Journal of Biomolecular NMR, **26:** 281–282, 2003.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C backbone assignment of the Green Fluorescent Protein (GFP)

Farid Khan[a], Katherine Stott[b] & Sophie Jackson[a*]
[a]*University of Cambridge, Centre for Protein Engineering, Department of Chemistry, Lensfield Road, Cambridge, CB2 1EW, U.K.;* [b]*Cambridge Centre for Molecular Recognition, Department of Biochemistry, University of Cambridge, 80 Tennis Court Rd, Old Addenbrookes Site, Cambridge, CB2 1GA, U.K.*

Received 29 January 2003; Accepted 6 March 2003

Key words: assignment, chromophore, GFP, NMR

Biological context

The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* has revolutionised many areas of molecular and cell biology research (Tsien, 1998). GFP has been used extensively as a marker for the expression, localisation and interactions of target proteins to which it is fused (Zimmer, 2002; Zhang et al., 2002). The usefulness of GFP is due to the post-translational formation of a unique chromophore that is responsible for its strongly visible green fluorescence, which requires no cofactors.

GFP consists of 238 residues, folded into an 11-stranded β-barrel which is wrapped around a central α-helix to form a β-can structure (Yang et al., 1996; Ormoe et al., 1996). The buried chromophore is in the centre of the α-helix and it is formed by an auto-catalytic cyclisation and subsequent oxidation of backbone residues Ser65, Tyr66 and Gly67. Although at least 22 crystal structures have been deposited in the protein database, there has been no NMR assignment data reported to date. Evidence from crystallographic studies shows that the chromophore is in a deeply buried and tightly packed region within the protein core and is, therefore, excluded from solvent. This gives rise to the high fluorescence efficiency (Helms et al., 1999). The chromophore has been shown to undergo a *cis-trans* photoisomerisation (Chen et al., 2001). GFP has been extensively modified through mutations in the chromophore or surrounding β-sheets to produce desirable properties such as maximum emission wavelength, improved quantum yield, rapid chromophore maturation, greater structural stability and also to respond to environmental factors such as pH and ion concentration (Zimmer, 2002). GFP has also been shown to undergo photodynamic reactions such as the photo-induced decarboxylation (van Thor et al., 2002). However, the mechanism of chromophore formation and its link to protein folding and dynamics, and the pronounced dependence of its properties on the chemical environment are still not fully understood. The backbone assignment of GFP is a key step towards a better understanding of these processes. Here we report the backbone ^{1}H, ^{15}N and ^{13}C assignment of a truncated (Met1-Ile229) form of GFPuv which has three mutations (F99S, M153T, V163A) that enhance solubility and fluorescence (Battistutta et al., 2000; Li et al., 1997).

*To whom correspondence should be addressed. E-mail: sej13@cam.ac.uk

Methods and experiments

The gene encoding GFPuv (Clontech) was cloned into a modified pRSET vector (Invitrogen) engineered without a hexahistidine tag. A stop codon was introduced at residue 230 using PCR to produce a truncated form of GFPuv. ^{1}H, ^{15}N (>95%), ^{13}C (>95%) and uniformly ^{2}H (∼75%), ^{15}N (>95%), ^{13}C (>95%) labelled protein was expressed in the *E. coli* strain BL21 (DE3) and purified by a combination of cation and gel filtration chromatography. Selective ^{15}N labelling was achieved by addition of 50 mg l^{-1} of either ^{15}N-Lys, ^{15}N-Tyr or ^{15}N-Phe to minimal media with no other isotopic enrichment, cells were harvested after 3 h of growth at 25 °C.

NMR measurements were made on samples containing 1 mM protein, 90% H_2O/10% D_2O in phos-

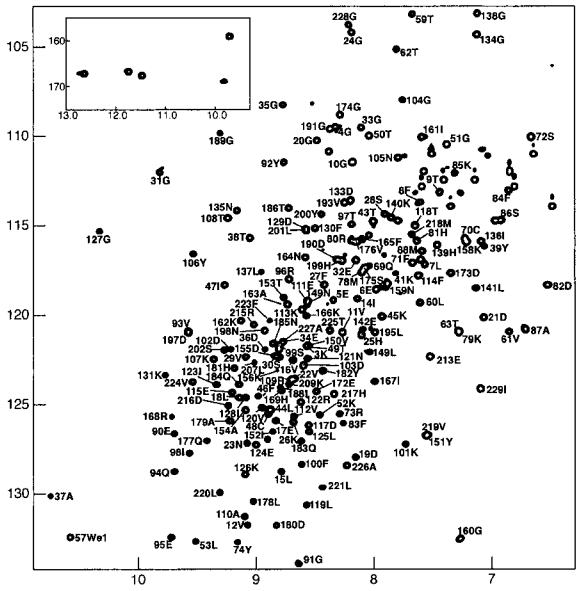

Figure 1. 800 MHz 2D ^{1}H-^{15}N HSQC spectrum of truncated GFPuv at 310 K. The insert shows five resonances from histidine sidechains.

