NMR assignments of the C-terminal domain of human polypeptide release factor eRF1

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Abstract We report NMR assignments of the protein backbone of the C-terminal domain (163 a.a.) of human class 1 translation termination factor eRF1. It was found that several protein loop residues exist in two slowly interconverting conformational states.

Keywords Termination of protein synthesis · Human polypeptide release factor eRF1 · NMR assignments

Abbreviations
RF Protein release factor
C-domain eRF1 C-terminal domain

Biological context
Termination of translation is governed by the cooperative action of two interacting polypeptide chain release factors, class-1 eRF1 and class-2 eRF3 (Alkalaeva et al. 2006). The human eRF1 in the crystal (Song et al. 2000) and in solution (Kononenko et al. 2004) consists of three domains. The N-terminal domain of the release factor eRF1 is involved in the recognition of one of the three stop codons, UAA, UAG or UGA (Bertram et al. 2000; Kisselev et al. 2003). The M-domain of eRF1 participates in the subsequent hydrolysis of the ester bond in peptidyl-tRNA (Song et al. 2000; Kisselev et al. 2003). The C-terminal domain of eRF1 interacts with the C-domain of eRF3 (Ito et al. 1998; Merkulova et al. 1999) and the binding of both factors is essential for fast kinetics of the termination of translation (Alkalaeva et al. 2006). At the same time, removal of this domain from human eRF1 enhances, rather than reduces, the termination activity in vitro (Merkulova et al. 1999; Frolova et al. 2000). The eRF3 is a ribosome- and eRF1-dependent GTPase, which is encoded by an essential gene and belongs to class-2 release factors (Frolova et al. 1996). The mechanism of the interaction of eRF1 and eRF3 and its role in translation termination requires further elucidation. It is known that residues GILRY (411–415) of eRF1 are crucial for the interaction of eRF1 with eFF3 (Merkulova et al. 1999). It is supposed, that the interaction between eRF3, GTP and eRF1 increases the affinity of eRF1 in forming the pre-termination complex (Alkalaeva et al. 2006). It is shown (Alkalaeva et al. 2006), that the binding of eRF1, eRF3 and GTP with the ribosome is accompanied by their cooperative interaction in vitro and it is suggested that this leads to significant conformational changes in the whole pretermination complex. These results are in good agreement with the fact, that both ternary (eRF1-eRF3-GTP) and quaternary complexes (eRF1-eRF3-GTP-Mg2+) exist in solution even in the absence of the ribosome (Mitkevich et al. 2006).

The structure of the C-domain of human eRF1 is still poorly known. The published crystal structure of eRF1 (Song et al. 2000) has relatively low resolution of 2.8 Å and the C-domain has vast unresolved fragments. These
unresolved highly mobile loops may have a functional role in interaction of eRF1 with eRF3. Earlier NMR assignments have been reported for the N-terminal domain (BMRB-6116) and the middle (M) domain (BMRB-6763) of the human eRF1. The solution structure and dynamics of the M-domain of human eRF1 have also been studied (Ivanova et al. 2007). In order to elucidate the structure, dynamics and function of the C-domain of human eRF1, NMR assignments for the protein backbone of the C-domain of human eRF1 have been obtained.

Methods and experiments

The full-length cDNA encoding human eRF1 with the C-terminal His6-tag fusion was cloned into pET23b(+) vector (Novagen) under the phage T7 RNA polymerase promoter. The C-domain (residues 276–437 of eRF1) was overproduced in E. coli, strain BL21(DE3), in M9 minimal medium and isolated using Ni-NTA resin (Qiagen). The protein was further purified by cation exchange chromatography using HiTrap SP columns (Pharmacia). For 13C and/or 15N-isotope labeling, [13C6]-D-glucose and/or 15NH4Cl (Cambridge Isotope Laboratories Inc.) were used as the isotope sources in M9 minimal medium.

The samples for NMR (approximately 1 mM) were prepared in either 95% H2O/5% D2O or in 100% D2O and 10 mM potassium phosphate, 50 mM KCl, pH 7.0. Mercaptoethanol (final concentration ~2 mM) was added to the final solution in order to prevent oxidation of the free cysteine residue Cys-302.

All spectra were acquired at 25°C on Varian INOVA 600 and 800 MHz and Bruker AVANCE 600 MHz spectrometers equipped with triple resonance z-gradient probes. Spectra were processed by NMRPipe (Delaglio et al. 1995), and analyzed using SPARKY (from Goddard and Kneller). Sequential assignments for the backbone were obtained using the following spectra: 3D HNCO, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNHAHB and HBHA(CO)NH.

Assignments and data deposition

In total, chemical shifts assignments were made for 89% of all the possible protein backbone amide resonances (Fig. 1); 89% of 1H, 15N and non-prolyl 15N resonances, 96% of 13Cα and 88% of 13Cβ resonances. It was found that several residues (between 333 a.a. and 362 a.a.) of protein loop that is invisible in crystal structure (Song et al. 2000), exist in two slowly interconverting conformational states. The 1H, 15N and 13C chemical shifts have been deposited in the BioMagResbank database (http://www.bmrb.wisc.edu) under the accession number BMRB-15366.

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