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Bioprotective effects of deuterium oxide solution on proteins' secondary structure against extremely low frequencies electromagnetic fields

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ABSTRACT

The effects of separated exposures to a static magnetic field at 200 mT and to a 50 Hz electromagnetic field at 2 mT on the secondary structure of a typical protein, the bovine serum albumin (BSA), in H_2O water solution and in deuterium oxide (D_2O) solution have been taken into account analyzing the changes of characteristic vibration bands by means of FTIR spectroscopy.

It was observed that after 3 h of separated exposure to the static magnetic field and to 50 Hz electromagnetic field, amide A and amide I vibration bands decreased in intensity for BSA samples in bidistilled H_2O water solution. In contrast, no significant change was observed in amide I and amide A modes after exposure of BSA in D_2O solution. This result can be explained by the hypothesis, advanced by other authors with respect to other stress agents, that H/D isotopic exchange can strengthen the hydrogen bond interaction providing that D_2O can protect the secondary structure of a protein against the effects of extremely low frequency electromagnetic fields.

KEYWORDS

Bovine serum albumin;
Deuterium oxide;
Electromagnetic field;
FTIR spectroscopy;
Amide I and amide A modes.

INTRODUCTION

The aim of this study was to highlight the effects of H/D isotopic exchange on the secondary structure of a typical protein, the bovine serum albumin (BSA) in H_2O and in D_2O solution, under two separated extremely low frequency electromagnetic fields (ELF-EMFs) exposures: a static magnetic field (SMF) and a 50 Hz EMF.

BSA is one of the most studied proteins utilized as a model for many and diverse biophysical and biochemical studies.

It is the most abundant of the proteins in blood plasma, and serves as transport protein for numerous compounds, as long chain fatty acids or bilirubin, which are bound with a high affinity to the protein.

This protein has interesting properties in binding a variety of hydrophobic ligands in the transport and deposition of a variety of endogenous and exogenous substances in blood due to the existence of a limited number of binding regions with different specificity.

The secondary structure of BSA is characterized

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by a high α-helix content (~60%), 10% turn, and 23% extended chain, and quasi non-β-sheet structure in the amide I and II modes.

The band shape of the amide I mode can be considered as overlapping bands representing α-helices, β-sheets, turns, and random structures. This band arises mainly from the CP%O stretching vibration with minor contributions of the C—N stretching vibration, and the N—H in-plane bend. The weak intense band around 1550 cm^{-1}, the amide II band, corresponds to a mixture of N—H in-plane bend and C—N stretch[1-3].

Another prominent spectral features is the amide A vibration in the IR spectra close to 3300 cm^{-1}, which is due mostly to the peptide linkage N—H stretching mode, which is present in the mid-infrared spectra of polypeptides and proteins.

Previous studies showed that the secondary structure of BSA can be influenced by external stress factors. For instance, the unfolding of BSA was showed to be induced by denaturants such as urea and surfactant micelles[4], by chaotropic reagents such as guanidinium chloride or ionic detergents such as sodium dodecyl sulfate[5] and by thermal stress up to 130°C[6].

Proteins secondary structure can be successfully investigated by means of Fourier transform infrared (FTIR) spectroscopy.

Indeed, FTIR can be considered as a valuable tool for analyzing protein structure in H_2O-based structure or in deuterated form, which can detect transitions between rotational and vibration energy levels, yielding interesting information on molecular structure[7-10].

The effects of the exposure of BSA to a SMF at 200 mT and to a 50 Hz EMF at 2 mT have been taken into account when analyzing the changes of the vibration bands of protein linkages in the mid-infrared region from 4500 to 1200 cm^{-1} by FTIR spectroscopy.

A careful analysis of EMF effects on the secondary structure of some proteins has been performed up to now[11-15].

