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Bioelectromagnetics: The State of the Science

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Abstract. *Electromagnetic exposure, from Extremely Low Frequency (ELF) to Radio Frequency (RF), at field intensity or Specific Absorption Rate (S.A.R.) values below the current safety standards, can affect biological processes. In this paper, we discuss a biophysical basis for assessing the effects of low-intensity ELF-RF fields, with specific emphasis on ion binding as a first interaction step. The actual endogenous potential energy (U_{end}) of the ion in the binding crevice must be consistent with the data provided by the protein data bank. We developed a method for evaluating U_{end} from the protein data, which has been incorporated in the Langevin-Lorentz (L-L) classic or in the Zeeman-Stark (Z-S) quantum modelling of a ligand ion-receptor protein system. The models include a metabolic force which emulates the basal state of any living cell.*

1. INTRODUCTION

The published experimental results suggest that electromagnetic exposure, from sub-Extremely Low Frequency (ELF) to Radio Fre-

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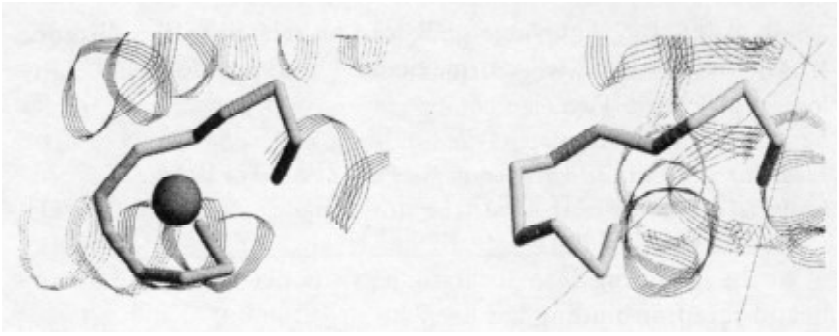
quency (RF), at field intensity or Specific Absorption Rate (S.A.R.) values below the current safety standards, can affect biological processes. Two elementary processes are good candidates to be the first interaction step: the binding of messenger ions to their receptor proteins and the transport of messenger ions along protein channels. In both cases the ion dynamics can be often described in terms of the classical Langevin-Lorentz (L-L) model [1-4] or the quantum Zeeman-Stark (Z-S) model [5-6]. The case of ligand-receptor binding has been studied in more detail. The actual endogenous potential energy of the ion in the binding crevice must be consistent with the data provided by the protein data bank. The model must incorporate a metabolic force which emulates the effect of the basal state of any living cell. When all the above features are included in the models, the observed biological effects of low-intensity e.m. exposure become biophysically plausible.

2. THE RECEPTOR ENDOGENOUS FIELD

The binding site of several metalloproteins is hydrophobic (it repels the solvent water molecules and strips away the ion hydration shell) so that the ligand metal ion (whose mass is M and charge is Q) is attracted by the receptor protein in an almost dehydrated environment [7-10]. Furthermore, the protein atoms are slightly displaced by the docking / sailing ion, with respect to their equilibrium positions obtained from the protein data bank. Therefore the actual net attracting force is lower than the value calculated on the basis of these fixed atomic positions because the actual protein conformation depends on the ion location (fig. 1).

Both features are necessary conditions for a ligand-protein system to be susceptible to low-intensity e.m. exposure. Once the resulting ion potential energy $U_{end}(\vec{r})$ is available versus the ion distance $\vec{r}$ from the centre of the binding site, a model (classical or quantum) of the binding process allows the prediction of its susceptibility to the e.m. exposure. A simple and isotropic relationship $U_{end}(r)$ has been developed which can be fitted to the characteristic of the ion-protein system obtained from the protein data



1.a 1.b

Figure 1. Backbone of one binding site of calmodulin, with (figure 1.a) and without (figure 1.b) bound ligand (Ca^{++}) (Brookhaven Protein Data Bank).

