A Zeeman-Stark/Markov Model Approach to Study the EM-RF Exposure of a Potassium Channel

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Abstract — **The extraordinary increase in the use of electromagnetic (EM) radiofrequency (RF) radiation has stimulated new researches concentrated on the study of the early steps of the EM interaction mechanisms. As most of the effects due to the exogenous exposure of biosystem has been associated with the cell membrane, these researches are mainly oriented toward the study of the molecular aspects of the interaction. In this paper we introduce an integrated methodology of analysis, which involves cascading steps such as quantum modelling of the system constituted by a ligand ion (Ca^{2+}) and a protein receptor (Calmodulin) of the cell membrane and analysis of the protein channel activity by means of a stochastic model of the channel.**

I. Introduction

The exposure of living systems to radiofrequency electromagnetic fields (emf) may produce bioeffects which lead to formulation of safety standards and to various applications, such as therapy, diagnosis, biotechnology [1, 2]. Basing on experimental results, it is possible to consider membrane protein channels as the principal sites of interaction between EM fields and biosystem [2]

In this paper we propose an integrated methodology of analysis. The methodology involves consequential and cascading steps: evaluation of emf distribution on a cell model; quantum modelling of the system constituted by a ligand ion, calcium ion Ca^{2+}, and a cell membrane protein receptor, calmodulin, under exogenous EM exposure; analysis of the protein channel activity by means of a stochastic model of the channel.

II. Ion Binding Model

The first step consists in resolving a dosimetric problem that is the evaluation of EM field at cellular level. Recently some authors have faced this problem [3 and reference herein]. In this work we don't develop a new microdosimetric procedure but refer to the model presented in [3]. We consider the EM-RF field at the level of the involved biological structure as an input of the following model. Anyway, the evaluation of the EM field in the binding sites is an essential prerequisite to perform the numerical analysis based on the developed models.

One elementary process is a good candidate to be the first interaction step: the binding of messenger ions to their receptor proteins in a cell membrane. In Fig.1 we sketched a portion of a cell membrane evidencing a particular channel protein. The sphere represents the site where ions can be adsorbed. The two chains around the center of the site represent the different folding conformation of the protein.

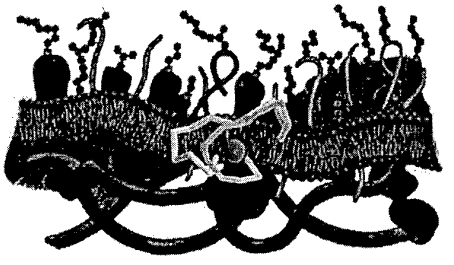

Fig. 1. Protein binding sites, with the ligand ion, in the cell membrane.

The ions dynamics can be often described in terms of classical Langevin Lorentz model or the quantum Zeeman Stark model [4]. The goal of these approaches is to analyse the variations in the motion of the ions with or without the applied field.

It is well known that many bioelectrochemical processes in the cells are controlled by some metallic ions the so called messenger ions; typically, these ions do not

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belong to the metabolic process of the whole cell but they control it by means of their chemical activity. These ions may be adsorbed or not to particular binding sites of proteins. From the microphysics point of view it is plausible that the exogenous EM field applied to the ion might change the dynamical state and, consequently, the whole system behaviour; a good approach to the study of ligand binding to a receptor protein, under EM-RF exposure, is based on quantum modelling of the process: the so called Zeeman-Stark model.

The problem is to find the reduced density operator ρ [4], [5] which describes the ion motion in the potential energy well U_{end} , which mimics the effects of the site charges on the messenger ion, in presence of exogenous potentials induced by EM fields.

A typical first order approximation for U_{end}(with spherical symmetry) can be obtained by fitting *ad hoc* parameters of relationship for this potential energy to the available data of the protein of interest, as obtained from a Protein Data Bank. A valid expression for this potential energy is:

$$U_{end}(r) = -\xi/r + \{\ \xi/r - U_0 + \xi/R_0 + \left(\xi/2R_0^2 - U_0/R_0\right)r +$$

$$+\left(M\omega_{end}^2/2 + \xi/6R_0^3 - U_0/2R_0^2\right)r^2\ \}\exp(-r/R_0) \qquad (1)$$

where the controlling parameters are U_0, ω_{end}, ξ and R_0. The time evolution of ρ must obey the following relationship:

$$\partial\rho/\partial t = \left(-j/\hbar\right)\left[H_{end} + H_1, \rho\right] +$$

$$-\left(j\beta/2\hbar\right)\sum_{i=1}^{3}\left[r_i, \Theta_{bm,i}\rho + \rho\Theta_{bm,i}\right] + \qquad (2)$$

$$-\left(\beta K_B TM/\hbar^2\right)\sum_{i=1}^{3}\left[r_i, \left[r_i, \rho\right]\right]$$

where r_1=x, r_2=y, r_3=z. Symbol [. , .] stands for the commutator, i.e. [S,R] = SR-RS for any couple of operators S,R.