The amide I vibration for proteins absorbs between 1600 and 1700 cm^{-1}, overlapping directly with the H_2O bending vibration band at 1645 cm^{-1}. The intensity of the water absorbance at 1645 cm^{-1} is approximately an order of magnitude higher than the amide I absorbance of proteins.

For IR study of protein in H_2O solution, water absorption in the 1600"1700 cm^{-1} region might be the biggest problem.

It is much easier if the spectroscopic study is carried out in D_2O solution because there is no absorption spectrum of D_2O in the region where the amide I and II bands are observed.

H_2O as a solvent is much more preferable than D_2O for studying protein structure[16-18]. In fact D_2O changes the protein properties somewhat in comparison with the native ones. In particular, the amide I band frequencies are strongly affected by the H-D exchanges in the peptide linkages[19-20].

The effect of H-D exchange on protein structural properties is not fully understood, and it has been used for the analysis of protein structure and structural dynamics of proteins[21-25]. It was shown that the structural dynamics of proteins are sensitive to the secondary structural composition and experimental conditions such as pH, temperature and pressure[21-23].

Hydrogen-deuterium isotopic exchange identifies sub-molecular motional domains, including fast exchanging protons of the protein surface, slow exchanging protons involved in secondary structures, exchange rate depending on accessibility to the solvent[26-31].

Slowly exchanging core has been related for several proteins to the initial folding core during the sequence of events leading to the protein folding[32].

In particular, the effects of H/D isotopic substitution on the structural stability of BSA in aqueous solution were studied over the temperature range of 5-90 °C[33]. It was found that the presence of D_2O retards the occurrence of irreversible thermal denaturation in BSA, as evidenced by a higher onset temperature of 58 °C, showing that D_2O exhibits a protective effect on the domain structure during the early stages of domain denaturation.

These results led us to investigate the protective effects of H/D isotopic exchange on the secondary structure of BSA with respect to another stress agent: the exposure to static and extremely low frequency EMFs.

MATERIALS AND METHODS

BSA samples

Lyophilized BSA was purchased from Sigma Chemical Co. Prior to infrared spectroscopy, the pro-

tein was dissolved in D_2O at 25 °C at a concentration of 60 mg/mL and lyophilized from D_2O. This procedure was repeated using H_2O for further exposure.

Experimental design

The exposure system consisted of a couple of Helmholtz coils, with pole pieces of round parallel

polar faces, to produce a uniform magnetic field at the center of the coils distance. This device was used to generate time-varying EMFs at a frequency of 50 Hz by means of an AC voltage regulating up to 230 V. An SMF was generated by another couple of Helmholtz coils that were powered by a DC generator, producing a uniform magnetic field intensity of 200 mT.

Exposed samples were placed at the center of the generated uniform field area between the coils.

The magnetic field was continuously monitored by a magnetic field probe (GM07 Gmeter of HIRST Magnetic Instruments, Ltd, UK).

Infrared spectroscopy

FTIR absorption spectra were recorded by a spectrometer (Vertex 80v) from Bruker Optics. The attenuated total reflection (ATR) method was chosen for spectrum collection.

For each spectrum, 64 interferograms were collected with a spectral resolution of 4 cm^{-1} in the range 7500-1200 cm^{-1}.

IR spectra of the water solution were subtracted from the spectra of BSA at the corresponding temperature. Each measure was performed under vacuum to eliminate minor spectral contributions due to residual water vapor. However, a smoothing correction for atmospheric water background was performed. IR spectra were baseline-corrected and area-normalized for exposed solutions and control samples.

The automatic baseline scattering correction function was used to subtract baselines from spectra, which allows getting spectra with band edges of up to the theoretical baseline.

ATR spectra were smoothed by the Loess algorithm and the deconvolved spectra fitted with Gaussian band profiles. Initial values for the peak heights and widths were estimated from the deconvolved spectra.

Either exposed and control samples were located in the same room at a temperature of 20 °C.

