Assessment of Potential Changes in Thermal Characteristics of β-lactoglobulin Exposed to RF-Electromagnetic Fields and ELF-Pulsed Magnetic Fields

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A direct effect on conformation of proteins is one of the existing hypotheses about a potential interaction mechanism of EMF with biological cell cultures and solutions. Effects of electromagnetic field (EMF) exposure on the folding kinetics of bovine β-lactoglobulin protein in terms of reduced relaxation times in response to temperature changes were reported in literature. Experiments with protein solutions are very demanding regarding uncertainties due to various potential side effects caused by the experimental setup. In small volumes of protein solutions (i.e. for solutions in cuvettes) a large temperature gradient can exist leading to significant differences between the temperature distribution of the exposed and the non-exposed case. One of the main concerns is that the observed changes might be due to localized thermal effects induced by electromagnetic fields. Thus, strictly controlled experimental conditions including temperature measurements within the sample are required. In this study potential changes in protein conformation and on the folding kinetics of β-lactoglobulin are investigated using an experimental setup for simultaneously exposing and recording protein solutions. The setup is based on exposure devices for radio frequency (RF-EMF) and extremely low frequency pulsed magnetic fields (ELF-pMF) placed in the measurement compartment of a circular dichroism (CD) spectrophotometer. An optical temperature sensor monitors the temperature in the center of the volume relevant for the measurement of the protein solution. The experiments carried out up to now cover sinusoidal RF-EMF at 2.0 GHz at different field strengths and a specific ELF-pMF signal. The results obtained so far suggest a potential effect of RF-EMF on the folding kinetics of β-lactoglobulin. However, the potential effect observed here is of a different characteristic compared to the one reported in literature before. The origin of the changes in relaxation time has to be further investigated by experimental means.

1. INTRODUCTION

Effects of electromagnetic field (EMF) exposure on the folding kinetics of bovine β-lactoglobulin protein in terms of reduced relaxation times in response to temperature changes were reported [1]. A direct effect on conformation or folding kinetics of proteins is one of the existing hypotheses about a potential interaction mechanism of EMF with biological cell cultures and solutions. Proteins are the key molecules for intracellular processes including intercellular signalling; their functionality is based on their conformational state. The experimental conditions in such studies have proved difficult to control, in particular, because the temperature at the site of interaction was not measured [3]. Therefore, one of the remaining concerns is that the observed changes might be due to localized thermal effects [4].

In this study the bovine protein β-lactoglobulin is exposed to RF-EMFs and ELF-pMF while simultaneously monitoring its potential structural changes via circular dichroism spectrophotometry. Thus, the point of observation becomes identical with the potential interaction site in space and time, a situation that is expected to allow for the detection of even small effects of EMFs.

In contrast to previous studies investigating effects of RF-EMF on the tertiary protein structure here potential direct effects of RF-EMF and ELF-pMF on the secondary structure of β-lactoglobulin and its folding kinetics are examined. The investigated signals are extended to ELF-pMF because these signals have previously shown effects on cell cultures and tissues [10,11,12]

2. EXPERIMENTAL EQUIPMENT AND MATERIALS

a) Circular Dichroism Spectrophotometer

A CD spectrophotometer JASCO J-715 is used. CD spectroscopy is a well established and widely used experimental method for the investigation of structural properties of biomacromolecules. In CD measurements the ellipticity of diluted protein solutions, i.e. the difference in absorption between left and right-handed polarised light is recorded. The measured CD signal of macromolecules provides information about their conformational equilibrium under certain environmental conditions (pH, temperature, etc.), i.e. information about the fractional population of different conformational states of macromolecules as function of the temperature within the measurement volume [13]. In particular the ellipticity signal is retrieved as an average from the total irradiated volume over a specified integration time. The different events expressed by instantaneous ellipticity are independent and superimposed by noise resulting from protein movement (brownian motion) and from thermal noise of the photodetector. CD radiation itself has shown to be not invasive, i.e. it does not alter the covalent and non-covalent structure of proteins and other biomacromolecules [5].
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Figure 1. CAD sketch of the RF exposure unit (left) and the unit mounted inside the measurement compartment of the spectrophotometer (right).

