on the conformation and the stability of γ- and α-crystallins. α-, β- and γ-crystallins are the main components of mammalian eye lenses and their structural and associative properties are responsible for lens transparency. γ are monomers (21 kDa, up to 80% sequence identity), whereas α are large hetero-oligomers of about 800kDa. The C-terminal domain of α belongs to the ubiquitous superfamily of sHSPs (small heat shock proteins): upon stress, they are able to incorporate the non-native proteins to prevent their aggregation.

High-pressure experiments performed with α-crystallins have shown a partially reversible change in size from 2 to 3kb at room temperature, and this effect was enhanced by the combination of temperature and pressure. In the case of γ-crystallins, pressure and temperature needed to be combined with pH, and the results depend upon the different γ itself. Crystallins are known to be exceptionally stable *in vivo* since they are synthesised to last for life. They therefore represent an extreme case of stability versus unfolding and these results have shown that these proteins (mainly beta strands) are also stable upon pressure.

Keywords: high-pressure SAXS, crystallins, conformation changes

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When Macromolecular Crystallography Meets high Pressure Techniques…

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Until recently, only two crystal structures of small proteins at high pressure below 200 MPa generated in a Be cell were published [1,2]. The lack of structural data at high pressure was due mainly to the cumulated complexities of high-pressure containment and crystallography. A technical breakthrough was achieved with a set-up at the ESRF ID30/ID27 beamline combining a diamond anvil cell, ultra-short wavelength (0.33 Å) X-rays from undulators and a large imaging plate [3]. The accessible pressure range was increased by nearly one order of magnitude. The quality of diffraction data collected under high pressure achieved usual standards.

We will present the technical advances as well as scientific results that we have obtained. In particular, scientific results will focus on the first crystal structure of a complex macromolecular assembly under high pressure, the Cowpea Mosaic Virus capsid at 330 MPa [4], demonstrating that high pressure macromolecular crystallography can now be considered as a mature and general technique.

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Keywords: high-pressure, X-ray crystallography, macromolecules

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High Pressure Cooling of Protein Crystals without Cryoprotectants

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The flash cooling of protein crystals is the best known method to effectively mitigate radiation damage in macromolecular crystallography. To prevent physical damage to crystals upon cooling, suitable cryoprotectants must usually be found, a process that is time-consuming and, in certain cases unsuccessful. Recently we have developed a novel method to cryocool protein crystals without the need for penetrative cryoprotectants. In the new method, each protein crystal is pressurized up to 200 MPa (2000 atm) in He gas at 10 ˚C. The crystal is then cyrocooled under pressure and the pressure was released while the crystal is kept cooled. Results are presented for two proteins that have been flash-cooled at ambient pressure and pressure-cooled, in all case without penetrating cryoprotectants. For glucose isomerase, the flash-cooled crystal diffracted to only 5.0 Å and mosaicity could not be estimated but the pressure-cooled one diffracted to 1.05 Å with 0.39˚ mosaicity. For thaumatin, the flash-cooled crystal diffracted to only 1.8 Å with 1.29˚ mosaicity but the pressure-cooled one diffracted to 1.15 Å with 0.11˚ mosaicity. The protein structures show that the structural perturbation by pressure is very small. A mechanism on the pressure cooling is proposed involving the dynamics of water at high pressure and high density amorphous (HDA) ice.

Keywords: high pressure cooling, cryocrystallography, crystallography of biological macromolecules

MS34 Advances in Computational Methods for Electron Density Studies

Chairpersons: Louis Farrugia, Tibor Koritsanszky

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Ab initio Quantum-mechanical Calculation of Electron Charge-density in Crystals

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The *ab initio* quantum-mechanical CRYSTAL code [1] is one of the tools available for the calculation of the electronic structure and properties of crystals. It is based on a description of the wavefunction in terms of linear combinations of atomic orbitals (LCAO), which permits an easy interpretation of the electronic structure and a direct comparison with molecular fragments.

A large variety of properties of matter in the condensed phase can be calculated with the present release of the code, CRYSTAL03, even for systems of considerable size: a calculation of the electronic structure of the crambin protein ($P2_1$, 92 amino acid residues per cell) has been attained recently [2].

Molecular crystals are an important area of application of CRYSTAL. The use of a basis set of atomic orbitals is convenient for the calculation of the lattice energy and the characterization of hydrogen bonds, where the modifications in the electron charge density of the molecules due to the formation of the crystal can be investigated, along with their effect on the structure factors [3].

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Keywords: charge density, ab initio calculations, molecular crystals.

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Beyond $\nabla^2\rho_b$: Chemical Bond Analysis using the Local Form of the Source Function

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The sign of the Laplacian of the density at the bond critical point, $\nabla^2\rho_b$, has been largely used for discriminating the closed-shell-like ($\nabla^2\rho_b>0$) from the shared-shell-like ($\nabla^2\rho_b<0$) interactions. This dichotomous bond classification has the merit of being simple, but it has also proved to be often inadequate. This is the case of bonding between heavy atoms missing the outermost regions of charge depletion and concentration in their atomic Laplacian distributions and/or the case of interactions having very low $|\nabla^2\rho_b|$, a fact which makes the sign of $\nabla^2\rho_b$ quite indeterminate and the use of $\nabla^2\rho_b$, as the only classification index, deceiving. Other quantities, based on the first and/or the second order density matrices, have in these cases been