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ScienceDirect
Physica B 385–386 (2006) 874–876
PHYSICA B
www.elsevier.com/locate/physb

Dynamics of an antibiotic oligopeptide

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Abstract

Neutron time-of-flight spectra were measured for an H_2O-hydrated and a nominally dry sample of a 15-residue antibacterial oligopeptide from 99 to 271 K. Proton mobilities, quasielastic broadenings, and changes in low-frequency inelastic intensities characterise the evolution of the peptide energy landscape as a function of momentum transfer and temperature.

PACS: 29.30.Hs; 87.14.Ee; 64.70.Pf

Keywords: Inelastic scattering; Peptide dynamics; Proton mobilities; Boson peak

1. Introduction

The evolution of the energy landscape of proteins and peptides from low to near-ambient temperatures is a subject of central interest in current work on biodynamics [1,2]. Neutron techniques are uniquely suited to contribute data on the proton dynamics, either in the form of window-integrated quasielastic intensities, $S_{qe}(Q;T)$, or as spectrally resolved dynamic structure factors, $S(Q,\omega;T)$ ($\hbar Q =$ momentum transfer, $\hbar\omega =$ energy transfer). Most experiments on the low-temperature dynamics of proteins have aimed to derive mean-square proton amplitudes, $\langle u_p^2 \rangle$, from effective or modified Debye–Waller (DW) factors extracted from $S_{qe}(Q;T)$ functions measured at intermediate to high Q between $\approx$30 and 300 K [2]. In recent work, the emphasis has been increasingly on (i) examining the spectral changes responsible for deviations from idealised behaviour and (ii) bridging the interpretational gap between $S_{qe}(Q;T)$ studies of small biomolecular building blocks and those of proteins. In parallel with quasielastic neutron scattering (QENS) experiments on di- and tripeptides of biomedical interest [3,4], we have begun to study time-of-flight spectra from a larger peptide. Here, we report initial results from an inelastic neutron scattering (INS) experiment on cecropin-mellitin, a biochemically and pharmacologically important 15-residue oligopeptide.

2. Materials and methods

This hybrid oligopeptide, called CM15 in the following, is a prominent member of a family of eukaryotic antimicrobial peptides which are being studied intensely by a variety of techniques to optimise potency and specificity, focusing in particular on their membrane interactions [5,6]. CM15 is a hybrid comprising seven amino acid residues from cecro/pin A and eight from mellitin: CA(1–7)M(2–9), with sequence H-KWKLFKK-IGAVLKVL-NH_2. A large sample (350 mg) of CM15 was synthesised at Porto, and H_2O-hydrated to a specific hydration h of 0.49 (g/g). The nominally dry sample contained residual, structurally essential water at a level $h = 0.068$, as determined by Karl Fischer titration. We used the spectrometer MIBEMOL at LLB (incident wavelength 5 Å, elastic Q-range 0.51–2.38 $Å^{-1}$) to record sets of neutron spectra over energy transfers $\hbar\omega$ from -2.3 to $\approx$200 meV with a resolution of 0.195 meV (FWHM) at $\hbar\omega = 0$. Standard programs were employed to correct the data and to normalise them with respect to monitor counts and a vanadium run.

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doi:10.1016/j.physb.2006.05.131

3. Results and discussion

Data analysed are from three temperature series—(1) a set of spectra for CM15 · H_2O from long runs (6–10 h each) at eight temperature points between 99 and 271 K, (2) a continuous 8-h scan for CM15 · H_2O from 196 to 269 K during which spectra were taken every 7 min, and (3) a set of 1–4 h runs for the 'dry' CM15 sample at temperatures corresponding to, or very near, those of (1). It was not possible in this experiment, for technical reasons, to record spectra below 99 K. The quality of the spectra from (1) is such that small inelastic intensity differences down to a few 10^{-4} of $S(Q,0)$ are analysable with confidence. By contrast, individual spectra from the continuous T-scan are statistically poor in the inelastic region proper, but their window-integrated quasielastic intensities are comparable in quality to data from scans using the backscattering spectrometer IN13 at ILL [2], providing $S_{qe}(Q;T)$ functions over a fine temperature grid in the region where the dynamics begins to be affected by softer modes and anharmonic interactions.