bank (fig. 2). The binding potential energy depends on four parameters only, i.e. U_0, ω_{end}, ξ and R_0. The minimum value of the potential energy at r = 0 is $U_{end}(0) = -U_0$: for small values of r ($r << R_0$) it is $U_{end} \cong -U_0 + M\omega^2_{end}r^2 / 2$ (harmonic oscillator), so that U_0 and ω_{end} can be evaluated by the protein data like those of fig. 1.a ; for large values of r ($r >> R_0$) it is $U_{end} \cong -\xi / r$ (Coulomb approximation), so that ξ can be evaluated by fitting data as

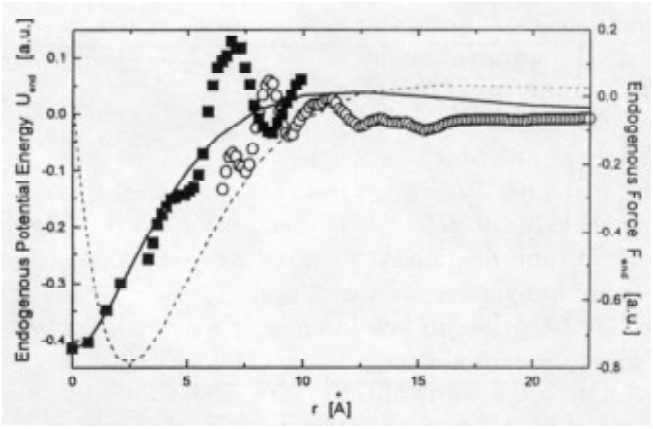


Figure 2. Example of the endogenous potential energy for Ca^{++} in one of the four binding sites of calmodulin, as obtained from the Brookhaven protein data bank. The squares refer to fig 1.a and the circles to figure 1.b. The dashed curve is the attractive endogenous force $-dU_{end}/dr$.

those of fig. 1.b. The value of R_0 can be related to the distance where the attracting force is maximal. The expression for $U_{end}(r)$ is:

$$U_{end}(r) = -\xi/r + \{\xi/r - U_0 + \xi/R_0 + (\xi/2R_0^2 - U_0/R_0)\, r + (M\omega_{end}^2/2 + \xi/6R_0^3 - U_0/2R_0^2)r^2\} \exp(-r/R_0) \quad (1)$$

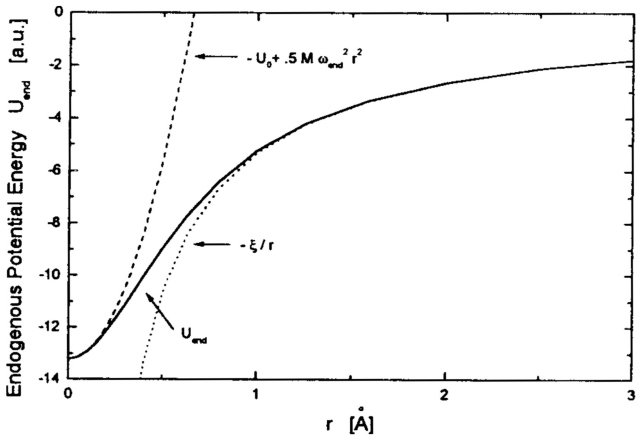


Figure 3. Example of the ligand endogenous potential energy versus r. The dashed line corresponds to an elastic attracting energy, while the dotted line corresponds to a Coulombic one.

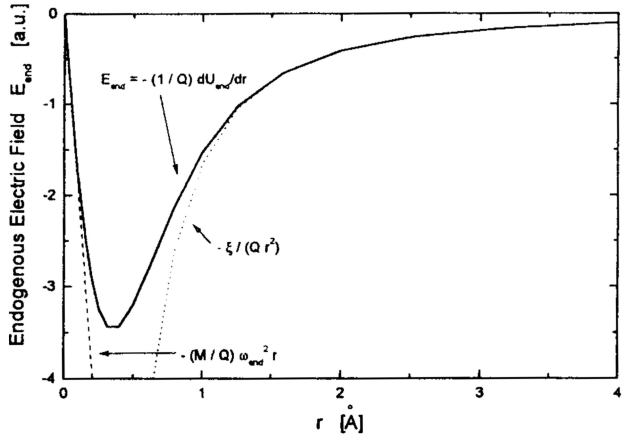


Figure 4. Example of the ligand endogenous attracting field versus r. The dashed line corresponds to an elastic field, while the dotted line corresponds to a Coulombic one.

