ABSTRACT

The carboxy-terminal domain (CTD) of eukaryotic initiation factor 5 (eIF5) plays a central role in the formation of the multifactor complex, an important intermediate for the 43 S preinitiation complex assembly. The IF5-CTD interacts directly with the translation initiation factors eIF1, eIF2ɓ, and eIF3c, thus forming together with eIF2 bound Met-$tRNA_i^{Met}$ the MFC. In this work we present the high resolution crystal structure of eIF5-CTD at 1.8 Å resolution. This domain of the protein is exclusively composed out of alpha-helices and is homologous to the carboxy-terminal domain of eIF2Ů (eIF2Ů-CTD). The most striking difference in the two structures is an additional carboxy-terminal helix. The binding sites of eIF2-ɓ, eIF3 and eIF1 were mapped onto the structure. eIF2-ɓ and eIF3 bind to non-overlapping patches of negative and positive electrostatic potential, respectively.

Data collection

Crystals were mounted by sequential transfer (5% steps) into the crystallization solution containing 20 % glycerol (v/v) and were flash-cooled in a nitrogen stream at 110 K prior data collection. Native Data were collected at beamline ID29 (ESRF, Grenoble, France) (Table 1). MAD Data were collected at the Swiss Light Source at 100 K on beamline X06SA with a mar225 CCD detector (MAR X-ray-research, Hamburg, Germany) (table 1). A second MAD experiments was performed at the Grenoble ESRF, beamline BM14 at 100 K, employing a mar225 CCD detector (MAR X-ray-research, Hamburg, Germany) All datasets were integrated and scaled with XDS. Data collection statistics is given in Table 1.

Structure solution and refinement

Heavy-atom derivative crystals were prepared by soaking the native crystals in mother liquor containing 20 mM potassium dicyanoaurate (I) for 2 days. Data were collected at ESRF, beamline BM14 and at SLS, beamline X06SA.

One gold position was determined by SHELXD. Phases were computed using SOLVE and improved by RESOLVE. Automatic model building was performed by RESOLVE. 164 out of 194 residues were built by the program. This initial model was transported into the unit cell of the native high-resolution data using MOLREP. Further density modification, phase extension and automatic model building was carried out by ArpWarp version using the resulting model from MOLREP and the

native high-resolution data set. Refinement was effected using REFMAC. Refinement statistics are given in Table 2.

Table 1

Grenoble ID 29	Native	**SLS PX I**	Inflection	High remote
Data collection (XDS)		*Data collectoion (XDS)*		
Wavelength (Å)	1.00655	Wavelength (Å)	1.0397	0.855
Resolution range (outer shell)	40-1.8 (1.91-1.8)	Resolution range (outer shell)	40-2.97 (3.1-2.97)	40-2.46 (2.6-2.46)
No. observations	104040	No. observations	26602	48023
No. unique reflections	17574	No. unique reflections	7131	12719
Completeness (%)	98.4 (97.4)	Completeness (%)	96.4 (72.1)	98.7 (93.2)
Rsym (%)	6.1 (8.5)	R_{sym} (%)	4.9 (8.6)	3.9 (7.2)
I/ů(I)	27.6 (18.2)	I/ω/I)	21.4 (11.9)	23.5 (14.8)

Table 2

Refinement **(REFMAC)**	
Resolution range (Å)	30-1.8
No. reflections working set	17303
No. reflections test set	228
No. non hydrogen atoms	1505
Solvent water molecules	139
R/Rfree (%)	19.5/24.4
RMSD bond length (Å)	0.006
RMSD bond angles (deg.)	1.188