RESULTS AND DISCUSSION

Samples of 200 µL of BSA in bidistilled H_2O water and in D_2O solutions were exposed separately to an SMF at 200 mT at a room temperature of 20 °C. Analogue unexposed samples at the same room temperature were used as the control.

ATR-FTIR analyses in the mid-infrared region were performed after 3 h of exposure as described in the preceding section, to detect eventual structural changes of vibration bands of BSA induced by exposure compared to unexposed samples.

Typical spectra acquired after 3 h of exposure of BSA in bidistilled water aqueous solution are represented in Figure 1 (A) and Figure 2 (A), where the amide A band, represented by a double peak close to 3293 and 3307 cm^{-1}, and the amide I mode are shown, respectively.

It appears that after 3 h of exposure to SMF, the intensity of the amide A and amide I bands decreased for BSA in bidistilled water solution.

Analogue exposure of BSA in D_2O solution showed a lower decrease in intensity of amide A after exposure to the SMF in comparison to not-exposed samples, as represented in Figure 1 (B), whereas no appreciable change was observed in the amide I mode (see Figure 2-B).

Exposure of samples of 200 µL of BSA in bidistilled H_2O water and D_2O aqueous solutions was carried out also with respect to a uniform 50 Hz EMF at 2 mT.

It produced a decrease in amide A and amide I intensities for exposed samples of BSA in bidistilled water aqueous solution, as represented in Figure 3(A) and Figure 4 (A), respectively.

H-D exchange represented by exposure of BSA in D_2O solution, provided only a low decrease in amide A (Figure 3-B) in comparison to not-exposed samples, whereas no appreciable change was detected in the amide I region, as can be observed in Figure 4(B).

A reasonable explanation of the observed amide A band is that it derives from an overtone of amide II (NH in-plane angle bend) or a combination of amide I and amide II that interacts with the strong NH s fundamental through a cubic anharmonic potential, acquiring enough intensity to be observable through a Fermi Amide A is a useful indicator of secondary structure and often

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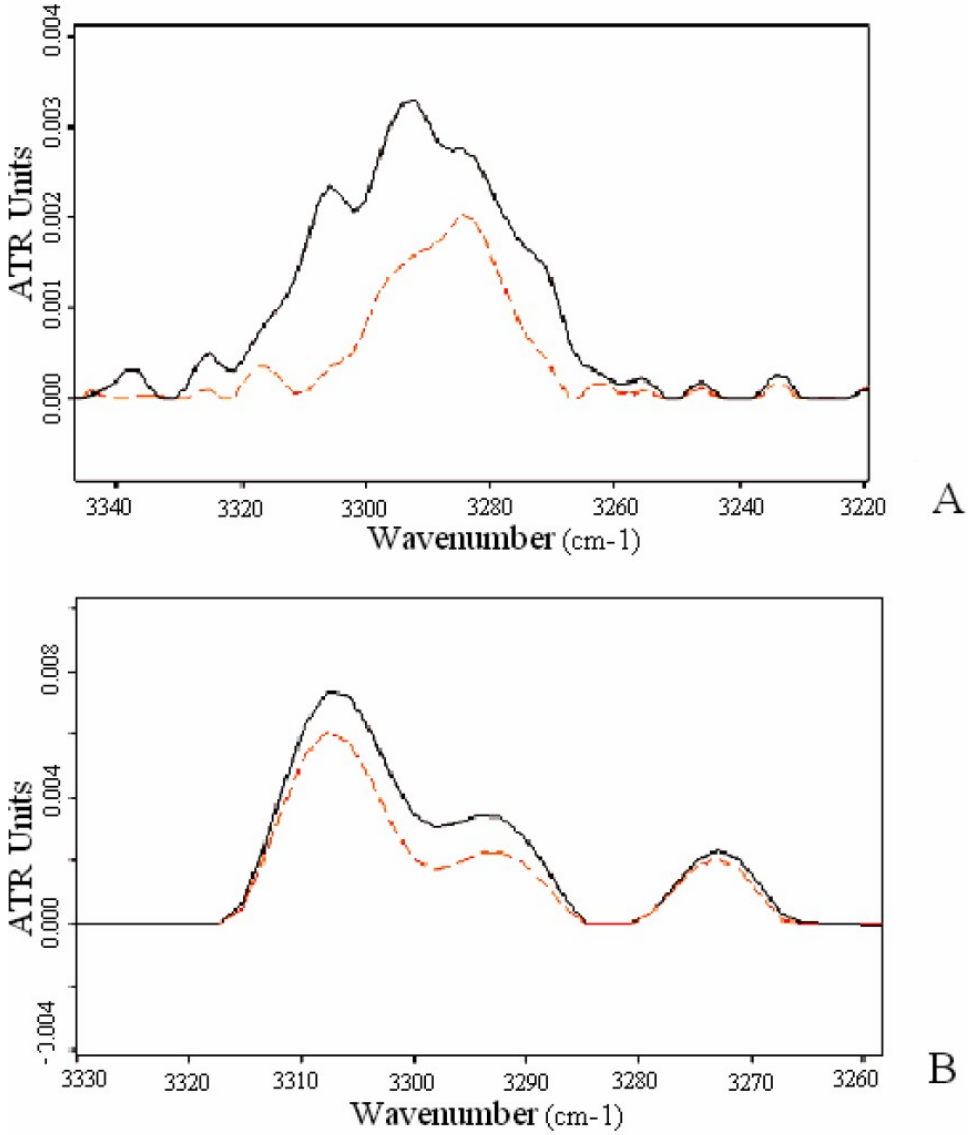

