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Atomistic simulation of hydrophobin HFBII conformation in aqueous and fluorous media and at the water/*vacuum* interface

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ABSTRACT

Hydrophobins are proteins of interest for numerous applications thanks to their unique conformational and surface properties and their ability to self-assemble at interfaces. Here we report fully atomistic molecular mechanics and molecular dynamics results together with circular dichroism experimental data, aimed to study the conformational properties of the hydrophobin HFBII in a fluorinated solvent in comparison with a water solution and/or at an aqueous/*vacuum* interface. Both the atomistic simulations and the circular dichroism data show the remarkable structural stability of HFBII at all scales in all these environments, with no significant structural change, although a small cavity is formed in the fluorinated solvent. The combination of theoretical calculations and circular dichroism data can describe in detail the protein conformation and flexibility in different solvents and/or at an interface, and constitutes a first step towards the study of their self-assembly.

1. Introduction

Hydrophobins are a family of small, film-forming proteins produced by filamentous fungi, endowed with exceptional surface activity features which make them effective coating or protective agents, adhesion promoters and surface modifiers [1–5]. Hydrophobins have possible interesting applications for instance in food technology [6,7], in the formation of biocompatible surfaces for biosensors [4], and in the surface modification of biomimetic composite materials [8,9]. It was recently shown that hydrophobins also effectively stabilize dispersions of fluorous materials in water by self-assembling at the interface [10–13]. Since protein surface activity and self-assembly at hydrophilic–hydrophobic interfaces is usually linked to denaturation of the individual molecules, in this work we study the stability of the hydrophobin HFBII in water, in a fluorinated solvent, and at the air/water interface, using Molecular Mechanics (MM) and Molecular Dynamics (MD) methods at a fully atomistic level, and adopting a simulation protocol formerly used by us [14–18] to model the conformational properties and stability of unlike proteins, in particular when adsorbed on solid biomaterial surfaces. The theoretical results will also be compared when possible with previous MD simulations, which used a coarse-grained model, yielding some chemical details for HFBI in water and at a water/octane interface [19], or a fully atomistic model in water and at a water/decane interface [20]. Furthermore, we report new circular dichroism (CD) data of HFBII in water and in an emulsion of a fluorinated solvent in order to compare these experimental data with the theoretical results.

2. Experimental methods

2.1. Materials

HFBII (molecular weight 7.5 kDa) was produced using recombinant strains of *Trichoderma reesei*, purifed by RP-HPLC as previously described [21] and freeze-dried before use. Galden® SV90 is a low molecular weight perfluoropolyether fluid (boiling point 90 °C) produced by Solvay Specialty Polymers, which was used without further purification.

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2.2. Circular dichroism experiments

Circular dichroism experiments were performed on an Applied Photophysics Chirascan spectrometer. Solutions of HFBI in milli-Q water (100 μg/ml) were treated in a sonicator bath for 30 min at 40 °C, followed by mixing in a thermomixer at 25 °C (1000 rpm, 1 h). The emulsions were prepared by addition of 25 μl of Galden® SV90 to 475 μl of the HFBI solution (ratio 1:20) and ultrasonication of the mixture with an ultrasonic probe (Soniprep150 MSE, 26 mm tip, 750 W, 20 kHz, 30% amplitude 30%, 5 × 50 s). Analyses were performed on freshly prepared samples in a 0.1 cm path length quartz cuvette at 25 °C, scanning the λ = 180–260 nm region, with a step size of 0.5 nm, a minimum time/step of 3.0 s with adaptive evaluation option enabled, bandwidth 1 nm, 10 repetitions/sample. Spectra were averaged and smoothed.

2.3. Molecular mechanics and molecular dynamics methods

The simulation studies based on MM and MD methods were performed with InsightII/Discover 2000, Materials Studio and Discovery Studio [22] using the COMPASS force field. The coordinates of the non-hydrogen atoms of HFBII were taken from the X-ray structure [3] deposited within the Protein Data Bank (2B97), and the hydrogen atoms were added in the calculated position. Using the periodic boundary conditions the simulation box to study the HFBII protein in water was a cubic cell of size 50 Å (more than twice the protein size), and to model the water/*vacuum* interface we considered a cell with dimensions of 50 × 50× 120 Å adopting an NVT statistical ensemble. The protein in the fluorinated solvent (Galden®, modeled as CF_3-O-(CF_2-CF(CF_3)-O)$_n$-(CF_2-O)$_m$-CF_3 with n, m= 1, density equal to 1.69 g/cm^3) was modeled within a cubic cell of 50 Å using the *Amorphous Cell Tools* Modulus of Materials Studio. All energy minimizations were carried out up to an energy gradient lower than 4×10^{-3} kJ mol^{-1} Å^{-1}. The MD runs were performed at a constant T (300 K) controlled through the Berendsen thermostat. Integration of the dynamical equations was carried out with the Verlet algorithm using a time step of 1 fs, and the instantaneous coordinates were periodically saved for further analysis or geometry optimization. The length of the MD run was 60 ns in aqueous solution and 60 ns in the fluorinated solvent to check the system stability. The system equilibration on the time-scale of the present simulations was monitored through the time changes of the total and potential energy and of their components (the electrostatic and van der Waals contribution, as well as the intramolecular terms), and of the geometrical parameters such as the molecular size and radius of gyration, which showed in any case a stationary behavior for most of the latter part of the MD run.

