Triplet-Selective Chemistry : a Possible Cause of Biological Microwave Sensitivity

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A hypothesis is proposed to explain *resonant* biological effects of microwaves. The interaction is thought to occur with low-frequency electronic transitions of transient triplet (or quartet etc.) molecules. As the transient molecules' substate populations can be assumed to be far from thermal equilibrium the effect of resonant microwaves would be to equalize the population of two substates. Since the overall chemical reactivity of a molecule depends on its substate the result is an overall change of the molecules' reaction rate which may cause further changes in the biochemical pathway.

The model can in principle account for fixed frequencies, narrow linewidths and low saturation thresholds as are reported to be characteristic of biological sensitivity to millimeter waves [1–4]. The interaction at the molecular level predicts identical resonances in widely different living objects. These resonances may offer a useful tool in biology and they can furthermore influence the discussion of microwave environmental impact.

Introduction

In the field of electromagnetic bioeffects, experimental claims of a resonant [1–3] or at least a nonthermal [4, 5] biological response to millimeter microwaves have been received with caution. The mere sharpness of resonances with Q-values of *e.g.* 5000 [3] did however create interest. In the absence of any explanation it was the prediction of Fröhlich's far-reaching conjecture regarding nonlinear vibrational excitations [6] which has guided much of the experimental activity.

Here we consider electronic excitations. The proposed model applies well-established concepts of electron spin resonance (ESR) spectroscopy as are for example used in studying chemically induced dynamic electron polarization (CIDEP) [7] or reaction yield detected magnetic resonance (RYDMR) [8]. We focus our attention to the possibility of high frequency ESR transitions in the absence of an external magnetic field ("zero-field-transitions"). Therefore we have to disregard free radicals but consider molecules or intermediate complexes having *two or more unpaired electrons.*

Theory

Triplet molecules are characterized by two unpaired electrons and possess three substates X, Y, Z which generally differ in energy-even in the absence of an external magnetic field – because of the mutual magnetic interaction of two unpaired electrons ("zero-field-splitting"). Any two of the substates are connected by magnetic dipole allowed transitions [9]. Typically the transition frequencies ν_{ij} are low (~ 3 GHz) in organic molecules where the spin-spin interaction is dominant. Higher frequencies in the millimeter microwave range are found, for example, in molecules with tight orbitals, *e.g.* 43.5 GHz in benzenesulfonitrene [10]. The strong spin-orbit interaction present in transition metal ions of porphyrin-type molecules can cause even very high splittings, *e.g.* 260 GHz in acid-met myoglobin [11]. Such metallo-proteins seem to be of special interest for our question because of the central importance such a metal ion may have in the enzymatic function.

In room-temperature thermal equilibrium the triplet substate populations n_i are nearly equal, $n_i/n_j = \exp(-h\nu_{ij}/kT) \approx 1 - h\nu_{ij}/kT$. As an example, $n_i/n_j = 0.995$ for $\nu_{ij} = 30$ GHz. Nonthermal populations relax toward equilibrium by radiationless "spin-lattice" processes on time scale T_1. The latter is known to be long, typically of the order of 1 s at low temperature. At higher temperature the time T_1 can generally be expected much shorter. Unfortunately only few data exist for temperatures of physiological in-

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terest [18]. Some of the very short values for T_1 are however obtained in high magnetic field. Note that under this condition a dominant contribution can arise from molecular reorientation, *i.e.* from the fact that the molecules rotate or librate with respect to the field direction, a contribution which certainly is absent in the case of zero external magnetic field.

Nonthermal populations quite generally appear when triplet molecules are formed. Of two formation mechanisms, chemical and optical, the optical one involves the radiationless decay ("intersystem crossing") of a photoexcited singlet molecule. This transition is substate-selective according to symmetry rules, and preferentially populates that substate where incipient spin momentum is most readily balanced by the generation of orbital momentum, since total angular momentum is conserved [7]. Analogous selection rules exist also for chemical routes leading to triplet molecules [8]. On the other end substate selectivity also occurs in triplet annihilation processes. For example, the rate of radiative return to a singlet ground state ("phosphorescence"), or the transition into chemical products, depends on the substate and hence renders the triplet lifetime substate-dependent. Therefore, nonthermal populations are likely whenever T_1 exceeds the triplet lifetime, a fact supported by the large body of literature on optically detected magnetic resonance (ODMR) [12]. In one single case only, to my knowledge, has it been possible to observe that resonant microwaves alter the overall reaction rate by disturbing substate populations of transient triplet molecules [13].