Its plot versus r is shown in fig. 3. The endogenous force, $\vec{F}_{end} = -\pi U_{end}(r) = Q\vec{E}_{end}$, acting on the ligand is:

$$\vec{F}_{end} = -\{\xi/r^2 + [\xi/r^2 - \xi/rR_0 - \xi/2R_0^2 + (M\omega_{end}^2 - \xi/6R_0^3) r - (M\omega_{end}^2/2 + \xi/6R_0^3 - U_0/2R_0^2)\, r^2/R_0] \exp(-r/R_0)\}\vec{i}_r \qquad (2)$$

The plot of the equivalent electric field $\vec{E}_{end} = \vec{F}_{end}/Q$ versus r is shown in fig. 4. In conclusion, a simple and effective procedure for fitting the protein characteristics has been developed and therefore for predicting the frequency ranges in which the ligand binding to a receptor protein can be affected by exogenous e.m. exposure. The endogenous non linear force is the basic mechanism which transduces the low intensity e.m. input signal into a large effect on the ligand displacement, e.g. the output variable, well above thermal noise. The necessary power is mainly provided by the constant metabolic force discussed in the next paragraph.

3. THE METABOLIC FORCE

To describe completely the site environment we have to take into account even the existence of a basal force $\vec{F}_{bm}$, which mimics the living state of the cell. The equivalent electric field, $\vec{E}_{bm} = \vec{F}_{bm}/Q$, plays the role of an effective basal electric field, assumed spatially uniform for sake of simplicity. In the absence of any exogenous exposure, the basal metabolism *per se* causes a macroscopic average steady ion velocity $\vec{v}_{bm}$. Classically speaking, $\vec{v}_{bm}$ can be related to the basal force by the relationship $\vec{v}_{bm} \cong (Q/M)\vec{E}_{bm}/ß$, where the coefficient ß is the Langevin collision frequency of the ion in the binding cleft [5-15]. This relationship holds almost true in r = 0, i.e., at the bottom of the potential energy well, and for large values of r.

The basal force $\vec{F}_{bm}$ is of paramount importance in explaining how low intensity exposure can affect biological processes. In fact, the power necessary to sustain the changes of ion position, induced by the exogenous e.m. field, can be provided by the cell basal metabolism, i.e., by $\vec{F}_{bm}$ via $\vec{v}_{bm}$. The e.m. average power transferred by the exogenous e.m. field to the ion is not directly

related to the observed bioeffects, as it is in the case of the thermal effects, i.e. of the induced tissue heating considered by the current safety standards. What mainly matters is the waveform of the e.m. field, which affect the ligand dynamics. The role of the exogenous e.m. power is limited to the detectability of the low-intensity signal at the first interaction step, above the thermal noise.

4. THE CLASSICAL MODEL OF INTERACTION: THE LANGEVIN-LORENTZ MODEL

We review the classical Langevin-Lorentz model, which mimics the interaction mechanism of low-intensity e.m. exposure with a ligand-receptor system [3-4]. The ligand dynamics inside the binding cleft of the receptor protein strongly depends on the endogenous force due to the protein atoms, which, as we have previously observed, is a non linear function of the ligand displacement.