b) Target protein bovine β - Lactoglobulin

β - lactoglobulin is a whey protein consisting of 162 residues, with an 18.4 kDa molecular weight. In order to achieve cold denaturation (larger rate of unfolded proteins at low temperature) and a high CD signal sensitivity a protein solution containing 4.3 M urea, 0.04 M KCl and 72.4 μM β – lactoglobulin is used. The pH of the solution is adjusted to 2.0 using HCl as reported in [14]. The CD signal of the protein solution is recorded at a wavelength of 222 nm that has shown to exhibit a high sensitivity regarding changes in secondary structure.

c) Radio Frequency Exposure Unit

The RF experimental unit shown in Figure 1 consisting of a temperature-controlled exposure chamber mounted in the measurement compartment of the spectrophotometer was already realized and tested for previous studies reported in [6-9]. The exposure chamber was designed and characterized using numerical simulation together with experimental tools. The applied electric field strength up to 3000 V/m is uniformly distributed over the illuminated volume with normalised standard deviation $\sigma < 0.1$ for all frequencies as recommended by [2]. The exposure chamber is fed using a signal generator together with an arbitrary waveform generator. The setup supports all commonly used study protocols and allows for arbitrary pulsed exposure durations with

Figure 2. CAD sketch of the ELF–pMF exposure unit (left), a figure of the measured magnetic flux density at the center of the tube (top right) and a photograph of the unit holding a Hellma QS 165 1 mm temperature controlled cuvette (right bottom).
user-defined signals including DVBT, GSM and UMTS signals. An optical temperature probe is directly inserted into the cuvette of path length 5 mm containing the protein solution. The position of the probe is adjusted according to the centre of the optical beam measuring the CD-signal. A small sensor monitors the electric field strength within the exposure chamber. A detailed description of the experimental unit and the evaluations performed can be found in [6,7].

d) Extremly Low Frequency Pulsed Magnetic Field Exposure Unit

An ELF-pMF experimental unit consists of a Helmholtz coil configuration mounted on a customized 3D printed cylindrical tube made of Polyethylene. A holder for a cylindrical temperature controlled cuvette (optical path length of 1 mm) is placed in the center of the tube as depicted in Figure 2. The setup was designed to fit into the measurement compartment of the spectrophotometer. The coil arrangement supports a uniformly distributed magnetic field within the protein solution. The magnetic field lines are oriented axially to the tube and therefore perpendicular to the optical beam. The two coils with a diameter of 110 mm placed 50 mm apart from each other consists of 37 windings each created of a 1.7 mm cooper wire to support currents up to 10 A without noticeable temperature increase (<0.1°C). An arbitrary waveform generator connected to a 2.4 kW audio amplifier and matched via a resistor network drives the coils.

An asymmetric pulsed magnetic field consisting of pulse sequences of 20 150µs on-off cycles resulting in a 6 ms interval at a repetition rate of 15 Hz were applied at a flux density of 5.0 mT as reported in [11]. Measurements of the mechanical vibrations of the holding structure resulted in values below the detection limit (<0.001 g). The relation between the feed current and the resulting magnetic flux density was calibrated using a commercial hall sensor.

In both exposure units a computer-controlled thermostat maintains the temperature of the water flow surrounding the protein solution. Furthermore, the signal generation units as well as the monitoring devices are all computer-controlled. All electronic devices as well as the thermostat are connected to the corresponding devices within the measurement compartment through light-tight feed through in the walls.