The effect of microwaves on substate populations n_x, n_y and n_z is adequately described in a two-level model using the Bloch equations [14]. The density matrix formulation useful for treating coherent phenomena such as free induction, nutation or echo is however not required when time scales (*e.g.* of microwave exposure as in [1–5]) by far exceed T_2, the transition dipole lifetime. Hence we come to simple rate equations,

$$\begin{aligned}\dot{n}_x &= S_x + w_{yx}n_y + w_{zx}n_z - (w_{xy} + w_{xz} + k_x + r_x)n_x\\ \dot{n}_y &= S_y + w_{zy}n_z + w_{xy}n_x - (w_{yz} + w_{yx} + k_y + r_y)n_y \qquad (1)\\ \dot{n}_z &= S_z + w_{xz}n_x + w_{yz}n_y - (w_{zx} + w_{zy} + k_z + r_z)n_z.\end{aligned}$$

Here S_i are formation rates, k_i are decay rate constants, r_i are chemical reaction rate constants, and w_{ij} ($\approx (\pi T_1)^{-1}$) are spin-lattice relaxation rate constants (Fig. 1). A resonant microwave field at frequency $\nu \approx \nu_{xy}$ adds two radiative terms, *viz.* $\mu_{xy}(n_y - n_x)$ to eq. $\dot{n}_x$, and $\mu_{xy}(n_x - n_y)$ to eq. $\dot{n}_y$; where μ_{xy} is the radiative rate constant for magnetic dipole transitions

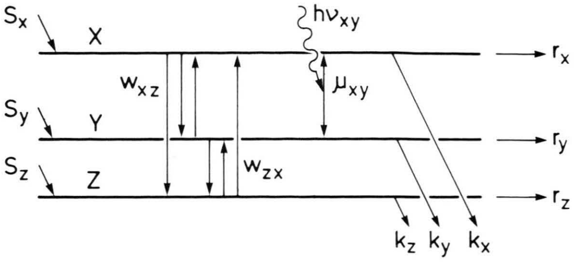

Fig. 1. Schematic representation of microwave-influenced population dynamics in the zero-field levels of chemically reacting triplet molecules.

$$\mu_{xy} = \frac{\pi}{4}\gamma^2\mu_0^2 H_0^2 f(\nu - \nu_{xy}). \qquad (2)$$

Here $\gamma = 4.912 \cdot 10^{11}\ \mathrm{m^2V^{-1}s^{-2}}$ is the electronic gyromagnetic ratio, H_0 is the amplitude of the magnetic field $H = H_0 \cos 2\pi\nu t$, and f is the resonance line shape normalized as $\int_0^\infty f(\nu)\mathrm{d}\nu = 1$.

To see the essence of the microwave effect, we seek a stationary solution of (1) by setting $\dot{n}_x = \dot{n}_y = \dot{n}_z = 0$. At room temperature we have $w_{ij}/w_{ji} = \exp(h\nu_{ij}/kT) \approx 1$. We consider the simplified case $w_{ij} = w$ and obtain

$$\begin{aligned}n_x\,(2w + k_x + r_x + \mu_{xy}) &= n_y\,(w + \mu_{xy}) + n_z w + S_x\\ n_y\,(2w + k_y + r_y + \mu_{xy}) &= n_x\,(w + \mu_{xy}) + n_z w + S_y \qquad (3)\\ n_z\,(2w + k_z + r_z) &= (n_x + n_y)w + S_z.\end{aligned}$$

Although the solution of Eqn. (3) is straightforward, we restrict the discussion to the limit $w \ll k_i + r_i$ and find for the total triplet reaction rate, defined as $R = \sum_i r_i n_i$

$$R_0 = \frac{S_x}{1 + \dfrac{k_x}{r_x}} + \frac{S_y}{1 + \dfrac{k_y}{r_y}} + \frac{S_z}{1 + \dfrac{k_z}{r_z}} \qquad (4)$$

in the absence of microwaves, and

$$R_s = \frac{S_x}{1 + \dfrac{k_x + k_y}{r_x + r_y}} + \frac{S_y}{1 + \dfrac{k_x + k_y}{r_x + r_y}} + \frac{S_z}{1 + \dfrac{k_z}{r_z}} \qquad (5)$$

in the presence of strongly saturating microwaves, defined by $\mu_{xy} \gg k_x + r_x$, $\mu_{xy} \gg k_y + r_y$. Thus a critical microwave intensity I_c exists, with the consequence that the microwave has no influence at low intensity $I \ll I_c$, while for $I \gg I_c$, the microwave influence is independent of intensity. The critical intensity I_c can be calculated from $\mu_{xy} = 1/\tau$, where τ is some effective triplet lifetime of the order of magnitude of $(k_i + r_i)^{-1}$. Using (2) and recalling that for plane waves in vacuum $I = c\mu_0 H_0^2/2$ we obtain $I_c = 2c\,(\pi\mu_0\gamma^2\tau\, f(\nu - \nu_{xy}))^{-1}$. For the case of a Lorentzian line shape,