Now we can describe each single term of this equation [4]. The Hamiltonian H_{end} refers to the ion motion in the endogenous potential energy. The Hamiltonian H_1 takes into account the contribution of the exogenous EM-RF potentials. The parameter β is the viscous friction coefficient. The operators $\Theta_{bm,i}$, i=x,y,z, play the role of appropriate quantum analogues of the classical friction terms in the Fokker-Plank equation, which gives the time evolution of the classical probability density of the ligand. They take into account the viscous friction effect and the contributions of the endogenous basal force, $\vec{F}_{bm}$ [5], which emulates the effects of the basal metabolism of the living cell on the ion receptor system. The need of such a force is consistent with the macroscopic evidence that across the membrane of any living cell it exists an excess voltage drop sustained by the biochemically driven ion pumps.

The main property of this approach is that equation (2) takes into account all the various aspects of the interaction of the quantum system with the thermal bath, as a function of temperature T and that is based on one fitting parameter only, i.e. β, which has a classical physical meaning.

Once a complete set of suitable orthonormal basis has been chosen, any quantum operator may be represented by means of a infinite matrix; the solution of equation (2) leads to the integration of a first order linear system of differential equations with periodically time-varying coefficients. The average value of any observable is computed by the evaluation of the trace expression

$$< R > = \mathrm{tr}\ (R\rho) \qquad (3)$$

where ρ is the reduce density matrix and R is the matrix associated to that observable. The main output of the Zeeman-Stark model is the relative change of the binding probability. Solving equation (2) we can compute $<P_{as}>$ that is the mean value of the asymptotic binding probability when the RF exposure is applied to the biological medium. The quantity P(0) is the binding probability without exposure. Fig. 2 represents some results of simulations which describe the variations in the relative change with an applied field of 900 MHz .

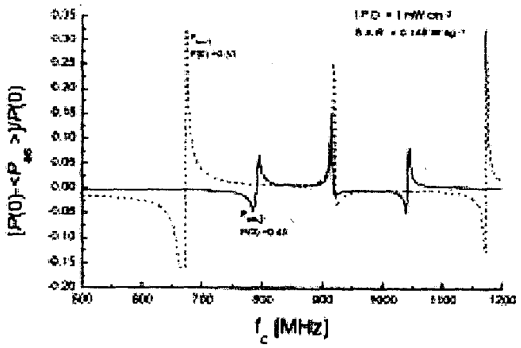

Fig. 2. Relative change of the binding probability vs. the carrier frequency of 900 MHz at the same exposure intensity, for two different values of the metabolic force (with identical cartesian components) equal to $5x10^{-17}$ N (continuous line) and 10^{-16} N (dotted line).

A link to a simpler binding model can be obtained by the following assumptions. A receptor site can be occupied by one ligand only, or it is empty. Letting P_B be the probability for a receptor to be occupied and L the concentration of the ligands near the cell surface, the simplest first order mass-action law gives the time course of P_B:

$$dP_B/dt \approx K^+ L(1-P_B) - K^- P_B \qquad (4)$$

where K^- and K^+ are, respectively, the so called dissociation and association rate "constants" in SI units.

In general, K^- and K^+ depend not only on the endogenous attractive force exerted by the binding site on the ligand, but they may depend also on the exogenous EM exposure. The complete Zeeman Stark model allows us to find out these parameters under such condition [4].

III. The Biological Bases Of The Integration Procedure

In the study of the interaction between EM fields and biosystems at cellular level, a fundamental role is played by Calcium: intracellular Ca^{2+} is an important messenger in most cell types. One pathway for Ca^{2+} to control cellular functions is by regulating the potassium, K^+, efflux through the cell membrane. In particular, we focus our attention to the small conductance potassium channels (SK channels) activated by micromolar concentrations of intracellular Calcium [6].

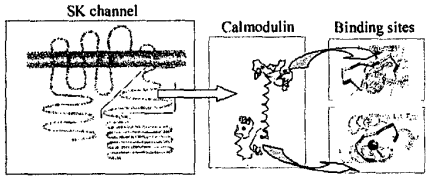

Fig.3. Basic idea of integration between ion binding and protein channel model.