3. METHODS

The potential effects of RF-EMF and ELF-pMF on the folding kinetic of β-lactoglobulin is investigated by conducting two different exposure protocols and comparing them to the non exposed case.

a) Folding Kinetics for Heating-Cooling cycle using Thermostat

In the first set of experiments the potential effects of different EMFs (non EMF, RF-EMF at 1.5 kV/m, RF-EMF at 3.0 kV/m and ELF-pMF at 5mT) on the folding kinetics is investigated by exposing for 5 s in the heating cycle at 10°C. For the heating-cooling cycle two different temperature change rates (0.6 K/min and 1.5 K/min) are applied so that in total 8 experiments are conducted without any repetition. In addition one experiment without EMF exposure is conducted with a temperature plateau at 13°C in order to estimate the shift in relaxation for the heating and cooling path.

b) Folding kinetics for rapid temperature changes using RF-EMF heating

The second set of experiments is conducted at a constant temperature of the water bath of 7°C while exposing the protein solution with EMFs for 5 min to either a sinusoidal RF signal at 2GHz of amplitude 1.5 or 3.0 kV/m or a ELF pulsed magnetic field

Figure 3. Folding kinetics of beta-lactoglobulin, heating and cooling cycles both shown (cooling is mirrored) with a temperature change rate of 0.6 K/min driven by the thermostat (left) and for the same conditions but additionally exposed to 5 s sinusoidal 2GHz RF-EMF at time t=0 with an electric field amplitude of 1500 V/m during the heating cycle at 10°C (right).
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4. Preliminary Results

In general, the cold denaturation process of β–lactoglobulin is a symmetric relaxation process as shown in Fig. 4 with nonlinear decreasing relaxation gradient (maximal 130 deg cm²/dmol at 1.5 K/min compared to 100 deg cm²/dmol at 0.6 K/min).

As a result, no difference in the folding kinetics of β–lactoglobulin exposed to ELF pulsed magnetic fields are observed neither for the first (Fig. 4) or the second case (not shown). Furthermore, also no effect on the long-term stability is observed.

As shown in Fig. 3 the CD signal of β–lactoglobulin follows immediately the RF induced temperature change without any detectable relaxation. It has to be noted that the uncertainty of the CD signal is high due to a high noise level. Nevertheless, beside the thermal increase the folding kinetic is not altered by the RF exposure of either field strength. For the RF heating case depicted in Fig. 5 the CD signal pursues an immediate temperature increase but relaxes with a different time constant than the temperature, thus following the relaxation curve in Fig. 3. An interesting effect is observed during the 5 min exposure. At an electric field strength of 1.5 kV/m an immediate reaction to the temperature jump is succeed by a linear decrease in CD signal value to the relaxation level of 4300 deg cm²/dmol as outlined in Fig. 3. In comparison an RF-EMF exposure of 3.0 kV/m results in a temperature jump up to 60°C without any observable relaxation process. Regardless of the applied electric field strength no potential effect of RF-EMF on CD signal degradation or alteration in the long term was detected.

Figure 4. Left: unexposed folding kinetics of beta-lactoglobulin with a temperature gradient of 1.5 K/min and an intermediate relaxation period of 5 min at 13°C to demonstrate symmetric relaxation process; right: same case without intermediate relaxation period, but exposed to 5 s ELF-MF at 5 mT with a peak field strength of the pulses of 5 mT. The experiments are conducted 6 times each with 10 min relaxation time in order to investigate the long term stability of the configuration of maximal 1.5 hours.

Figure 5. CD signal of beta-lactoglobulin exposed to an harmonic RF signal at 2.0 GHz of 1500 V/m (left) and 3000 V/m (right) electric field strength for 5 min duration
5. CONCLUSION

The CD signal response of $\beta$–lactoglobulin in solution to different temperature changes caused by conventional and RF induced heating was analysed at an optical wavelength of 222 nm. An exposure of $\beta$–lactoglobulin in solution to a sinusoidal signal at 2GHz and an amplitude of 3.0 kV/m results in a temperature jump up to 60°C without any observable relaxation process.

No observable response of the folding kinetics of $\beta$–lactoglobulin to the exposure of extremely low frequency pulsed magnetic fields at 5mT was detected for different protocols.

Further experiments will be conducted to investigate the folding kinetics of the tertiary structure at 293 nm as suggested by [14]. Also the relaxation time of the CD signal in response to a constant temperature gradient generated by conventional heating in combination with RF heating will be further examined in order to evaluate the observed differences.

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