In order to better describe the possible conformational changes of the HFBII protein, in particular the stability of its secondary structure in water, at the water/*vacuum* interface and in the fluorinated solvent, we used (i) the Ramachandran maps to monitor the changes in the (ψ,ϕ) torsion angles adjacent to the C_α atoms in the different environments, hence the conformational changes related to the protein secondary structure, and (ii) the root-mean-square distance (RMSD) maps [14–18] to determine the average distances of the same backbone atoms at different times within an MD trajectory at room temperature irrespective of its diffusional behavior. In particular, significant conformational changes in the secondary structure are shown by RMSD values of the order of 1 Å or more, whereas purely rigid-body diffusive (either translational or rotational) motion displays much smaller RMSD values, thus allowing to separate local from global motion. The RMSD map is constructed by calculating for a given set of n instantaneous conformations, or frames, the $n \times n$ root-mean-square distances among selected atoms (for instance the backbone atoms), and plotting them as a function of the frame indices as a bi-dimensional map with an appropriate color coding. The above-described RMSD between two frames is the minimum value of the function calculated with respect to the position of the centers of mass and the orientation of the principal axes of the molecule in the a and b frames

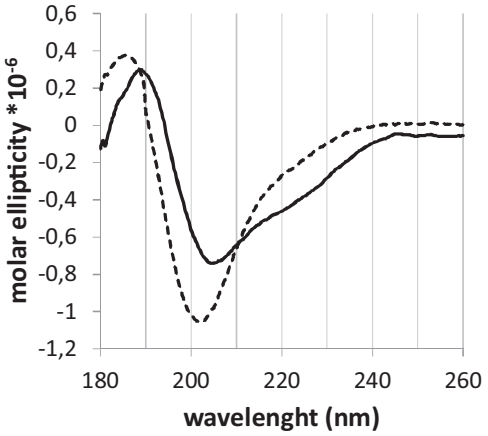

Fig. 1. Circular dichroism spectra of HFBII in water (dashed line) and in a 5% v/v emulsion of Galden® SV90 in water.

$$\left\{ \frac{1}{N} \sum_{i=1}^{N} \left(\boldsymbol{r}_i^a - \boldsymbol{r}_i^b \right)^2 \right\}^{1/2} \quad (1)$$

where the index i runs over the N selected atoms, the a, b superscripts indicate two different frames, and r is the vector position of the given atom, while the minimum value is chosen to remove trivial effects related to rigid-body translations or rotations of the whole system, thus getting rid of the above-mentioned diffusive motion. Therefore, similar conformations have a small RMSD, and unlike conformations a large RMSD.

Moreover, in order to describe the distribution of the solvent molecules around HFBII we used the Pair Distribution Function (PDF) [14–18], giving the probability density of finding a given set of atoms as a function of their distance r from another set within an MD run: for instance, in the case of solvation we considered the outer atoms of the solvent in the first set, and the protein atoms as the second set.

3. Results and discussion

CD spectroscopy was employed to experimentally investigate possible changes in the secondary structure of HFBII in fluorous/aqueous emulsions. To this end, we compared the CD spectrum of HFBII in water to that of a 5% v/v emulsion of the perfluoropolyether oil Galden® SV90 in aqueous HFBII solution (protein concentration: 0.1 mg/ml in both cases). Both spectra are reported in Fig. 1. It should be remarked here that the emulsions were stable for the entire timescale of the experiment, as confirmed by the absence of significant changes in the spectra obtained in subsequent measurement replicates on the same sample.

The negative band, originally centered around 202 nm in the HFBII spectrum in water, becomes clearly red-shifted to about 206 nm when analyzing fluorous oil-in-water emulsions. In addition, the onset of a broad negative shoulder is observed in the 225 nm area for the emulsified sample.

These changes are diagnostic of a small increase in the α-helical content within the protein structure, and suggest that the presence of the fluorinated solvent has some minor effect on it. Indeed, such observations appear to be consistent with those reported by Askolin et al. [23], who performed CD analysis on aqueous solutions of HFBII