The X-ray crystal structures of perdeuteriated and protiated enzyme elongation factor Tu are very similar

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X-Ray structural comparisons show that perdeuteriation does not affect the overall fold or domain arrangement of elongation factor Tu.

Perdeuteriation involves replacing all the non-exchangeable hydrogens in a molecule with deuterium. For proteins this can be achieved by growing a bacterial expression system on a fully deuteriated medium, which is a technique used for protein NMR[1] and has potential for protein neutron scattering studies.[2,3] Whilst the effects of D_2O on proteins are well documented and indeed is a method used in the study of enzyme mechanisms,[4] there is a paucity of structural information on the impact of perdeuteriation. Most of the published information on perdeuteriation derives from the elegant work of the groups of Crespi and Berns in the 1960s.[5,6] Their experiments, as well as more recent data, indicate that perdeuteriation has a destabilising effect on proteins. Conversely biological properties such as ligand binding do not appear to be drastically changed.[7–9] This leads to the possibility of preparing perdeuteriated proteins with similar activities but with modified physical properties relative to the parent protiated protein. As part of a larger study on the effects of perdeuteriation on proteins we compare the X-ray crystal structure of perdeuteriated with that of native *i.e.* protiated, elongation factor Tu (EFTu) from *Escherichia coli*.

Both forms of the enzyme were prepared using an *E. coli* over-expression system, with the perdeuteriated form utilising a deuterium adapted *E. coli* strain, MRE600D.[10] Once cells were grown all further processes were carried out in an aqueous (H_2O) environment, so only the deuterons unable to exchange with the solvent remained attached to the protein. The native and perdeuteriated enzymes were purified identically. Analysis by electrospray mass spectrometry showed that the protein was 95% deuteriated on carbon, and that 78 of the 668 non carbon bound deuterons did not exchange with protium during isolation (*ca.* 24 h).

Crystals of both native and perdeuteriated EFTu were obtained by utilising conditions similar to those of Kjeldgaard and Nyborg.[11]‡ Both forms of the enzyme were subjected to limited cleavage with 1% trypsin for 1 h on ice. In this period a 14 peptide fragment, between Ala45 and Arg58, is excised and no further proteolysis occurs. Cleavage does not affect enzyme activity, as assessed by GDP binding assays. Crystals grew within a few days, to a typical size of 0.3 × 0.3 × 0.5 mm but deteriorated after approximately one week, as determined by visual examination. Within each droplet the crystals displayed a range of (roughly) bipyramidal habits. Protiated crystals were found to diffract to *ca.* 3.5 Å using Cu-Kα radiation from a rotating anode with an R-AXIS IIc area detector. The diffraction resolution was extended to 2.5 Å at station PX7.2 SRS Daresbury.§ Many of the crystals were found to diffract poorly or were twinned. A 4.0 Å data set of a perdeuteriated EFTu crystal was measured on the R-AXIS IIc area detector using rotating anode Cu-Kα radiation.¶ A second perdeuteriated crystal was measured at station PX7.2 SRS Daresbury and although it diffracted to 2.8 Å was subsequently found to be adversely affected by twinning. All the crystals were of space group $P4_32_12$ with small but significant variation in the unit cell dimensions. We are not certain whether this variation is the result of perdeuteriation, but it is most probably simply due to the variety of crystal morphologies, as also observed previously.[11]

We have solved both the 2.5 Å protiated and 4.0 Å perdeuteriated structures by molecular replacement using GDP-EFTu coordinates based on a 2.6 Å analysis, kindly given to us by Professor M. Kjeldgaard of Aarhus University.[11]‖ The native and perdeuteriated models have been refined to R factors of 0.230 and 0.217 respectively.** The lower resolution of the perdeuteriated structure deems it unrealistic to draw any very detailed comparisons between the two structures, *i.e.* particularly with reference to differences in bonding distances. However it does readily allow a comparison between the overall domain to domain arrangement, as well as secondary structure. EFTu is an ideal candidate to examine the importance of perdeuteriation on proteins as it is a large (393 amino acid) multiple domain protein, the only other perdeuteriated X-ray structure available to date being a small (149 amino acids) single domain protein.[3] EFTu comprises three domains, and therefore has a large potential for conformational flexibility. The two refined X-ray models were aligned with respect to each other and also to the original Aarhus model,[11] using the program LSQMAN.[12] The rms distance deviations between protein C_α atoms of these alignments are, in ascending order of disagreement: 0.56 Å Aarhus protiated to our protiated; 0.79 Å Aarhus protiated to perdeuteriated and 0.83 Å perdeuteriated to our protiated. The protiated to protiated value of 0.56 Å represents the 'control' rms against which the other two should be compared. The rms overlap value of 0.56 Å between protiated and protiated EFTu models corresponds well with the Luzzati (0.55 Å) and Cruickshank (0.29 Å) estimates for the errors in our protiated model.†† Thus subtle structural differences between perdeuteriated and protiated EFTu cannot be ruled out yet and clearly requires confirmation.

