, $\lambda_{Ox} = 1.5 \times 10^{-7}$: (●) $K_R = 10^3$, $K_{Ox} = 10^4$; (○) $K_R = 10^3$, $K_{Ox} = 10^6$.

Comparison with selected experimental results

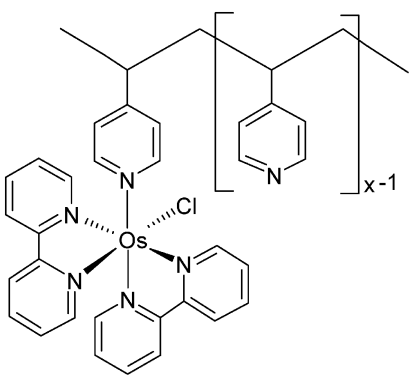
Dependence of the apparent formal potential with the binding species concentration. As a first example of the dependence of the potential on the concentration of the binding species in solution, we will consider the dependence of the redox potential of two synthetic redox polymers such as Os-PVP and Os-PVI.[39] In this case the binding species are the protons in the presence of 1 M KCl as supporting electrolyte. We will consider that this concentration is high enough to completely screen the interaction between the charged sites. The oxidation of this polymer involves the loss of one electron from the Os ion:

$$\text{Os(II) (env)} \rightarrow \text{Os(III) (env}') + \text{e} \qquad (29)$$

where (env) and (env′) refer to the environment around the Os(II) and Os(III) ions, respectively (see Scheme 3 for the environment of the Os(II) in the complex). Os-PVI is similar to Os-PVP but with imidazole groups instead of pyridyl in the polymer backbone and with the Os remaining coordinated to the two bipy.

This reaction involves no direct participation of protons in the electron transfer reaction; however, the formal potential depends on the pH as depicted in Fig. 5.

The results shown in Fig. 5a and b should be compared with those calculated in Fig. 2 for $K_{Ox} < K_R$ (Fig. 5a) and $K_{Ox} > K_R$ (Fig. 5b).



Scheme 3 Representation of the polymer Os-PVP.

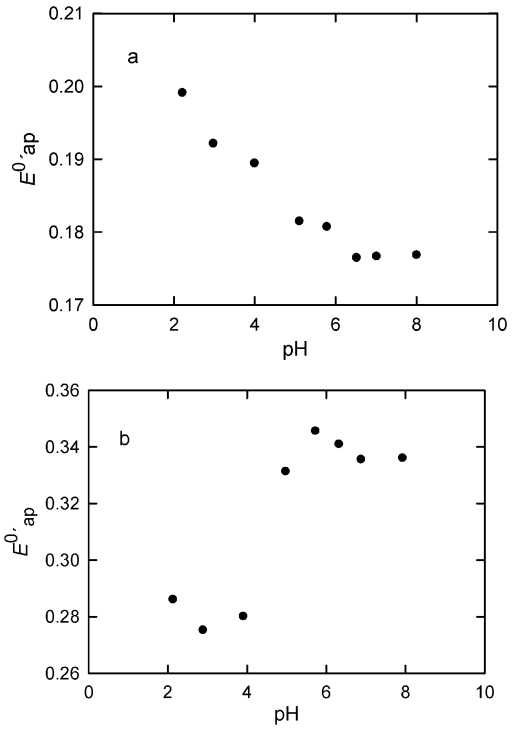


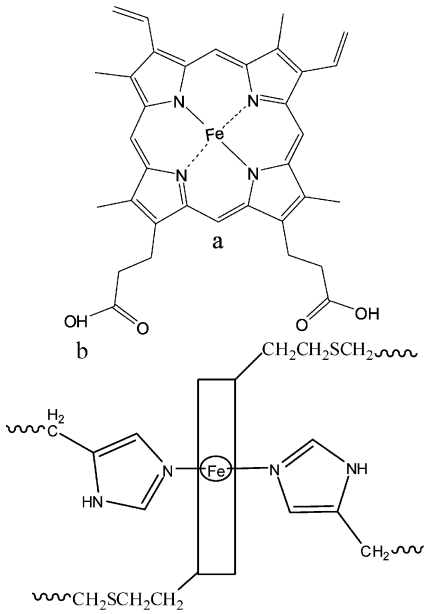
Fig. 5 pH dependence of the apparent formal potential, $E^{0\prime}_{ap}$ for (a) Os-PVI and (b) Os-PVP taken from ref. 39. Supporting electrolyte 1 M KCl. Potentials are referred to the saturated calomel electrode (SCE).