$$f(\nu - \nu_{xy}) = (\pi^2 \Delta\nu(1 + 4((\nu - \nu_{xy})/\Delta\nu)^2))^{-1} \tag{6}$$

with halfwidth (FWHM) $\Delta\nu$, we obtain on resonance $\nu = \nu_{xy}$,

$$I_c[\mathrm{W/m^2}] = 6.217 \cdot 10^{-9}\, \Delta\nu[\mathrm{s^{-1}}]/\tau[\mathrm{s}]. \tag{7}$$

Discussion and Consequences

When we apply the hypothesis to the effects of microwave irradiation of living objects our first remark is that we should primarily expect modest changes of some metabolic reaction rate (rather than a drastic effect like mutation or death). This is in fact observed [1–5]. In our model, the size of the relative reaction rate change, $(R_s - R_0)/R_0$, can be calculated from (4) and (5) to be for example 10% when it is assumed that the triplet molecules form dominantly in one substate X or Y and that furthermore there is a 50% difference in the "branching ratioes" r_i/k_i of X and Y (all r_i, k_i being of similar order of magnitude, $\gg w$). An experimental value is 10% for the change of the growth rate of yeast [3]. Large biological amplification or damping factors can however be generally expected to relate primary molecular effects to final biological expression.

The hypothesis predicts resonant sensitivity paired with a threshold-like intensity dependence. Both features have been observed in two cases [2, 3]. The resonance width is 8 MHz, and the critical intensity is ≈ 1 mW/cm^2 for the yeast growth effect in [3]. Taking both quantities into Eqn. (7) we can evaluate an effective triplet lifetime, $\tau \leq 5 \cdot 10^{-3}$ s, of the unidentified target triplet molecule*. This value is in the order of magnitude expected for chemical and decay lifetimes τ, and thus, both the striking experimental numbers are not at variance with but rather support the hypothesis.

In further consequence of the hypothesis, the experimental resonance frequency should be apt to serve as a fingerprint to identify the target molecule. Unfortunately, spectroscopic data on large zero-field-splittings are however scarce. Also, difficulties may come from chemical shifts as are reported, for example, for conformational substates of proteins [15]. Note that satellite resonances observed in [2, 3] may be due to chemical shifts at specific sites, or to interaction with nuclear magnetic dipoles. The total absorption cross section for magnetic dipole transitions is $\int_0^\infty \sigma[\mathrm{cm^2}]d\nu = 1.05 \cdot 10^{-20}\, \nu_{xy}$ (2). On resonance of a Lorentzian (6) with $\Delta\nu = 8$ MHz and $\nu_{xy} =$ 42 GHz we expect therefore an absorption coefficient $\alpha[\mathrm{cm^{-1}}] = 5.6 \times 10^{-18}\, \varrho[\mathrm{cm^{-3}}]$, where ϱ is the net triplet concentration. This absorption (or emission [16] is probably too small to be observable in an *in vivo* experiment.

Finally it may turn out that the hypothesis might best be tested by measuring the microwave bioeffects in the presence of an external magnetic field. A rather specific behaviour is predicted. Only at high field strengths $H \gg H_c = 2\pi\nu_{xy}/\mu_0\gamma$ will there be Zeeman tuning of the resonance frequency ν_{xy}, at a rate of the order of 3 MHz/Gauss. In our example, a critical field of 15 Kilogauss would be required for $\nu_{xy} =$ 42 GHz. At much lower fields, however, we expect a complete disappearance of the microwave bioeffects rather than a shift of the resonance frequency, because of magnetic mixing of triplet substate wave functions which drastically reduces substate selectivity. Indeed, the latter mechanism provides the basis of many chemical and biological effects of ~ 100 Gauss d.c. magnetic fields [17]. Note that, for a given target molecule, the effect of such a magnetic field will be related if not equal to a resonant microwave effect [13]. Both types of external perturbations offer interesting diagnostic tools for biochemistry. Regarding these applications it might turn out that resonant microwaves provide the selectivity necessary to pinpoint specific target molecules or sites in complex *in vivo* systems.

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* The $\leq$ sign allows for the possibility that the observed linewidth may not be fully resolved and may contain inhomogeneous or power broadening contributions.

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