The closed form integration of the L-L equations, under *ad hoc* simplifying conditions was performed in [1-3], for the first time. This rather comprehensive approach includes, as particular cases, all the classical models so far published. In order to analyse or predict the experimental results, the values of the various parameters which enter the L-L equation must be physically plausible, keeping the number of fitting parameters as low as possible. Moreover, their value must be consistent with the effectiveness of the low value of the exposure intensity. Toward such goals, the following features are included. The binding or channel crevice is hydrophobic. If the gradient of the ion endogenous potential energy U_{end} inside the crevice is a highly non linear function of the spatial coordinates, the water dipoles can be drifted away from the protein crevice by the resulting endogenous dielectrophoretic force (negative dielectrophoresis). Therefore, the ion moves in the ballistic Knudsen regime and its Langevin collision frequency can be several order of magnitude lower than in bulk water [5]. The resulting energy losses are small, so that the ion dynamics become more sensitive to low-intensity e.m. exposure. However, the exogenous signal is typically unable to overcome the Langevin random force (thermal noise) if the ion-protein system, in the absence of the

e.m. exposure, is at thermal equilibrium. On the other hand, the basal state of any ion-protein systems in a living cell is maintained out of thermal equilibrium by the cell metabolism, as already pointed out.

The purpose of this section is to review the complete L-L model, thus offering a comprehensive framework for the classical analysis of the ion dynamics under e.m. exposure:

$$d\vec{r}/dt = \vec{v}\ ;\ \ d\vec{v}/dt = \gamma\ (\vec{E}_{end} + \vec{E} + \vec{E}_{bm} + \vec{v} \times \vec{B}) - ß\vec{v} + \vec{n} \qquad (3)$$

where $\vec{v}$ is the ion velocity, $\vec{r}$ its displacement, $\gamma = Q/M$ and ß is the Langevin collision frequency [5]. The metabolic force which mimics [5] the cell living state is $Q\vec{E}_{bm}$. The initial conditions for the differential equations are $\vec{r}(0) = 0$ e $\vec{v}(0) \approx \gamma\vec{E}_{bm}/ß$. The exogenous magnetic field $\vec{B}$ includes also the effects of the earth static magnetic field. It is obtained by summing two terms. The first is constant and the second is slowly time-varying:

$$\vec{B} = [B_{0,x} + B_{1,x}f(t)\ \vec{i}_x + [B_{0,y} + B_{1,y}\ f(t)]\ \vec{i}_y + [B_{0,z} + B_{1,z}f(t)]\ \vec{i}_z \qquad (4)$$

If one considers the magnetic field produced by Helmholtz coils, often used in laboratory experiments at ELF, the electric field induced by the time-varying $\vec{B}$, in a Petri dish, has the following expression:

$$\vec{E} = [(\vec{r} + \vec{d}) \times (d\vec{B}/dt)]/2 = [(\vec{r} + \vec{d}) \times \vec{B}_1\ (df/dt)]/2 \qquad (5)$$

where $\vec{d}$ is the distance vector of the binding site from the dish axis, parallel to the z axis.

The up-to-dated discussion of the Langevin white noise term $\vec{n}$, whose expectation value is $\varepsilon\{n_i(t)\} = 0$, can be found in [4] for the harmonic oscillator approximation. The noise autocorrelation expected value is

$$\varepsilon\{n_i(t_1)\ n_j(t_2)\} = 2\ \frac{K_B T}{M}\ ß\delta\ (t_1 - t_{2)},\ (i = x, y, z),$$

where K_B is the Boltzmann constant, T is the absolute temperature and δ is the Dirac delta function.

The integration of eq. (3) gives the expectation value $\varepsilon\{\vec{r}(t) \cdot \vec{r}(t)\}$ as function of the e.m. exposure. Therefore it is possible to evaluate the desorption time of an adsorbed ion or its transit time along a membrane channel.

5. THE QUANTUM MODEL OF INTERACTION: THE ZEEMAN-STARK MODEL

The Zeeman-Stark model mimics the interaction mechanism of low-intensity e.m. exposure with a ligand receptor system [6-13] adopting the quantum approach. The e.m. exposure is described by means of the magnetic vector potential $\overline{A}$ and the electric scalar potential ϕ, under the Coulomb gauge $\pi \cdot \overline{A} = 0$. The related binding probability of the ligand is computed via its density matrix. The time evolution of the density matrix depends on the characteristic lifetimes, which model the ligand-receptor interaction with the thermal bath. These lifetimes are related to the classical Langevin-Lorentz collision frequency of the ligand, but their values have been so far arbitrarily chosen [6-13-14]. Starting from the first principles, a closed form relationship, which allows to compute the quantum lifetimes in terms of K_BT of the aforesaid collision frequency, has been developed [15]. So doing, a useful link is established among a classical parameter, e.g. the Langevin collision frequency which enters measurable quantities like the ligand adsorption and desorption constant, and the quantum lifetimes of the Zeeman-Stark model.

