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Crystallization of the yeast elongation factor complex eEF1A–eEF1Bα

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Crystals of the *Saccharomyces cerevisiae* elongation factor eEF1A (formerly EF-1α) in complex with a catalytic C-terminal fragment of the nucleotide-exchange factor eEF1Bα (formerly EF-1β) were grown by the sitting-drop vapour-diffusion technique, using polyethylene glycol 2000 monomethyl ether as precipitant. Crystals diffract to better than 1.7 Å and belong to the space group $P2_12_12_1$. The unit-cell parameters of the crystals are sensitive to the choice of cryoprotectant. The structure of the 61 kDa complex was determined with the multiple anomalous dispersion technique using three selenomethionine residues in a 11 kDa eEF1Bα fragment generated by limited proteolysis of full-length eEF1Bα expressed in *Escherichia coli*.

1. Introduction

The eukaryotic protein-synthesis elongation factor 1 (eEF1) is one of the major participants in the flow of genetic information from DNA to RNA to protein. Its major role is in the elongation cycle of protein synthesis, which involves the sequential addition of residues to the carboxy-terminal end of the nascent protein (Merrick & Nyborg, 2000). In S. cerevisiae, eEF1 consists of the three subunits eEF1A (50 kDa), eEF1B (23 kDa) and eEF1B (48 kDa). The eEF1A subunit is a classic G-protein that binds aminoacylated tRNA (aa-tRNA) in a GTP-dependent matter and this ternary complex then binds to the elongating ribosome. The correct match of codon with anticodon triggers the hydrolysis of GTP to GDP and releases eEF1A–GDP from the ribosome. The aa-tRNA in the A site is now competent to react with the peptidyl-tRNA in the P site, thus forming the peptide bond. For eEF1A to perform another cycle of aa-tRNA binding, the GDP must be exchanged with GTP. This reaction is facilitated by the eEF1B subunit, which functions as a guanine nucleotide-exchange factor in vitro (Slobin & Moller, 1978). The role of the eEF1B-associated subunit eEF1B is not yet clear, although it stimulates the exchange activity of eEF1B (Janssen & Moller, 1988). eEF1A probably has several other functions besides its function as carrier of aa-tRNA. One widely accepted function is the binding of eEF1A to elements of the cytoskeleton (Condeelis, 1995). eEF1A forms a complex with both monomeric G-actin and polymerized F-actin (Yang et al., 1990).

Here, we report the purification of a catalytic C-terminal fragment of eEF1Bα and the crystallization of the complex between eEF1A and the C-terminal fragment of eEF1Bα. We also present the X-ray diffraction results for the eEF1A–eEF1Bα crystals together with the initial results from the structure determination.

2. Materials and methods

2.1. Cloning and expression

The intron of the *S. cerevisiae TEF3* gene (SwissProt accession number P32471) encoding eEF1Bα was removed by site-directed mutagenesis, producing the plasmid TKB105. The intronless full-length *TEF3* gene was cloned with an N-terminal histidine tag in the pET11-d expression vector (Novagen). The construct was transformed into electro-competent B834(DE3) (Novagen) *E. coli* cells and selected clones were grown aerobically in LB medium containing 100 mg ml^{-1} ampicillin at 310 K to an OD_{600} of 0.6. The expression of eEF1Bα was induced by addition of isopropyl-1-thio-D-galactoside to a final concentration of 0.5 m*M* for 3 h. Selenomethionine-substituted eEF1Bα was prepared by growing the eEF1Bα-expressing *E. coli* strain in minimal media containing 1 mg l^{-1} vitamins (riboflavin, niacinamide, pyridoxine monohydrochloride and thiamine), 40 mg l^{-1} of all amino acids except methionine, 40 mg l^{-1} seleno-L-methionine, 25 mg l^{-1} $FeSO_4$, 0.4% glucose, 2 m*M* $MgSO_4$, 2 g l^{-1} NH_4Cl, 6 g l^{-1} KH_2PO_4, 25.6 g l^{-1} $Na_2HPO_4 \cdot 7H_2O$. Expression was allowed to continue for 7 h after induction.

