Poster Presentation

MS073.P01

Humidity induced phase transitions of HEWLysozyme investigated by powder diffraction

Detlef Beckers[1], Thomas Degen[1], Gwilherm Nenert[1], Stefanos Saslis[2], Souzana Logotheti[2], Fotini Karavassili[2], Alexandros Valmas[2], Irene Margiolaki[2], Sofia Trampari[3]

[1]PANalytical B.V., AIM Department, Almelo, Netherlands, [2]University of Patras, Patras, Greece, [3]Kapodistrian University of Athens, Athens, Greece

E-mail: detlef.beckers@panalytical.com

The simplicity of XRPD data collection and the uniqueness of the diffraction pattern that each polymorph shows, marks powder diffraction as the most suitable technique for quick and accurate characterization of microcrystalline suspensions. However, for protein samples, the intense synchrotron beam for ultrahigh-resolution XRPD data collection, typically causes radiation-damage effects. The damage rapidly appears during the measurement, affecting both angular (FWHM) and d-spacing resolution. As radiation damage can be a serious obstacle for collecting high quality data, measurements on a laboratory system have a lot to offer. Here, we present our analysis results of well known tetragonal and of a new monoclinic HEWL polymoph on a laboratory X-ray powder diffractometer including in situ measurements under variable relative humidity conditions for both polymorphs.

Proteins often crystallize in microcrystalline precipitates. The protein molecules are then surrounded by solvent and their packing arrangement is retained by limited intermolecular contacts. A change in the crystal environment first affects the bulk solvent that fills the intermolecular space, with resulting changes in the crystal structure. In literature it is reported that protein crystals in controlled humidity environments show a large change in unit-cell parameters when the humidity is decreased [1-2]. When a protein crystal is carefully dehydrated, it is in a metastable state in which the crystal initially still retains the original packing structure [2]. Further dehydration may cause the collapse of the crystal lattice: the crystal no longer maintains its packing structure because of the loss of a large amount of bulk solvent. However in some crystals, the dehydration induces a molecular arrangement change resulting in a new crystal structure. This has been already reported for hen egg-white (HEW) lysozyme [3]. While dehydration can induce structural changes, this is also likely to happen upon hydration of the same crystals.

The observed gradual structural changes during our experiments as well as phase transitions upon dehydration and hydration of HEWL are analyzed in the relative humidity range 50% - 95%. Dehydration and hydration processes are reversible in humidity cycles in the range of 95% rH to 75% rH. Without stabilizing PEG the lower limit for dehydration of tetragonal HEWL is around 75% rH. With PEG the tetragonal HEWL samples remain crystalline below 75% rH, but show phase transitions and larger variations of the cell parameters. Below 75% rH another new tetragonal polymorph was discovered.

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Keywords: microcrystalline, humidity induced phase transitions, XRPD