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Discussion

Direct spectroscopic evidence for competition between thermal molecular agitation and magnetic field in a tetrameric protein in aqueous solution

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ABSTRACT

Samples of a typical tetrameric protein, the hemoglobin, at the concentration of 150 mg/ml in bidistilled water solution, were exposed to a uniform magnetic field at 200 mT at different temperatures of 15 °C, 40 °C and 65 °C. Fourier Transform Infrared Spectroscopy was used to analyze the response of the secondary structure of the protein to both stress agents, heating and static magnetic field. The most relevant result which was observed was the significant increasing in intensity of the Amide I band after exposure to the uniform magnetic field at the room temperature of 15 °C. This result can be explained assuming that protein's α-helices aligned along the direction of the applied magnetic field due to their large dipole moment, inducing the alignment of the entire protein. Increasing of temperature up to 40 °C and 65 °C induced a significant reduction of the increasing in intensity of the Amide I band. This effect may be easily explained assuming that Brownian motion of the protein in water solution caused by thermal molecular agitation increased with increasing of temperature, contrasting the effect of the torque of the magnetic field applied to the protein in water solution.

1. Introduction

In this study the opposite effects of thermal molecular agitation and of static magnetic field (SMF) on a typical tetrameric protein in bidistilled water solution, the hemoglobin (Hb), were analyzed by means of Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectroscopy is a versatile and accurate technique that can provide interesting information on the secondary structure of proteins in aqueous solutions as largely demonstrated up to now [1–4].

Thermal molecular agitation is a phenomenon which consists on the continuous random movement of atoms and molecules in a liquid or gas due to thermal energy, producing the so-called Brownian motion, a visible consequence of thermal molecular agitation [5]. It is an irregular agitation of small particles or molecules suspended in a liquid or gas. This phenomenon was studied by Robert Brown who showed that this motion affects organic and inorganic particles [6]. In the second half of the nineteenth century the kinetic theory developed and Brownian motion was considered as a visible consequence of thermal molecular agitation. Thermal molecular agitation derives from the kinetic energy of the system whose average value is $k_B T/2$ per degree of freedom of the system, where k_B is the Boltzmann constant and T is the temperature of the system. In the early twentieth century, accurate quantitative explanation of this phenomenon was carried out. In particular, Einstein showed that the Brownian motion of particles in a fluid is produced by their continue collisions with molecules of the fluid, developing new statistical methods of measuring this motion. He proposed to measure the mean displacement of particles instead of measuring their individual velocities showing that the mean displacement of a particle on x direction, calculated in a period of

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time t, is proportional to the square root of t as expressed in the following equation:

$$\lambda_x = \sqrt{t}\sqrt{\frac{RT}{N}\frac{1}{3\pi kP}}, \quad (1)$$

in which N is the number of molecules in a mole, T is the absolute temperature, R is the gas constant, k the viscosity of the fluid and P is the radius of the particle. Thus, the mean displacement in a period of time can be computed as a function of previous parameters and further investigations of Brownian motion confirmed evidence in favor of the kinetic theory of heat [7].

Regarding the second stress agent to which was exposed Hb, that is a SMF, appreciable values of SMFs can be generated in transport systems [8–10], smoothing, line filter inductors and inventers [11], industrial processes and telecommunications switching rooms [12], small magnets such as audio speakers components, battery-operated motors, refrigerator magnets [13]. Such and other devices can generate SMFs in living beings nearby, inducing some effects such as nausea and vertigo [14,15].

Given that we do not know if most relevant harmful effects can be induced in humans by SMFs, exposure limits have been recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [14,15]. Also, typical cells, proteins and polymers have been used as a model of simple organic systems to analyze the effects of SMFs [16–21]. The most relevant effect that was highlighted by means of FTIR spectroscopy was the orientation of molecular chains along the direction of the applied SMF. In particular, the orientation of proteins α-helix structure towards the direction of a magnetic field was evidenced by the increasing in intensity of the Amide I vibration band. This result was highlighted also in typical proteins exposed to high frequencies electromagnetic fields (HF-EMFs) [22]. Nevertheless, in order that such effect can be detected, the torque induced by an applied magnetic field should overhead the Brownian motion due to thermal molecular agitation.