2.2. Purification and characterization

eEF1A was purified by phosphocellulose chromatography essentially as described by Thiele *et al.* (1985). After dialysis against

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buffer [100 mM Tris–HCl pH 8.0, 2 mM $MgCl_2$, 25% glycerol, 0.5 mM dithiothretitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF)] overnight at 277 K, the phosphocellulose pool was loaded onto a Source-S column (Pharmacia) equilibrated in buffer A (20 mM Tris–HCl pH 7.6, 50 mM KCl, 5 mM $MgCl_2$, 25% glycerol, 0.5 mM DTT, 0.1 mM PMSF). Elution was carried out with a linear gradient from 0 to 65% buffer B (20 mM Tris–HCl pH 7.6, 700 mM KCl, 5 mM $MgCl_2$, 25% glycerol, 0.5 mM DTT, 0.1 mM PMSF).

The eEF1Bα-expressing cells were resuspended in lysis buffer [50 mM Tris–HCl pH 7.8, 50 mM KCl, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol (β-ME), 0.1 mM PMSF], ruptured in a French press and the homogenate was subjected to centrifugation at 90 000g for 1 h. eEF1Bα was purified from the supernatant by chromatography on a Hi-Trap nickel-chelate column (Pharmacia) equilibrated in buffer N (50 mM Tris–HCl pH 7.8, 100 mM KCl, 5 mM $MgCl_2$, 5 mM β-ME, 0.1 mM PMSF). The protein was eluted with 120 mM imidazole in buffer N. The catalytically active C-terminal fragment of eEF1Bα was prepared by dialysing the eluted protein against buffer (20 mM Tris–HCl pH 7.6, 100 mM KCl, 0.5 mM DTT) overnight at 277 K to remove the protease inhibitors before digestion with 0.018%(w/w) trypsin for 12 h on ice. The reaction was stopped by the addition of 0.5 mM PMSF, after which the digest was loaded onto a Superdex 200 HR gel-filtration column (Pharmacia) equilibrated in buffer (20 mM HEPES pH 7.2, 100 mM KCl, 0.5 mM DTT, 0.1 mM EDTA). The protein eluted in three peaks (Fig. 1a). Only protein from the second peak was able to form a complex with eEF1A (data not shown). Sequencing of material in the second peak identified the N-terminus as starting at Lys110.

The complex was formed by mixing eEF1A with a surplus of eEF1Bα while concentrating in a Centricon YM-10 (Amicon). The concentrated protein solution was then loaded onto a Superdex 200 HR (Pharmacia) gel-filtration column equilibrated with buffer (20 mM HEPES pH 7.2, 100 mM KCl, 0.5 mM DTT, 0.1 mM PMSF). 0.5 ml fractions were collected and 5 ml aliquots were analysed by SDS–PAGE. The complex was judged to be more than 95% pure (Fig. 2). Purification of the selenomethionine eEF1A–eEF1Bα complex was identical to that of the native protein, except that oxidation of selenomethionine was prevented by adding DTT to a final concentration of 5 mM to all buffers. Gel-filtration experiments with a complex between eEF1A and full-length eEF1Bα indicate formation of an eEF1A_2–eEF1Bα_2 dimer (data not shown), while the complex between eEF1A and the C-terminal fragment of eEF1Bα elutes as a 1:1 heterodimeric complex (Fig. 1b).

