Journal of Biomolecular NMR **30:** 385–386, 2004.

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignment of human γ*S*-crystallin, a 21 kDa eye-lens protein

Carine Baraguey[a], Fériel Skouri-Panet[b], François Bontems[c], Annette Tardieu[b], Gérard Chassaing[a] & Olivier Lequin[a,*]

[a]*UMR 7613 CNRS, Université Pierre et Marie Curie, case 45, 4 place Jussieu, F-75252 Paris Cedex 05, France;* [b]*(LMCP), UMR 7590 CNRS, Université Pierre et Marie Curie, case 115, 4 place Jussieu, F-75252 Paris Cedex 05, France;* [c]*ICSM-RMM, F-g1128 Palaiseau Cedex, France*

Received 1 July 2004; Accepted 2 September 2004

Key words: cataract, γ*S*-crystallin, NMR assignment

Biological context

Cataract is an opacification of the eye-lens, which is often due to age-related alterations of its bulk structural proteins called crystallins (Bloemendal et al., 2004). These proteins play a central role in maintaining the optical properties of the lens and are characterized by an exceptional stability since they need to last a life time. They fall into two major families, α-crystallins, which belong to the small heat-shock protein superfamily, and β/γ-crystallins, consisting of two β-sandwich domains, each containing two Greek key motifs.

γS-crystallin is a major component of the adult mammalian lens. It is more distantly related compared to its γA-γF counterparts and also differs by its spatio-temporal expression within the lens. It lacks the temperature-induced opacification typically observed with other γ-crystallins and known as cold cataract. γ*S*-crystallin is especially sensitive to oxidation, which is thought to contribute to cataract (Skouri-Panet et al., 2001). Furthermore, several mutations of the mouse γ*S*-crystallin gene have been found to be responsible for cataract (Sinha et al., 2001; Bu et al., 2002).

Although the X-ray structures of several β-and γ-crystallins are known, full length γ*S*-crystallin has been resistant to crystallisation so far. Only the crystal structures of the C-terminal domain of human and bovine γ*S*-crystallins have been determined (Basak et al., 1998; Purkiss et al., 2002).

*To whom correspondence should be addressed. E-mail: lequin@ccr.jussieu.fr

We have initiated an NMR study of human γ*S*-crystallin to investigate the structure and the interactions between the two domains in the context of the full length protein in solution. Since residue Cys24 was shown to be involved in dimer formation under mild oxidative conditions (Skouri-Panet et al., 2001), the NMR study was carried out on a C24S mutant.

Methods and experiments

Recombinant human γ*S*-crystallin (C24S mutant) was produced in *E. coli* strain BL21(DE3) pLysS from a pET3a expression vector. Cells were grown at 37 °C in 4 l of LB medium. When an OD_{600} of 0.8 was reached, cells were harvested and resuspended in 1 l of M9 minimal medium, supplemented with $^{15}NH_4Cl$ and ^{13}C-glucose, as required for the ^{15}N and $^{15}N/^{13}C$ labellings. Protein expression was induced by addition of 3 mM isopropyl-β-D-thiogalactoside (IPTG). After overnight incubation, cells were harvested by centrifugation, resuspended in a lysis buffer (50 mM Tris–HCl, pH 7, 100 mM glucose, 10 mM EDTA, 1.5 mM PMSF) and sonicated. The recombinant protein was purified from the clear supernatant fraction by gel filtration, using an FPLC system, a Superdex S-200PG column, and a 40 mM phosphate buffer, pH 6.8, containing 70 mM KCl, 1 mM EDTA and 1 mM DTT, for the elution. About 30–40 mg of protein were obtained from a 4 l LB culture. NMR samples (U–^{15}N and U-$^{15}N/^{13}C$) contained ~1.5 mM protein in 13.3 mM sodium phosphate buffer, pH 6.0, containing 30 mM KCl,

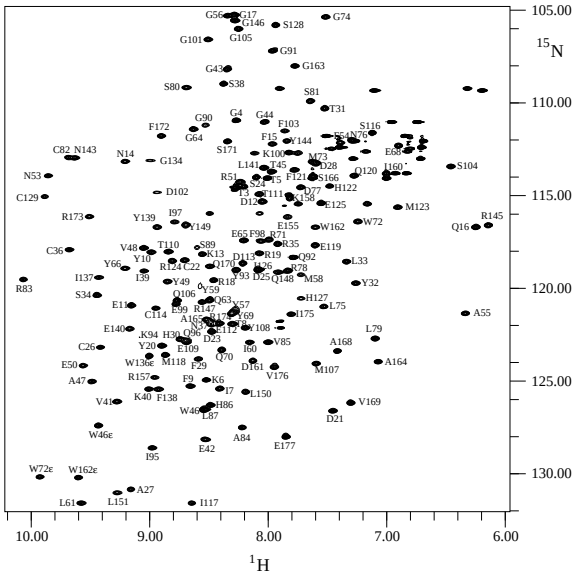

Figure 1. 2D ^{15}N, ^{1}H HSQC spectrum of ^{15}N-labelled human γ*S*-crystallin recorded at 500 MHz and 37 °C. The assignment of backbone and Trp side chain HN resonances is indicated.