Figure 1 : Infrared spectra in the region of Amide A of BSA in bidistilled H_2O solution (A) and in D_2O aqueous solution (B) after 3 h of exposure to a SMF at 200 mT. The red dashed lines refer to exposed sample spectra.

appears as a doublet band arising from two different structures, the stronger of the two components being associated with the standard α-helical structure in the chain[34,35].

The secondary structure of BSA is characterized by amide I and II regions, that can be considered as overlapping bands representing α-helices, β-sheets, turns, and random structures.

The amide I arises mainly from the C–O stretching vibration with minor contributions of the C–N stretching vibration, and the N–H in-plane bend. The weak intense band around 1550 cm^{-1}, the amide II band, corresponds to a mixture of N–H in-plane bend and C–N stretch[3].

The band shape of the amide I mode can be considered as overlapping bands representing α-helices, β-sheets, turns, and random structures. A high α-helix content (around 60%), 10% turn, and 23% extended chain, and quasi non-β-sheet structure characterize the amide I band of BSA[1,2].

Hence, H-D isotopic exchange resulted to preserve BSA from effects of SMFs and ELF-EMFs.

The significant decrease in intensity of amide A and amide I vibration band after exposure to SMF and to ELF-EMF of BSA samples in H_2O aqueous solutions, can be explained considering that a loss of CP%O and C–N stretching vibrations and NH bending linkages occurred in the secondary structure of the protein after

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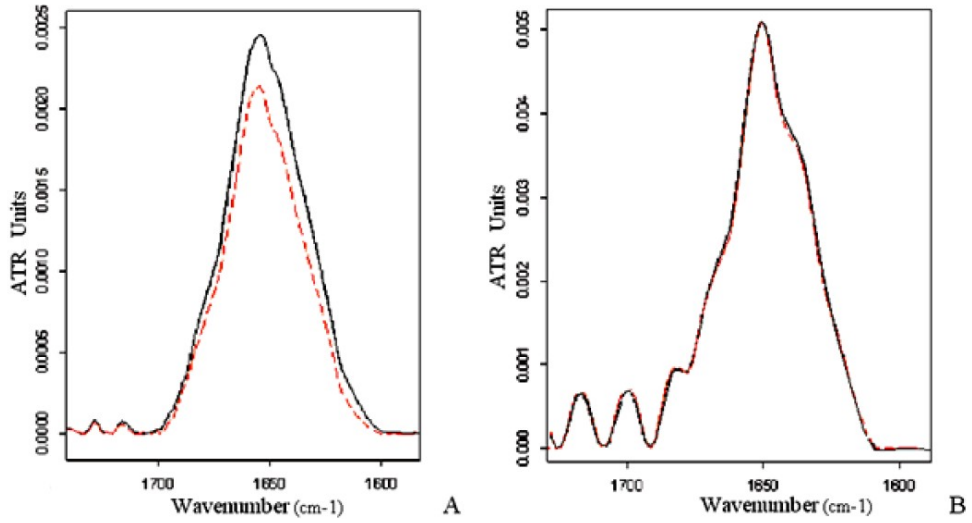