As a second example of this coupling we will analyze an electrochemically active biological macromolecule. We choose a metalloprotein in which the metal follows a simple electron transfer with no explicit participation of protons. Examples of these type of proteins are hemoglobin and the cytochromes. Cytochrome b_5 contains four heme groups in which four of the six coordinating positions are occupied by porphyrinic nitrogens (see Scheme 4a). The two other positions of the central iron are occupied by the nitrogen of the imidazole groups of histidine residues of the protein chains (see Scheme 4b). Although it is well known that the midpoint potential in the course of the redox titration of these macromolecules depends on the pH,[31,40–44] not many studies in a wide range of pH have been done. One of the more complete in this sense is that of Qian *et al.* on cytochrome b_5.[44] This is shown in Fig. 6 both for the wild type cytochrome and its mutants (see the reference for more details about the mutants).

The case of Fig. 6 can be compared with those of Fig. 2 for $K_{Ox} < K_R$.

Change of volume as a function of the potential

For this example we will consider two polymers, one a so-called conjugated polymer as poly(*o*-toluidine) (POT)[45] and the other a redox polymer such as Os-PVP.[46] In Fig. 7 the volume changes of these two polymers are shown as a function of the applied potential. It is seen in Fig. 7b that the change in volume has a characteristic symmetric sigmoid dependence on the potential as predicted by the model (Fig. 4). The results for POT, however, although they show a sigmoid curve, are not symmetrical. This fact must be related to the asymmetrical



Scheme 4 (a) Heme group and (b) diagram showing the coordination of the histidine residues to the heme group.

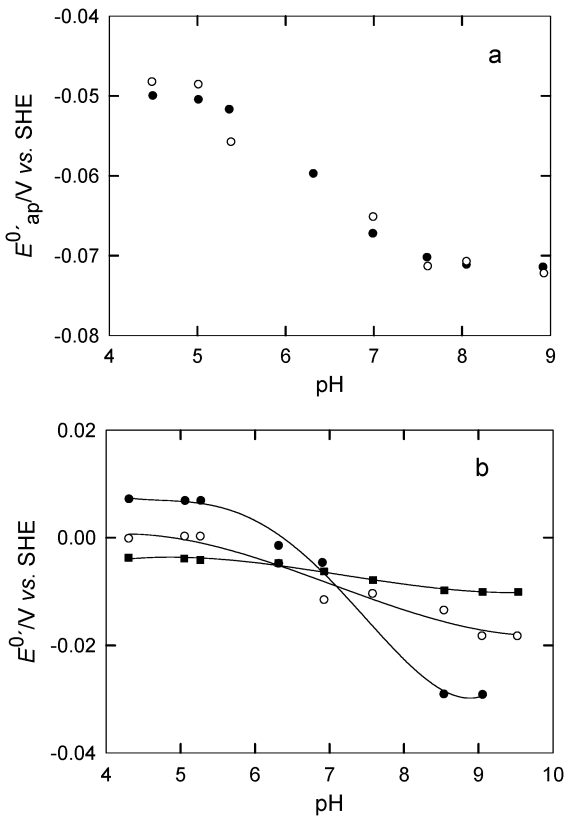


Fig. 6 pH dependence of the apparent formal potential of cytochrome b_5 and its mutants: (a) (●) wild type and (○) F35Y; (b) (●) E56A, (○) E44/56A, (■) E44A (see ref. 44 more details about the mutants).

shape of the current response for that system.[45] The reasons for the potential dependence of relative changes in volume for these two systems are discussed in ref. 45 and 46, respectively.