phate buffered saline (PBS) consisting of 137 mM NaCl, 2.7 mM KCl, 6 mM phosphate at pH 7.2. Truncated GFPuv tends to aggregate at concentrations higher than 1 mM. The peak linewidths decreased at 310 K relative to 298 K. All experiments for assignment were recorded on a Bruker DRX 500 spectrometer at 310 K. Both $^{1}H/^{13}C/^{15}N$ and ^{2}H (75%)/$^{13}C/^{15}N$ labelled samples were used for triple resonance NMR experiments. Assignments were derived from both standard and CT-versions of HN(CO)CA, HNCA, HNCO, HN(CO)CACB and HNCACB experiments, and the assignments were both assisted and confirmed with reference to 2D ^{1}H-^{15}N HSQC spectra collected on protein selectively labelled with ^{15}N-Lys, ^{15}N-Tyr or ^{15}N-Phe. ^{1}H, ^{15}N and ^{13}C chemical shifts were referenced relative to the frequency of the ^{2}H lock resonance of water.

Extent of assignment and data deposition

The assignments comprise 90% of all $^{1}H^{N}$, ^{15}N, $^{13}C'$ and $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances, not including the ^{15}N of proline or the resonances of residues 66 and 67 which lack the $^{1}H^{N}$ after post-translational modification to produce the chromophore. The backbone secondary chemical shifts are broadly consistent with the secondary structure of the crystalline protein. Five $^{1}H^{N}/^{15}N$ resonances from histidine sidechains were also observed, four of which could correspond to His 148, His 169, His 181 and His 199 which are buried within the beta-barrel in the crystal structure. Almost all of the remaining resonances were broadened beyond detection in the three-dimensional experiments. Among these missing peaks are residues 144–148 and 203–208 which lie in adjacent beta-strands close to the chromophore, and show distortions from an ideal beta-barrel in the crystal structure. Figure 1 shows the 2D ^{1}H-^{15}N HSQC spectrum. Chemical shifts have been deposited in the BMR data bank under accession number 5666.

Acknowledgements

This work was supported by the Medical Research Council (FK), The Royal Society and The Welton Foundation. The Cambridge Centre for Molecular Recognition is supported by the BBSRC. We would like to thank Stefan Freund (Centre for Protein Engineering, Cambridge) for running preliminary experiments.

References

Battistutta, R., Negro, A. and Zanotti, G. (2000) *Proteins*, **41**, 429–437.

Chen, M.C., Lambert, C.R., Urgitis, J.D. and Zimmer, M. (2001) *Chem. Phys.*, **270**, 157–164.

Helms, V., Straatsma, T.P. and McCammon, J.A. (1999) *J. Phys. Chem. B.*, **103**, 3263–3269.

Li, X., Zhang, G., Ngo, N., Zhao, X., Kain, S.R. and Huang, C. (1997) *J. Biol. Chem.*, **272**, 28545–28549.

Ormoe, M., Cubitt, A.B., Kallio, K., Gross, L.A., Tsien, R.Y. and Remington, S.J. (1996) *Science*, **273**, 1392–1395.

Tsien, R. (1998) *Annu. Rev. Biochem.*, **67**, 510–544.

Van Thor, J.J., Gensch, T., Hellingwerf, K.J. and Johnson, L.N. (2002) *Nat. Struct. Biol.*, **9**, 37–41.

Yang, F., Moss, L. and Phillips, G. (1996) *Nat. Biotechnol.,* **14**, 1246–1251.

Zhang, J., Campbell, R.E., Ting, A.Y. and Tsien R.Y. (2002) *Nat. Rev. Mol. Cell Biol.*, **3**, 906–918.

Zimmer, M. (2002) *Chem. Rev.*, **102**, 759–782.