The functional SK channels are complexed with Calmodulin (CaM), which is constitutively associated with the alpha-subunits in a calcium-independent way [6], [7]. Ca^{2+}-activated CaM contains four Ca^{2+}-binding sites Two globular domain are evidenced in the terminals of the protein chain: two sites are in correspondence to the C-terminal of the protein chain, the other two are close to the N-terminal (see Fig. 3, central picture). SK channels are coassembled complexes of pore-forming alpha-subunits and four chains of CaM. Experimental data support a model for channel activation in which Ca^{2+} binding to CaM induces conformational rearrangements in CaM and the alpha-subunits that result in channel gating [7]. Experimental results reported in [6] revealed a modular strategy in which one of the two globular domain of CaM is responsible for Ca^{2+}-induced conformational changes in the channel, whereas the other one mediates constitutive interactions [7]. SK channel seems elective in order to implement the integration for the two modelling levels, due to the role played by CaM and the well established knowledge about its gating modalities.

IV. Channel State Machine Model

In the past authors have solved the item of modelling ion channels by means of stochastic automata, simulating activity under physiological conditions as well as under exogenous EM fields exposure. Particularly they focussed their attention on finite state zero order ergodic Markov models [3]. Particularly microscopic models have been set-up for most common proteinic channels: Sodium, Potassium and Calcium (voltage dependent) and for acetylcoline (ligand dependent) [3].

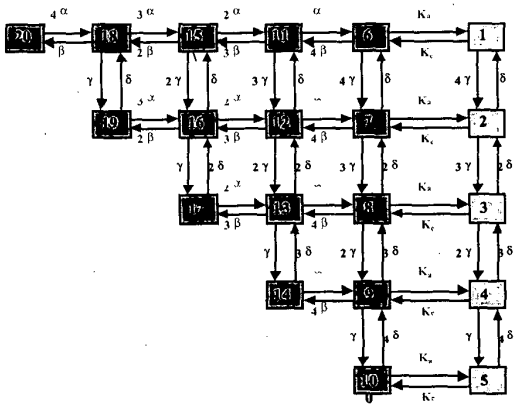

Fig. 4. State machine representation for SK channel

Here is considered the calcium-controlled small conductance potassium channel (SK). The chosen modelling lattice has been a twenty state model (Fig.4), obtained in [7] from experimental data of patch-clamp.

It has been possible to assign an association level for the Ca^{2+} ion in each of the twenty states of the model. Remembering the channel is controlled by four CaMs' and basing on what stated in the beginning about this protein, it is possible to refer to a kinetic diagram for a single CaM (Fig. 5) to understand the whole channel [9], where the kinetic coefficients α e β are the same of the SK channel model.

Basing on knowledge about CaM-Ca^{2+} sequential association mechanism, the C-terminal binding sites are supposed already bound, from this scheme it is possible to obtain information only about association of N-terminal

ones. Passing from State 1 to State 2 has been identified as the binding of the first Ca^{2+} ion, and kinetic coefficients α and β are referable to the association and dissociation coefficients K^+ and K^- introduced in the Z-S model.

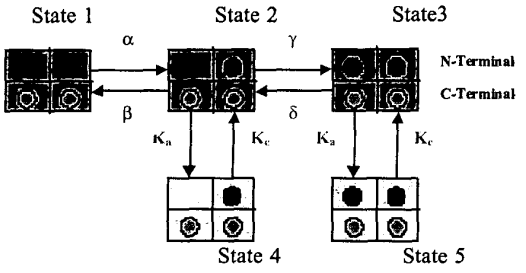

Fig. 5. Kinetic scheme for a single CaM in the SK channel.

The twenty states model for the SK channel has been numerically analysed performing a sort of sensitivity study: Fig. 6 represents the opening probability of the channel versus the main parameters of the system.

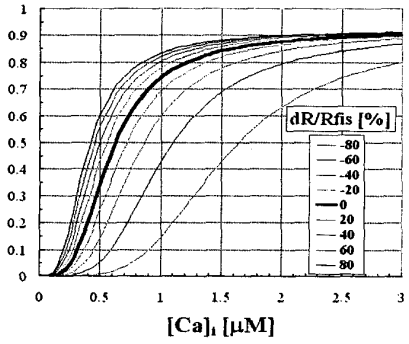

Fig. 6. Opening probability of the SK channel versus the Calcium concentration and the ratio $R=\alpha/\beta$ of the two characteristic parameter of the state model.

V. Conclusions

In this paper it has been discussed a biophysical basis for describing the effects of low-intensity radio frequency fields on ion binding and channel behaviour.

We have considered a quantum Zeeman-Stark model to describe the ion binding to a receptor protein, and then a zero-order Markov chain to describe the channel behaviour.

Authors perform an integration between the two models: considering exogenous EM field, temperature, and metabolic force as inputs for the quantum model it is possible to calculate the absorbing probability for the ion and kinetic parameters describing the ion state. The association and dissociation parameters K^+ and K^- become inputs of the channel model because they are related to the kinetic coefficients α and β. Finally we have obtained the dependance of the opening probability of a SK channel on the EM field applied to the biological structure.

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

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