The quantity of immediate interest in experiments of this kind is $S_{qe}(Q;T)$. Since for hydrogenous biomolecules the scattering is incoherent to better than 95%, $S_{qe}(Q;T)$ is essentially proportional to a proton-weighted DW factor in regions where the dynamic behaviour is adequately represented by a system of harmonic oscillators. Up to at least 150 K, a m.-sq. displacement $\langle u_p^2 \rangle$ averaged over all protons can be derived from the slope of $\ln S_{qe}(Q;T)$ vs. Q^2 for $Q > 1\,\text{Å}^{-1}$, and this $\langle u_p^2 \rangle$ increases essentially linearly with temperature (except at very low T, not covered in our experiment). At higher temperatures, usually around and above 200 K, all hydrated biomolecules show significant nonlinear increases in $\langle u_p^2 \rangle$. These may be modelled analytically as products of effective DW factors and barrier-jump terms [3], as integrals over a distribution of $\langle u_p^2 \rangle$ values [7], or fitted by Plazcek-type expansions $\sim \exp(-\langle u_p^2 \rangle Q^2 + cQ^4 + \cdots)$.

A comprehensive analysis is not possible on the basis of the data we have collected so far, but it seems that in CM15 · H_2O, we are observing three rather than two mobility regimes (Fig. 1) : first one up to $\approx$150 K, then a 'ledge' region with a roughly linear dependence up to $\approx$235 K, and finally a steep nonlinear rise similar to that in some globular proteins. Quantitative analysis of the latter two regions in terms of the models mentioned requires data up to at least $5\,\text{Å}^{-1}$ to allow extraction of parameters additional to the Gaussian DW term which dominates for $T < 235$ K. Comparisons with high-resolution backscattering data for small proteins [1–3] show that overall the $\langle u_p^2 \rangle$ values for H_2O-hydrated CM15 are larger by factors between 1.1 and 1.3, due in part to the wider proton time scale range sampled in our experiment (quasielastic window $\pm$0.2 meV), and in part to the fact that relative to tightly folded globular proteins (such as crambin [3] or BPTI), our hybrid oligopeptide is likely to retain more hydration-induced sidechain mobility at low temperatures

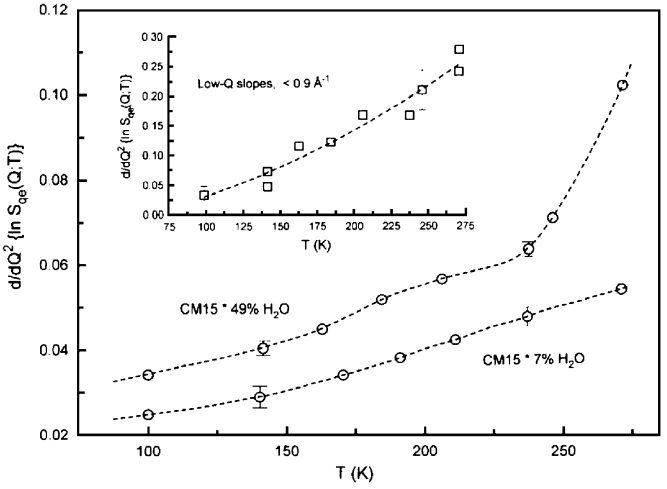

Fig. 1. Temperature dependence of $|d\ln S_{qe}(Q;T)\,dQ^2|$ for hydrated and 'dry' CM15, as determined from $1.5 < Q^2 < 4\,\text{Å}^{-2}$ (main graph) and for low Q from $0.3 < Q^2 < 0.85\,\text{Å}^{-2}$ (insert, hydrated CM15 only). In the harmonic and quasiharmonic regions (<230 K for hydrated CM15) where the Gaussian (i.e. DW) component dominates, the ordinate may be identified with $\langle u_p^2 \rangle/3$ and the resulting proton displacements $\langle u_p \rangle$ range from 0.27 to 0.43 Å.

(5 of the 15 amino acid residues are lysine, and there are 13 CH_3 groups). This qualitative interpretation appears to be corroborated by the fact that we observe $\langle u_p^2 \rangle$ differences between the hydrated and the 'dry' sample that are larger than in crambin.