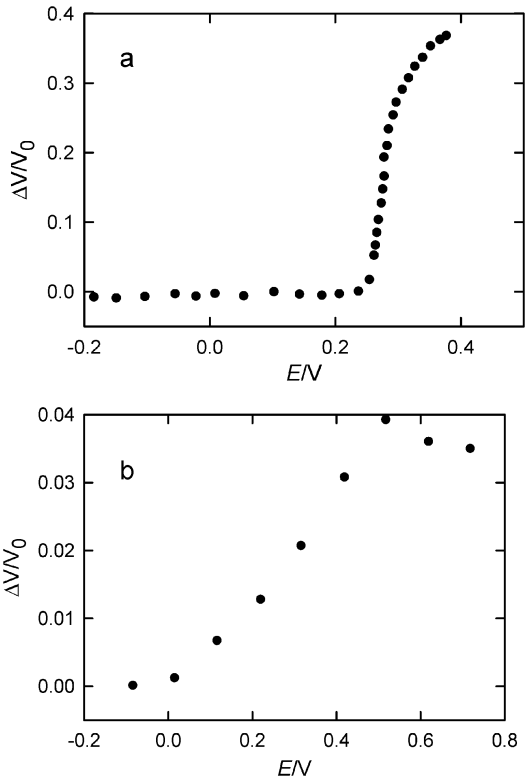


Fig. 7 Relative volume change with the potential during the oxidation of (a) POT in 1 M $HClO_4$ and (b) Os-PVP in 1 M KCl, pH = 7. Potentials are referred to the saturated calomel electrode (SCE).

Change of the binding species concentration in the external solution as a function of the oxidation fraction

As an example about the changes of the binding species during the oxidation of metalloproteins, we discuss the change of pH of the solution during the redox titration of hemoglobin measured by Wyman *et al.* (Fig. 8).[31] The pH change during the titration is defined as: $\Delta pH = pH_{Hb^+} - pH_{Hb}$ (Hb and Hb^+ being the reduced and oxidized form of hemoglobin). As shown in Fig. 8, ΔpH increases with the initial pH of the solution. Also, at pH values around 8.25, the ΔpH increases linearly with the titration fraction. At lower pH values, ΔpH first decreases and then increases as the titration fraction increases. This complex behavior must be expected because proteins are composed of different amino acids that have, in general, different proton binding sites with different affinities. In the present model (Fig. 3) we have assumed only one class of binding sites and, furthermore, we have also assumed no interactions (*i.e.* non-cooperativity) between different sites.

4. Conclusions

In this work we show that in electrochemically active macromolecules there is a coupling among the deformation, the state of binding, the state of screening and the redox potential. The coupling is so that the change in one of these conditions causes the others to change. We present a thermodynamic analysis in terms of the characteristic magnitudes of these systems. Then, we develop a simple statistical thermodynamic model and show the change of the magnitudes as predicted by the model.

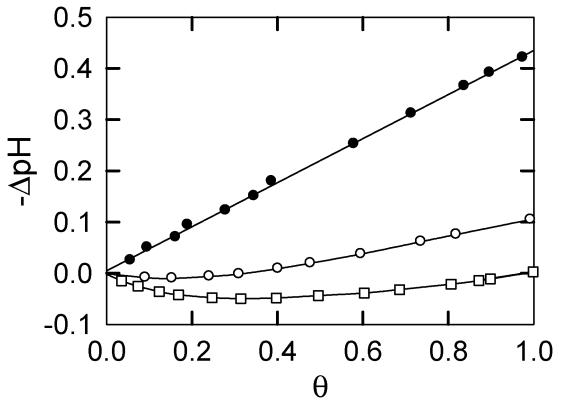


Fig. 8 Change of pH as a function of the titrated fraction for human hemoglobin with $K_3[Fe(CN)_6]$, at 20 °C: pH_{Hb} = (●) 8.25, (○) 6.75, (□) 6.33; $\Delta pH = pH_{Hb^+} - pH_{Hb}$, where pH_{Hb} and pH_{Hb^+} are the pHs corresponding to the reduced and oxidized forms, respectively. Data taken from ref. 31.

Finally, we discuss several examples of both synthetic and natural electrochemically active macromolecules showing this type of couplings. The comparison between the experimental results and the predictions of the model shows a qualitative agreement.

Acknowledgements

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