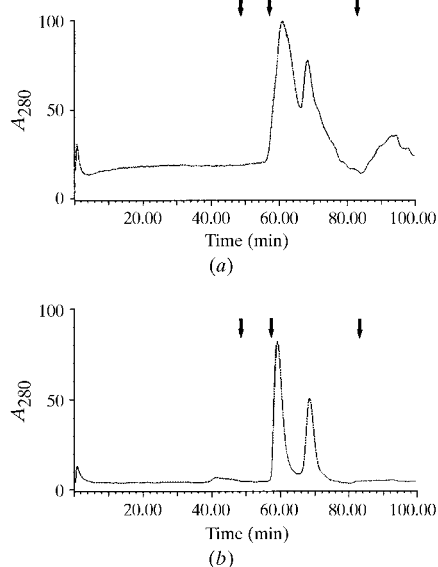

Figure 1
Elution profiles of gel-filtration runs performed with eEF1Bα and the eEF1A–eEF1Bα complex on a Superdex 200 HR gel-filtration column. The molecular weights of the proteins used to calibrate the column are 150 (left), 66 (middle) and 12.6 kDa (right) and are indicated with arrows. (a) eEF1Bα digested with trypsin elutes in three peaks, with the C-terminal fragment in peak 2 eluting at 30 kDa. Peak 1, eluting at 50 kDa, represents partially aggregated digested eEF1Bα, while peak 3 represents small peptides from the trypsin degradation. (b) The elution profile of eEF1A in complex with the eEF1Bα C-terminal fragment displays two peaks corresponding to the eEF1A–eEF1Bα complex, eluting at 62 kDa, and a surplus of eEF1Bα fragment, eluting at 30 kDa. The apparent molecular weight of 30 kDa for the eEF1bα fragment may indicate dimer formation, but it may also be explained by its highly elongated shape (Andersen *et al.*, 2000).

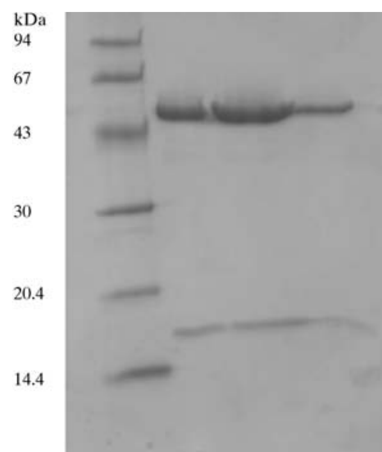

Figure 2
The gel was stained with Coomassie Blue. The left lane contains markers with molecular weights indicated in kilodaltons.

Figure 3
Orthorhombic crystal of the eEF1A–eEF1Bα complex grown with mmE 2K as precipitant. The scale bar represents 500 μm.

2.3. Crystallization and data collection

All crystallization trials were carried out at 277 K using the sitting-drop vapour-diffusion method by mixing 4 μl of the complex of eEF1A with the C-terminal fragment of eEF1Bα with 4 μl of reservoir solution. The complex concentration used for crystallization was 8 mg ml^{-1} in buffer (20 mM HEPES pH 7.2, 100 mM KCl, 0.5 mM DTT). The reservoir solution contained 100 mM KCl, 100 mM Tris–HCl pH 8.5, 3 mM DTT and 15–18% polyethylene glycol 2000 monomethyl ether (mmE 2K).

Crystals appeared after 2–3 d and grew to maximum dimensions of 500 × 150 × 100 μm within one week (Fig. 3). The native and selenomethionine-substituted complexes were crystallized under the same conditions.

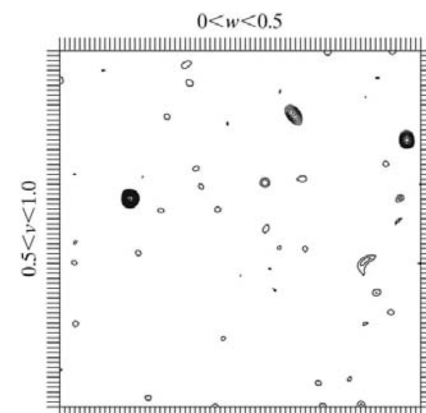

Figure 4
Harker section (u = 0.5) of the anomalous Patterson map. The contour level is 3σ with 1σ increments. Figure produced with *CNS* (Brunger *et al.*, 1998).

Table 1
Statistics for data collection and phase determination.

$R_{\rm merge} = \sum_h \sum_{j=1,N} |I_h - I_h(j)| / \sum N I_h$ for the intensity of reflection h measured N times; MAD = multiple anomalous dispersion; mmE = polyethyleneglycol monomethylether. Values in parentheses are for the outer resolution shell.

(*a*) Data collection.