Figure 2 : (A) Representative infrared spectra of amide I region of BSA in H_2O solution and in D_2O solution (B) after 3 h of exposure to a SMF at 200 mT (the red lines represent the exposed sample spectra).

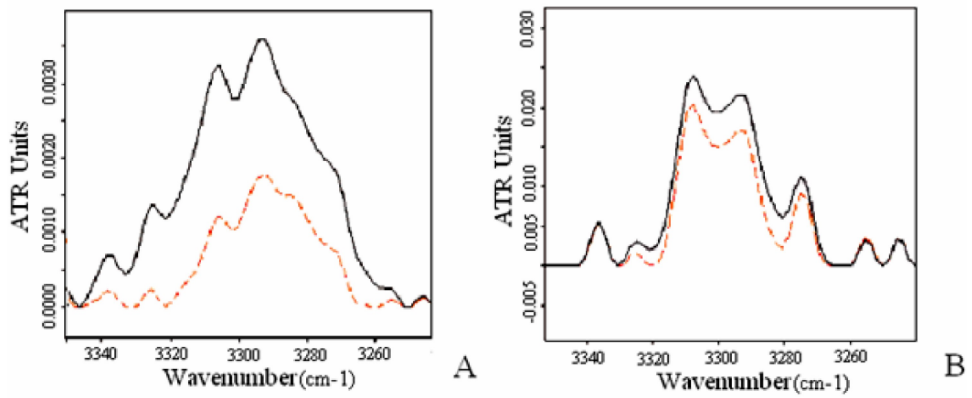

Figure 3 : Infrared spectra in the region of amide A of BSA in bidistilled H_2O solution (A) and in D_2O aqueous solution (B) after 3 h of exposure to a 50 Hz EMF at 2 mT. The red dashed lines refer to exposed sample spectra.