In this study it was shown that the amount of the increasing in intensity of the Amide I band in Hb samples in bidistilled water solution exposed to a SMF decreased with the increasing of temperature, demonstrating that there is a competition between the Brownian motion due to thermal agitation and an applied SMF also at a low intensity value of the applied magnetic field, that is 200 mT.

Proteins in aqueous solution represent a model of organic system that fit well to the environment in which some biological functions of livings occur. In particular, Hb in bidistilled water solution was chosen for this study because of previous large literature concerning the effects of EMFs on human and in vitro red blood cells, such as erythrocyte hemolysis, diminution of sedimentation speed, and globulin fraction changes [23–25].

Hb is one of the most important proteins in living organisms, because of its fundamental properties in cellular functions and in particular its main property represented by the oxygen transport to tissues and cells. It is a tetrameric protein, due to a quaternary structure with four identical subunits linked by non-covalent forces such as hydrogen bonding and electrostatic interactions. This equilibrium can be changed by exposure to EMFs, such as it was shown in previous research [26–30].

2. Materials and methods

2.1. Hemoglobin samples

Twenty healthy human subjects (10 males and 10 females) were recruited for this study in order to obtain blood samples by venipuncture. In order to obtain pure Hb solutions, blood samples were treated using the techniques accurately described in [13,31]. After the treatments reported in [13,31], Hb solutions were placed in twenty sterile microcentrifuge tubes, polypropylene, 1.5 ml capacity (BR780400, Sigma-Aldrich, Milan, Italy) at 150 mg/ml concentration in bi-distilled water solution and immediately subjected to the following assays.

2.2. Experimental design

The exposure system consisted of a couple of Helmholtz coils, with pole pieces of round parallel polar faces, in order to produce a uniform magnetic field at the center of the coils distance, following Helmholtz's theory. Thus, a SMF at the intensity of 200 mT was generated powering the Helmholtz coils by a DC generator. In the first part of the experiment, Hb samples were placed at the center of the generated uniform field area between the coils at the room temperature at 15 °C. In the second part of the experiment, Hb microcentrifuge tubes were embedded in a recipient with water heated at temperature up to 65 °C, which was placed at the center of the generated uniform field area between the coils. Analogue Hb microcentrifuge tubes were embedded in another equal recipient with water heated at the same temperature of exposed samples, far from the exposure system and were used as the control.

The magnetic field intensity was continuously monitored by a magnetic field probe (GM07 Gaussmeter of HIRST-Magnetic Instruments Ltd – UK).

2.3. Infrared spectroscopy

FTIR absorption spectra were recorded at room temperature by a spectrometer Vertex 80v of Bruker Optics.

Hb samples (150 mg/ml) of 250 µl, were placed between a pair of CaF_2 windows separated with a 25 µm Teflon spacer. For each spectrum, 128 interferograms were collected with a spectral resolution of 4 cm^{-1} in the range from 3500 cm^{-1} to 1300 cm^{-1}. IR spectra of water solution were subtracted from the spectra of Hb at the corresponding temperature. Measurements were performed under vacuum to eliminate minor spectral contributions due to residual water vapor and smoothing correction for atmospheric water background was performed. In addition, interactive baseline concave rubberband correction was used for the acquired spectra. This method uses a rubberband that is stretched from one spectrum end to the other, and the band is pressed onto the spectrum from the bottom up with varying intensity. It performs iteratively, depending on the number of iterations in the algorithm and the baseline as a frequency polygon consisting of n baseline points. In this study we used the default value of $n = 64$ baseline points and a number of 50 iterations.

3. Results

The secondary structure of proteins depends greatly on the environment in which proteins are embedded. Hence, the response of vibration bands in human Hb in water solution induced both by SMF and heating were studied by FTIR spectroscopy.

Hb samples in bi-distilled water solution, prepared as described in the preceding section, were exposed for 3 h to a uniform magnetic field at 200 mT at room temperature of 15 °C. In the second part of the experiment, beside exposure to SMF, analogue Hb samples were exposed to heating at the temperatures of 40 °C and 65 °C. Analogue unexposed samples were used as the control, at the same temperature values.

Representative exposed and unexposed spectra obtained after 3 h exposure of Hb in bidistilled water solution at the room temperature of 15 °C are represented in Fig. 1 (the red line represents exposed sample spectrum).