The $d\{\ln S_{qe}(Q;T)\}/dQ^2$ slopes reported in Fig. 1 (main graph) were derived from linear regression fits in the interval $1.5 < Q^2 < 4\,\text{Å}^{-2}$. Between $\approx$0.9 and 1.1 Å^{-1}, the behaviour of $S_{qe}(Q;T)$ is affected by uncertainties in the edge-on slab correction, but below $0.9\,\text{Å}^{-1}$, the temperature dependence of $d\{\ln(Q;T)\}/dQ^2$ shows a systematic trend which differs significantly from the $Q^2 > 1.5\,\text{Å}^{-2}$ region, although it is less well defined statistically (Fig. 1, inset). The main feature here is the apparent absence of a 'knee' in the 220–240 K region, an observation suggesting substantially different proton dynamics over distances of $\approx$0.7–12 Å. Little work has been done as yet on the interpretation of biomolecular $S_{qe}(Q;T)$ data at low Q. This is a challenging preliminary result well worth following up in the future.

The levels of proton mobility inferred from $S_{qe}(Q;T)$ are reflected in the quasielastic broadening (Fig. 2). At the resolution of MIBEMOL for $\lambda_0 = 5$ Å, the scattering around $\hbar\omega = 0$ is effectively elastic until $\approx$ 230 K and gives rise to better defined quasielastic wings only towards 270 K. The small broadening at 242 K seems to saturate for $Q \gg 1\,\text{Å}^{-1}$ near a level of 4 μeV, which on the basis of commonly used jump diffusion models corresponds to a characteristic time of about 150 ps. It will be of interest in future QENS experiments with μeV resolution to detect and quantify any broadening between 170 and 235 K; this would aid greatly in the interpretation of the dynamics in the 'ledge' region of $\langle u_p^2 \rangle$.

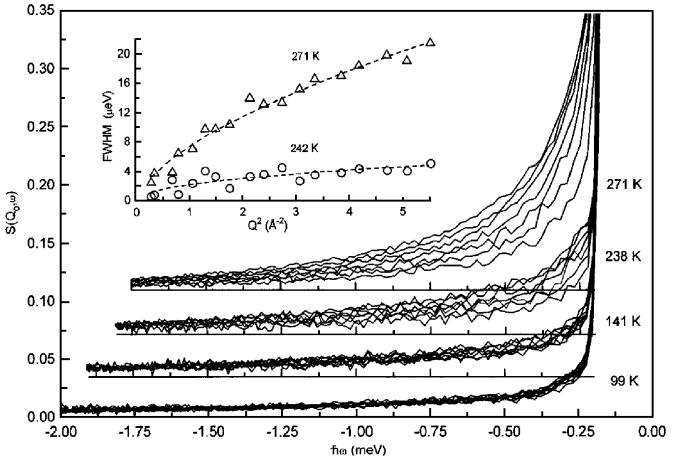

Fig. 2. Evolution of quasielastic intensities on the energy-loss side of spectra for H_2O-hydrated CM15. Every third spectrum from sets of 19 (binned down from 71 raw spectra, no smoothing) is shown; q.e. peak heights for the 238 and 271 K spectra are between 9 and 16. Inset: quasielastic broadenings (EISF model with single Lorentzian) for the two highest temperatures studied.

The temperature dependence of spectra in the 0.1–10 meV region relates to a number of basic questions about specific relaxation processes known from homo-polypeptides and the way in which they mix or couple in a more complex oligopeptide. A full discussion of the low-frequency inelastic region is beyond the scope of this paper, but in view of the much debated (but rather enigmatic) 'Boson peak' observed in a variety of biomolecular systems, we have fitted Debye density of states $\sim\omega^2$ to appropriately reduced spectra. We find that for $T \lesssim 200$ K, the low-frequency region is well modelled by Debye curves, with a conspicuous band of 5–15% 'excess' intensity extending from ≈ 2.2 to 4.5 meV. As T increases, the total 'excess' intensity decreases and the upper boundary shifts to slightly lower values (centroids shifting from 3.45 to 3.2 meV).

Acknowledgment

Experiments at LLB were supported by EU programmes NMI3 and FP6.

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