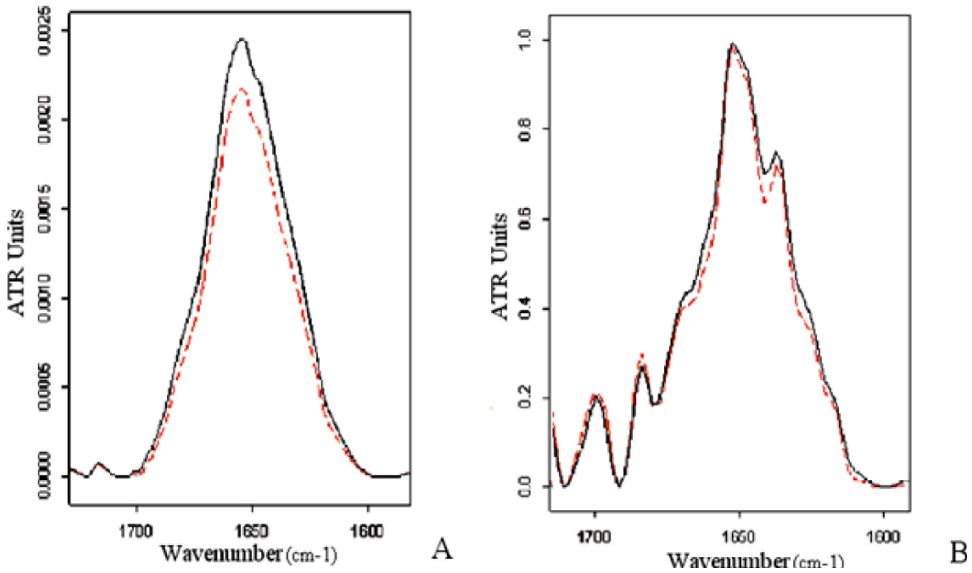

Figure 4 : (A) Representative infrared spectra in the region of amide I of BSA in H_2O solution and in D_2O solution (B) after 3 h of exposure to a 50 Hz EMF at 2 mT (the red lines represent the exposed sample spectra).

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exposure. In addition, the significant shifts of the amide A mode towards lower energies after exposures (shown in Figure 1-A and Figure 3-A) lead us to consider that protein's aggregation in BSA aqueous solution may be induced by exposure to EMFs.

In contrast, the infrared spectra of BSA in D_2O solution turned out to be almost unchanged after the exposure to SMF and ELF-EMF, suggesting that D_2O solution can preserve the protein from EMFs.

However, previous literature evidenced the effect of D_2O on both intra- and intermolecular protein interactions in solution[36-38].

Ref.[33] studied the effects of H/D isotopic substitution on the structural stability of BSA in aqueous solution over the temperature range of 5-90 °C, showing that the presence of D_2O retards the occurrence of irreversible thermal denaturation in BSA, exhibiting a protective effect on the protein's secondary structure.

Also[39] investigated lower critical solution temperature of elastin-like polypeptides as a function of elastin-like polypeptides chain length in both D_2O and H_2O solutions, observing that hydrogen bonding of β-turn structure is stabilized in D_2O solution.

Indeed, the secondary structure of proteins is defined as the local conformations of the primary backbone, which is characterized by regular repeating structures such as α-helices. The structural stability of these repeating structures is the result of hydrogen bonding between the amide hydrogen and the carbonyl oxygen within the alpha helix and between beta sheets, so that the hydrogen bonding is the primary stabilizing force in all of these structures.

Hence, the observed stabilization of BSA secondary structure under EMFs exposure produced by D_2O solution is in agreement with previous studies that have shown that hydrogen bonding configurations are stabilized in D_2O solution.

Also ref.[40] suggested that the H-bond in proteins not only mirrors the motion of hydrogen in its own atomistic setting but also finds its origin in the collective environment of the hydrogen bond in a global lattice of surrounding H_2O molecules. The authors tested their model by changing to D_2O as the surrounding medium resulting in a substantial solvent isotope effect, demonstrating the important influence of the environment on the individual hydrogen bond.

Also this result confirms that H/D isotopic exchange may strengthen the hydrogen bond interaction.

The increased stability of the secondary structure of BSA which was observed in FTIR spectra of samples in D_2O solution exposed to EMFs can find its explanation in this scenario. Indeed, amide A and amide I modes intensities are related to the α-helix component and therefore it depends on the formation of ligand-protein complexes related to hydrogen bonding.

CONCLUSION

An alteration of the secondary structure of BSA, represented by a clear decrease in amide A and amide I vibration band intensities, was observed exposing separately samples of protein in H_2O aqueous solutions up to 3 h to a 200 mT SMF and to a 50 Hz EMF at 2 mT, leading us to consider that a loss of C=O and C—N stretching vibrations and N—H bending linkages occurred in the secondary structure of the protein.

Analogue exposure of BSA samples in D_2O solution did not evidence appreciable change in intensity of amide A and amide I vibration bands, showing that H/D isotopic exchange can preserve a protein from effects ELF-EMFs. This result can be explained by the hypothesis advanced by other authors that D_2O solution can strengthen the hydrogen bond interaction with respect to H_2O solution.

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