Crystal	Selenomethionine	Native
Cryprotectant	37.5% mmE 650	28% glycerol, 18% mmE 2K
Solvent (%)	37.0	39.6
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters		
a (Å)	63.85	60.89
b (Å)	91.81	92.95
c (Å)	92.98	102.92
Unique reflections		39937
Peak	37326	
Inflection	37400	
Remote	63666	
Wavelength		0.9793
Peak	0.9792	
Inflection	0.9797	
Remote	0.9184	
Resolution		20.0–2.0 (2.07–2.0)
Peak	25.0–2.0 (2.07–2.0)	
Inflection	25.0–2.0 (2.07–2.0)	
Remote	25.0–1.67 (1.73–1.67)	
Completeness		99.3 (99.6)
Peak	99.3 (93.9)	
Inflection	99.2 (92.8)	
Remote	99.3 (93.5)	
Redundancy		6.4 (5.4)
Peak	3.5 (3.2)	
Inflection	3.6 (3.3)	
Remote	3.6 (3.4)	
Mean $I/\sigma(I)$		34.8 (12.7)
Peak	31.5 (14.4)	
Inflection	40.3 (16.7)	
Remote	36.1 (10.8)	
Reflections with $I/\sigma(I) > 3\sigma(I)$ (%)		92.4 (83.2)
Peak	92.2 (82.0)	
Inflection	93.0 (82.2)	
Remote	88.7 (69.8)	
$R_{\rm merge}$ (%)		6.7 (14.0)
Peak	4.1 (9.6)	
Inflection	3.5 (8.4)	
Remote	3.8 (15.5)	

(*b*) Sites in MAD phasing.

Occupancy	*B* factor (Å^2)
0.9088	16.9
0.8622	15.0
0.9296	21.3

(*c*) Phasing.

Program	Figure of merit	Resolution (Å)
SOLVE (MAD phasing)	0.53	20.0–2.0
DM (density modification)	0.66	25.0–1.67

To reduce decay during data collection, the crystals were soaked stepwise in two different cryoprotectants (Table 1) before flash-cooling of the crystals. Data from both native and selenomethionine-substituted crystals were collected at beamline BW7A at EMBL, Hamburg on a MAR CCD detector (Table 1). The data were reduced with the *HKL* package (Otwinowski & Minor, 1997).

3. Results and discussion

The choice of cryoprotectant was found to be important for the unit-cell parameters, with the *c* axis of the crystals shrinking by 10 Å in mmE 650 compared with glycerol; this improved diffraction from 2.0 to 1.67 Å (Table 1). The native data set was initially used in an attempt to solve the structure by molecular replacement using various X-ray models of EF-Tu and a NMR structure of a human eEF1Bα C-terminal fragment. Since this approach proved unsuccessful, a three-wavelength MAD experiment was performed in order to obtain experimental phases. Initial phases were calculated with the program *SOLVE* (Terwilliger & Berendzen, 1999) from data sets collected at wavelengths optimized for selenomethionine (Table 1). In order to determine the peak (maximum for f'') and the inflection (minimum for f') wavelengths, a fluorescence scan was recorded on a single SeMet-substituted crystal. Since only the eEF1Bα fragment was expressed in *E. coli*, there were only three selenomethionines per 61 kDa in the asymmetric unit. However, the anomalous Patterson map was interpretable, with three clear peaks in each Harker section (Fig. 4). Subsequent density modification with the program *DM* (Cowtan & Zhang, 1999) produced an experimental electron density of high quality (Andersen *et al.*, 2000).

Despite the fact that only the eEF1Bα fragment was expressed in *E. coli* and contained only three methionine residues, phase determination by the multiple anomalous dispersion technique (MAD) was a success. Replacement of the three methionine residues in the eEF1Bα fragment with selenomethionine did not appear to change the physical properties of the protein. The eEF1Bα fragment is clamped between two domains of eEF1A (Andersen *et al.*, 2000) and the three methionines have well defined density in agreement with the low *B* factors calculated by *SOLVE* (Table 1). Recently, phases were calculated with four selenomethionines in 506 residues, also using data collected at three wavelengths (Izard & Blackwell, 2000). Thus, it appears possible in general to determine phases from crystals containing one ordered selenomethionine in an asymmetric unit of 15–20 kDa and possibly even 20–30 kDa using high-brilliance synchrotron radiation.

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