To examine possible structural differences in more detail we also aligned each domain individually, *i.e.* our protiated EFTu domains 1, 2 and 3 with perdeuteriated EFTu domains 1, 2 and 3 in turn, and thereby accentuate any domain shifts. The rms distance deviations (for only the C_α atoms within the relevant domains) are; domain 1, 0.76 Å; domain 2, 0.99 Å and domain 3, 0.80 Å. The rms distance deviations for all C_α atoms in the above alignments are; 0.96 Å based on domain 1; 1.15 Å based on domain 2 and 0.99 Å based on domain 3. The greatest deviation is within the loop or turn regions, as can be seen in Fig. 1. However, considering typical temperature factors of $\geqslant$40 for these regions, a coordinate displacement of *ca.* 0.7 Å^2 would be expected in any case. Overall it appears that there are no major domain shifts (*i.e.* none > 1.5 Å) and both structures have the same fold and global arrangement of domains. Thus the X-ray crystal structure analyses clearly show that perdeuteriation has no effect on the tertiary structure of EFTu. This is a significant finding for structural techniques that utilise perdeuteriated proteins such as NMR and neutron diffraction. It appears that the observed physiochemical differences[7] between protiated and perdeuteriated EFTu are not of an overall structural nature. X-Ray studies are underway on a number of

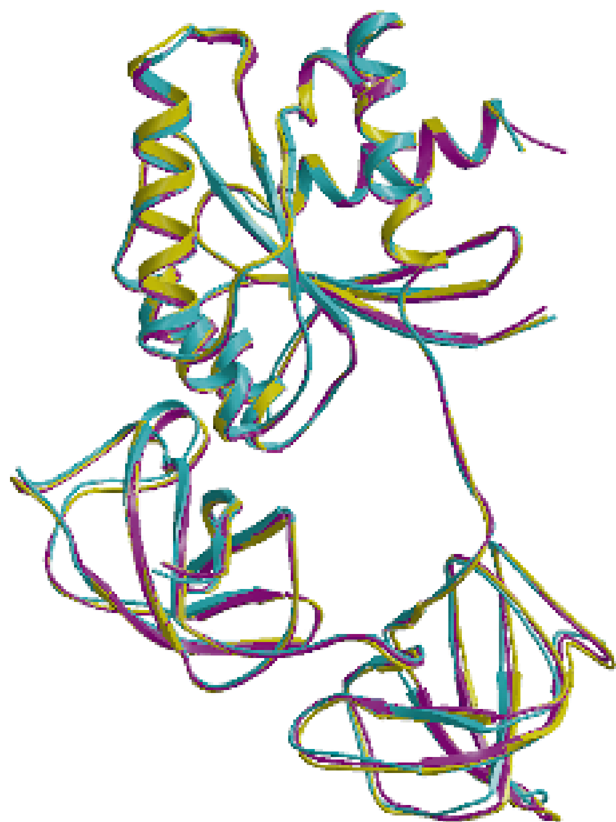

Fig. 1 Overlay of perdeuteriated and protiated EFTus indicating the similarity of the domain domain arrangements. Key: yellow, our protiated structure; magenta, Aarhus protiated structure, reproduced with permission;[11] cyan, perdeuteriated structure. Figure created using MOLSCRIPT.[13]

other proteins and will allow further analysis of the potential structural changes due to perdeuteriation to compare with the EFTu results presented here.

We thank The Wellcome Trust for a Sir Henry Wellcome Commemorative Award for Innovative Research and Ms Lu Yu and Professor Simon Gaskell (UMIST) for collaboration with the electrospray mass spectrometry work. We thank Professor M. Kjeldgaard of Aarhus University for the 2.6 Å GDP-EFTu coordinates. We thank Professor D. W. J. Cruickshank for discussion on coordinate precision based on his Data Precision Indicator (DPI). J. R. H. thanks the BBSRC who funded molecular graphics and computer workstations and partial (33%) salary support for J. R. and The Wellcome Trust who funded the R-AXIS and SERC who funded the rotating anode generator.

Notes and References

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‡ Crystals were grown by vapour diffusion in 20 μl sitting drops, determined by screening as: 10 mM $MgCl_2$, 50 mM Tris-HCl at pH7, 0.5 mM dithiothreitol (DTT), 1 mM NaN_3, 10 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM guanosine diphosphate (GDP) and either 4.7 mg ml^{-1} protiated EFTu with 3.5–4.25% PEG (6000 Da) or 1.9 mg ml^{-1} perdeuteriated EFTu with 3–4.5% PEG (6000 Da). All drops were equilibrated with a reservoir of 200 μl 10% (6000 Da).