The first goal is to find the expected value of the reduced density operator ρ [6-11-12-14] which describes the ion motion in the attracting potential energy well $U_{end}(r)$ in the presence of exogenous e.m. potentials, i.e. the scalar potential ϕ and the vector potential $\vec{A}$ and of thermal noise. The time evolution of ρ must obey the following relationship [6-11]:

$$\partial\rho/\partial t \cong (-j/\hbar)\,[H_{end} + H_{bm}, \rho - \rho_0] - \Sigma_S[T_S,[T_S, \rho - \rho_0]] - (j/\hbar)\,[H_1, \rho] \qquad (6)$$

where t is the time variable and $\hbar$ is the Plank constant divided by

2π.

The Hamiltonian $H_{end} = -[\hbar^2/(2M)]\pi^2 + U_{end}$ refers to the ion motion in the potential energy U_{end}. The Hamiltonian H_{bm} takes into account the living state of the cell [6], via the contribution of the basal force $-\pi H_{bm} \cong \vec{F}_{bm}$. We observe that, at thermal equilibrium, it is $\vec{v}_{bm} = \vec{F}_{bm} = 0$ and ρ_0 reduces to the Boltzmann relationship ρ_{th} [6-11-12]. We point out that H_{bm} contributes to the Stark effect in the equation governing the density time evolution (6).

The Hamiltonian H_1 takes into account the contributions of ϕ and $\vec{A}$: $H_1 \cong j\hbar(Q/M)\vec{A} \cdot \pi + Q\phi$. It provides the Zeeman and Stark effects in the equation. A typical assumption is that $\vec{A}$ is small enough so that the term proportional to $\vec{A} \cdot \vec{A}$ in H_1 can be neglected. The commutator [P,R] in equation (6) means, by definition, PR-RP. The sum of double commutators of the lifetimes operators T_S in the same equation takes into account the interaction of the ion-protein system with the thermal bath [12-14]. The density operator ρ_0 is the value of ρ in the basal steady state which occurs when $H_1 = 0$, so that it provides also the boundary condition $\rho(0) = \rho_0$ at the onset ($t = 0$) of the exogenous e.m. field. The solution of the equation (6) can be obtained choosing a complete base of orthonormal functions $\Psi_n(x, y, z)$. We use the eigenfunctions of the hydrogenoid Hamiltonian $(H_{end} - U_{end} - \xi/r)$, which formally refers to the idealized ion motion in the coulombic potential energy $(-\xi/r)$. The corresponding eigenvalues are ε_n. Therefore ρ and, in general, any linear operator R can be represented, in the aforesaid base, by a matrix whose elements are $R_{mn} = \int\Psi^*_m R\Psi_n dxdydz$, where * means complex conjugate and the integration is performed over the whole space. Hence, the equation can be interpreted in terms of the corresponding matrices. The five eigenfunctions corresponding to the smallest eigenvalues ε_n of the hydrogenoid Hamiltonian are the most important ones because they are adequate for evaluating $\rho_{mn}(t)$, whereas the contribution of the others can be neglected. So doing, we adopt the so called n-state formalism of quantum mechanics [6-12], letting $n = 5$. The ground eigenvalue is $\varepsilon_1 = -(M\xi^2)/(2\hbar^2) = -4\hbar\omega_0/3$. The radian frequency ω_0 is a characteristic parameter of the binding site, because $(\hbar\omega_0)$ is the energy to be supplied in order to induce the ion transition from the ground energy level ε_1 to the first ex-

cited energy level $\varepsilon_2 = \varepsilon_3 = \varepsilon_4 = \varepsilon_5 = -\hbar\omega_0/3$. In quasi-classical terms, $\hbar\omega_0$ is a quantum measure of the depth of the binding potential energy well, as opposed to the classical depth U_0. The value of ω_0 is obtained from ξ, which is evaluated by fitting U_{end} to the protein data (see fig. 2). In the case of a potential energy well whose characteristic value of ω_0 falls in the RF range, we predict that the receptor may be susceptible to RF e.m. exposure.

