Nanosecond structural dynamics of intrinsically disordered β-casein micelles using neutron spectroscopy

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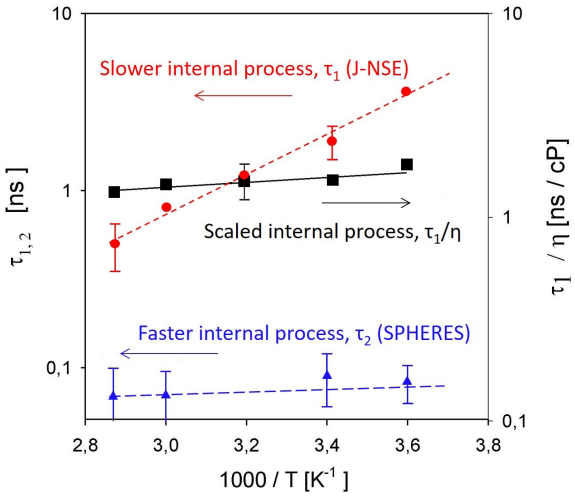
The structural flexibility of β-casein micelles was characterized by small-angle neutron scattering, spin echo spectroscopy and backscattering spectroscopy. Two relaxation processes on a nano-second and a sub-nano-second time scale were observed. The slower process involves density fluctuation of the solvent, and the faster one requires hydration water. The flexibility of a β-casein monomer is preserved in the micelle.

β-casein undergoes a reversible endothermic self-association with increasing temperature, forming micelles of limited size. In its functional state, a single β-casein monomer is unfolded, creating a high structural flexibility, which is supposed to play a major role in preventing the precipitation of calcium phosphate particles. The structural flexibility in terms of nano-second molecular motions was characterized, depending on the temperature, by a combination of small-angle neutron scattering, spin echo spectroscopy and backscattering spectroscopy using KWS-1, J-NSE and SPHERES.

Two relaxation processes can be distinguished

Two relaxation processes on a nano-second and a sub-nano-second time scale were observed for β-casein in solution. Both processes are analyzed via the Brownian Oscillator model, by which the spring constant can be defined in the isotropic parabolic potential. The slower process, which is analyzed by neutron spin echo, seems a characteristic feature of the unfolded structure, and dividing the relaxation time by the solvent viscosity removes most of the temperature dependence (see figure), indicating that the process involves density fluctuations of the solvent. It requires bulk solvent and is therefore not seen in hydrated protein powders. The faster process, which is analyzed by neutron backscattering, has a smaller amplitude and requires hydration water, which is also observed with folded proteins in the hydrated state. The self-association had no significant influence on internal relaxation, and thus a β-casein protein monomer flexibility is preserved in the micelle. In contrast, this type of internal structural dynamics is changed upon the folding and unfolding of a globular protein due to the change of the atomic packing. The dynamical change upon the folding or unfolding might also be related to the change of the hydration state since the protein folding is accompanied by dehydration of the polypeptide chain. The small dynamical change of the β-casein monomer upon micellization is compatible with the highly hydrated state inside the casein micelle..

[1] H. Nakagawa et al., Nanosecond structural dynamics of intrinsically disordered β-casein micelles by neutron spectroscopy, Biophys. J. 120, 5408 (2021)



Arrhenius plots: red circles: internal relaxation time $\tau_1(T)$ by neutron spin echo, dashed line: Fit to Arrhenius law, pre-exponential: τ_0 = 0.3 (±0.03) ps, activation enthalpy, H1 = 21.8 (±0.5) kJ/mol. Black squares: relaxation time divided by the solvent viscosity: τ_1/η. The full line is the Arrhenius fit, the activation enthalpy is now reduced to 4 (±1.5) kJ/mol, blue triangles: internal relaxation time $\tau_2(T)$ by neutron backscattering spectroscopy.