§ 2.5 Å data were collected at station PX7.2 Daresbury from a single protiated crystal. A total of 27° of data yielded an R_{merge} of 0.078 for 14 002 reflections with a completeness of 89%, whereby 63% of the reflections have $I \geqslant 3\sigma(I)$. Unit cell dimensions are $a = b = 70.8$ $c = 163.4$ Å.

¶ 60° of 4.0 Å resolution data from a single perdeuteriated EFTu crystal were collected on the Manchester R-AXIS IIc, which yielded an R_{merge} of 0.22 for 5230 reflections, 60% of which have $I \geqslant 3\sigma(I)$, for the strong reflections the merging R is 0.05 and the overall R_{merge} only increases above 0.25 for data higher than 4.2 Å resolution. The data are 97% complete. The unit cell dimensions are $a = b = 69.8$ $c = 160.5$ Å.

‖ Molecular replacements were carried out using AMORE.[14] Both using the 2.6 Å resolution Aarhus coordinates with our X-ray data to 4 Å. The R factors at this stage for the protiated and perdeuteriated are 0.345 and 0.348 respectively.

** Subsequent to molecular replacement both structures were subjected to further rigid body refinement and simulated annealing followed by positional and B-factor refinement using XPLOR.[15] After inspection on the graphics and insertion of the Mg^{2++} and GDP co-factors into the protiated model it was put through further rounds of positional and B-factor refinement using the maximum likelihood method in REFMAC.[14] A round of refinement is defined as positional and B-factor refinement followed by model inspection/rebuilding. The perdeuteriated model was also further refined using REFMAC.[14] A bulk solvent correction was applied. The GDP and Mg^{2++} co-factors were added in the second round of refinement. R_{free}[15] was used as a guide for both protiated and perdeuteriated refinements, final values are 0.341 and 0.335, respectively.

†† The precision of protein model coordinates can be estimated using the Luzzati approach[16–18] and more recently that of Cruickshank,[19] who has considered the various protein cases in detail, including a medium resolution refined model based on initial better resolution coordinates. The Luzzati coordinate error estimates for the protiated and perdeuteriated EFTu models are 0.55 Å (5–2.45 Å) and 0.51 Å (8–4 Å) respectively. Likewise the Cruickshank estimates are 0.29 and 0.72 Å, respectively, with due account taken of the use of restraints in these refinements.

1 L. E. Kay and K. H. Gardner, *Curr. Opin. Struct. Biol.*, 1997, **7**, 722.
2 A. Wlodawer, *Prog. Biophys. Mol. Biol.*, 1982, **40**, 1.
3 T. R. Gamble, K. R. Clauser and A. A. Kossiakoff, *Biophys. Chem.*, 1994, **53**, 15.
4 K. B. Schowen and R. L. Schowen, *Methods Enzymol.*, 1982, **87**, 551.
5 D. S. Berns, *Biochemistry*, 1963, **6**, 1377.
6 A. Hattori, H. L. Crespi and J. J. Katz, *Biochemistry*, 1965, **4**, 1213.
7 D. J. Brockwell, *The Inhibition of Elongation Factor Tu by Kirromycin*, PhD Thesis, University of Manchester, 1996.
8 J. P. Derrick, L. Y. Lian, G. C. K. Roberts and W. V. Shaw, *Biochemistry*, 1992, **31**, 8191.
9 R. J. Brennan, A. Awan, J. Barber, E. Hunt, K. L. Kennedy and S. Sadegholnejat, *J. Chem. Soc., Chem. Commun.*, 1994, 1615.
10 D. Bloor, *Production of Deuteriated Elongation Factor Tu for Use in NMR Studies*, PhD Thesis, University of Manchester, 1992.
11 M. Kjeldgaard and J. Nyborg, *J. Mol. Biol.*, 1992, **223**, 724.
12 G. J. Kleywegt and T. A. Jones, *Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography*, 1994, **31**, 9.
13 P. J. Kraulis, *J. Appl. Crystallogr.*, 1991, **24**, 946.
14 Collaborative Computational Project, Number 4, S. Bailey, *Acta Crystallogr., Sect. D*, 1994, **50**, 760.
15 A. T. Brünger, *X-PLOR v3.1 Manual—A System for Crystallography and NMR*, Yale University Press, New Haven, CT, USA, 1992.
16 P. V. Luzzati, *Acta Crystallogr.*, 1952, **5**, 802.
17 G. J. Kleywegt and A. T. Brunger, *Structure*, 1996, **4**, 897.
18 G. Kleywegt, T. Bergfors, H. Senn, L. P. B. Gsell, K. Shudo and T. A. Jones, *Structure*, 1994, **2**, 1241.
19 D. W. J. Cruickshank, in *Macromolecular refinement, Proceedings of the Daresbury Study Weekend*, Daresbury Laboratory, Warrington W4A 4AD, 1996, pp. 11–22.

Received in Cambridge, UK, 9th February 1998; 8/01152D