0.3 mM EDTA, 0.3 mM DTT, 0.1 mM DSS and 0.02% (w/v) NaN_3, in H_2O/D_2O (95/5, v/v). An ^{15}N/^{13}C γ*S*-crystallin sample was also freeze-dried and resuspended in 100% D_2O.

NMR spectra were acquired at 37 °C on a Bruker Avance 500 MHz spectrometer equipped with a 5-mm z-gradient triple resonance (^{1}H/^{15}N/^{13}C) probehead. Data were processed with XWINNMR and analyzed with XEASY. DSS was used for direct ^{1}H and indirect ^{15}N and ^{13}C chemical shift referencing.

Backbone resonance assignments were obtained from a set of 3D TOCSY-HSQC and NOESY-HSQC recorded on the ^{15}N-labelled sample, and 3D HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH, HBHA(CO)NH recorded on the ^{15}N/^{13}C doubly labelled sample, using water flip-back pulses where appropriate. The 2D ^{15}N,^{1}H-HSQC spectrum of γ*S*-crystallin is shown in Figure 1. Side chain aliphatic ^{1}H and ^{13}C resonances were assigned from a 3D HCCH-TOCSY spectrum recorded in D_2O. Aromatic side chain resonances were mainly assigned using a 3D ^{13}C NOESY-HMQC experiment optimized for the detection of aromatic signals, 2D CT-HMQC-TOCSY (Zerbe et al., 1996) and 2D (HB)CB(CGCD)HD experiments (Yamazaki et al., 1993). The ^{15}N resonances of His side chains were assigned from a 2D ^{15}N,^{1}H-HSQC experiment optimized for the detection of heteronuclear ^{2}J and ^{3}J couplings. Side chain carbonyl groups were assigned from a 2D H_2(C)CO spectrum. The ^{13}CO–$^{15}NH_2$ moieties of Asn and Gln residues were also assigned from NHD scalar connectivities in HNCO, HN(CO)CA and CBCA(CO)NH spectra. The $^{13}C^{\zeta}$–$^{15}NH^{\varepsilon}$ groups of Arg residues were assigned from HNCO, HNCACB and ^{15}N-edited TOCSY experiments.

Extent of assignments and data deposition

Backbone HN groups were assigned for 159 out of the 169 non-proline residues, excluding S1 and K2 in the N-terminus, and residues V131-E133, W136, D152-K154 and Y156, located in the C-terminal domain. A few ^{15}N resonances were not assigned (^{15}N of Pro, $^{15}N^{\zeta}$ of Lys, $^{15}N^{\eta}$ of Arg and 3 $^{15}N^{\varepsilon}$ of Arg). The majority of aliphatic resonances were assigned (>96%). The assignment of aromatic resonances encompasses all His and Trp residues, 10 Tyr out of 14, and six Phe out of nine, some resonances could not be assigned owing to strong overlap. Assignment of ^{13}CO resonances is nearly complete, except for six backbone and seven side chain resonances. The ^{1}H, ^{15}N and ^{13}C chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6253.

Acknowledgements

This work was supported by a BIOMED European contract, 'Ageing vision'.

References

Basak, A.K., Kroone, R.C., Lubsen, N.H., Naylor, C.E., Jaenicke, R. and Slingsby, C. (1998) *Protein Eng.*, **11**, 337–344.

Bloemendal, H., de Jong, W., Jaenicke, R., Lubsen, N.H., Slingsby, C. and Tardieu, A. (2004) *Prog. Biophys. Mol. Biol.*, **86**, 407–485.

Bu, L., Yan, S., Jin, M., Jin, Y., Yu, C., Xiao, S., Xie, Q., Hu, L., Xie, Y., Solitang, Y., Liu, J., Zhao, G. and Kong, X. (2002) *Genomics*, **80**, 38–44.

Purkiss, A.G., Bateman, O.A., Goodfellow, J.M., Lubsen, N.H. and Slingsby, C. (2002) *J. Biol. Chem.*, **277**, 4199–4205.

Sinha, D., Wyatt, M.K., Sarra, R., Jaworski, C., Slingsby, C., Thaung, C., Pannell, L., Robison, W.G., Favor, J., Lyon, M. and Wistow, G. (2001) *J. Biol. Chem.*, **276**, 9308–9315.

Skouri-Panet, F., Bonneté, F., Prat, K., Bateman, O.A., Lubsen, N.H. and Tardieu, A. (2001) *Biophys. Chem.*, **89**, 65–76.

Yamazaki, T., Forman-Kay, J.D. and Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 11054–11055.

Zerbe, O., Szyperski, T., Ottiger, M. and Wüthrich, K. (1996) *J. Biomol. NMR*, **7**, 99–106.