The matrix elements of the summation of the double commutators on the right hand side of the density equation contain suitable lifetimes θ_{mn}, τ_{mn}, which are the quantum-mechanical counterparts of the aforesaid classical Langevin collision frequency ß of the ion. The lifetimes allow the system to relax to steady state when the exogenous field is reduced to zero. The integration of the density equation (6) leads to the evaluation of the matrix components $\rho_{mn}(t)$ so that the observed value R of any linear operator R corresponding to a dynamical variable can be computed from the trace expression $R = \mathrm{Tr}(R\rho)$. In particular, the binding probability of a ligand ion to its receptor can be evaluated under e.m. exposure.

6. CONCLUSION

We have discussed a biophysical basis for assessing the effects of low-intensity ELF-RF fields, with specific emphasis on ion binding as a first interaction step. The ion binding to a receptor hydrophobic site is the process most widely studied, but a similar approach can be applied to ion transport through a protein channel by choosing a suitable dependence of the endogenous potential energy U_{end} on $\vec{r}$. We developed a method for evaluating U_{end} from the protein data, which has been incorporated in the L-L classic or in the Z-S quantum modelling of a ligand ion-receptor protein system. The mechanistic insight provided by the analysis outlined in this paper offers a guideline for linking the e.m. susceptibility of the binding process to the information provided by the protein data bank, and suggests novel possibilities for modulating the physiological function of receptor proteins by means of e.m. fields.

In conclusion, we have offered a plausible biophysical basis for potential biological effects of low-intensity e.m. exposure at ELF or at RF, which could lead to novel clinical applications and should also be considered, in the future, by the safety standards regulators.

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BIOELETTROMAGNETISMO: LO STATO DELLA SCIENZA

Riassunto

Campi elettromagnetici di bassa intensità (al di sotto degli standards di sicurezza) e nelle gamme dalle bassissime frequenze (Extremely Low Frequency - ELF) alle frequenze radio (Radio Frequency - RF) possono interferire con i processi biologici. Tale interferenza può esercitarsi innanzitutto nel processo di legame di ioni messaggeri ai rispettivi recettori proteici, o nel trasporto degli ioni messaggeri attraverso proteine canale. In entrambi i casi la dinamica degli ioni viene spesso descritta utilizzando il modello classico di Langevin-Lorentz o il modello quantistico di Zeeman-Stark. In questo lavoro viene discusso in particolare il caso del legame ione-recettore. Lo ione durante il processo di adsorbimento-desorbimento sposta leggermente gli atomi della proteina recettrice dalle loro posizioni di equilibrio indicate nella banca dati delle proteine. Dal momento che la reale conformazione della proteina dipende dalla localizzazione dello ione, la reale forza di attrazione è minore del valore calcolato sulla base delle posizioni atomiche ricavate dalla banca dati. Ciò rende il sistema più suscettibile alla influenza di campi magnetici di bassa intensità. Una volta nota l'energia potenziale dello ione in funzione della distanza dello ione stesso dal centro del sito di legame, è possibile predire, con il modello classico o quantistico, la suscettibilità ai campi

elettromagnetici. I modelli includono una forza metabolica che emula lo stato basale di ogni cellula vitale. Gli autori hanno sviluppato un metodo per valutare tale energia potenziale a partire dalle informazioni disponibili nella banca dati delle proteine. Ciò può risultare utile sia per le applicazioni in ambito clinico sia nella determinazione degli standards di sicurezza.