Crystal Structure of the N-terminal Segment of Human Eukaryotic Translation Initiation Factor 2α*

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Eukaryotic translation initiation factor 2α (eIF2α) is a member of the eIF2 heterotrimeric complex that binds and delivers Met-tRNA^Met to the 40 S ribosomal subunit in a GTP-dependent manner. Phosphorylation/dephosphorylation of eIF2α at Ser-51 is the major regulator of protein synthesis in eukaryotic cells. Here, we report the first structural analysis on eIF2, the three-dimensional structure of a 22-kDa N-terminal portion of human eIF2α by x-ray diffraction at 1.9 Å resolution. This structure contains two major domains. The N terminus is a β-barrel with five antiparallel β-strands in an oligonucleotide binding domain (OB domain) fold. The phosphorylation site (Ser-51) is on the loop connecting β3 and β4 in the OB domain. A helical domain follows the OB domain, and the first helix has extensive interactions, including a disulfide bridge, to fix its orientation with respect to the OB domain. The two domains meet along a negatively charged groove with highly conserved residues, indicating a likely site for protein-protein interaction.

Eukaryotic translation initiation factor 2 (eIF2)^1 is a GTP-binding protein that plays a central role in initiating translation. It binds charged initiator tRNA (Met-tRNA^Met) in a GTP-dependent manner to form the ternary complex, eIF2-GTP-Met-tRNA^Met. This ternary complex binds to the 40 S ribosomal subunit, and additional initiation factors (including eIF1α and eIF1A) join to form the 43 S preinitiation complex and assist in recognizing the start codon (reviewed in Refs. 1–5).

Recognition of the translational site is accompanied by eIF5-mediated hydrolysis of eIF2-bound GTP, which releases an eIF2-GDP binary complex along with several other initiation factors. For another round of initiation, the GDP in the eIF2 binary complex must be exchanged for GTP in a reaction catalyzed by the multimeric protein factor eIF2B (previously called guanine nucleotide exchange factor) (6). The 40 S-mRNA-Met-tRNA^Met complex joins the 60 S ribosomal subunit to form an 80 S initiation complex that can enter the elongation phase of protein synthesis.

In eukaryotes, eIF2 is a heterotrimer composed of α (36 kDa), β (38 kDa), and γ (52 kDa)-subunits, which appear to remain associated throughout the initiation cycle. Cross-linking and genetic studies have suggested that both β- and γ-subunits are implicated in guanine nucleotide and Met-tRNA^Met binding (7–9). In addition, the γ-subunit was shown to interact specifically with eIF5 during GTP hydrolysis and also to bind mRNA (10–12). The γ-subunit participates in the recognition of the start site for protein synthesis (13).

The phosphorylation/dephosphorylation of a conserved serine (Ser-51) in the α-subunit is the major regulator of protein synthesis in eukaryotes (reviewed in Ref. 2). Phosphorylation of Ser-51 shuts off protein synthesis (14). Three protein kinases, HRI (heme-regulated inhibitor), PKR (RNA-dependent protein kinase), and GCN2, have been identified that specifically phosphorylate Ser-51 in response to a variety of cellular stresses including viral infection, heat shock, heavy metals, and deprivation of amino acids or serum.

The phosphorylated eIF2-GDP complex released during protein synthesis initiation binds eIF2B with much higher affinity than does eIF2-GTP, and binding to eIF2B does not allow the exchange GDP for GTP that is necessary for further rounds of initiation (reviewed in Refs 15–17). A recent paper by Nika and co-authors (18) has shown that unphosphorylated eIF2α also mediates nucleotide exchange. The rate of eIF2B-catalyzed nucleotide exchange increases in the absence of the α-subunit compared with wild type, which suggests that nucleotide exchange requires direct interaction between eIF2α and eIF2B and that phosphorylation strengthens this interaction. There are no structural data on any of the eIF2 monomers to provide a structural context for these important regulatory interactions.

Here we report the structure of human eIF2α determined by x-ray crystallography using multiple anomalous dispersion (MAD) on a selenomethionine (SeMet)-labeled baculovirus-expressed eIF2α at 1.9 Å resolution. The crystallization of eIF2α was achieved only by using limited proteolysis techniques, and the final structure comprises residues 3–182, roughly the N-terminal two-thirds of full-length human eIF2α.

**EXPERIMENTAL PROCEDURES**

Protein Expression and Purification—Baculovirus expressing human eIF2α was kindly provided by Dr. Jane-Jane Chen (MIT). Human eIF2α was overexpressed in SF9 insect cells grown in Cyto-SF9 medium (Kemp Biotechnologies, Frederick, MD). A cell pellet from 1 liter of culture was lysed in 100 ml of 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 5 mM dithiothreitol, and 1 ml of protease inhibitor mixture set III
Crystallographic data

| Data set | Wavelength | Resolution | Unique reflections | Completeness | I/σ(I) | Rsym |
|---------|------------|------------|--------------------|--------------|-------|-------|
| Edge    | 0.9795     | 30.0–1.9   | 14727              | 89.8         | 8.4   | 5.8   |
| Peak    | 0.9793     | 30.0–1.9   | 14713              | 89.7         | 9.5   | 5.9   |
| Remote  | 0.9640     | 30.0–1.9   | 14922              | 90.9         | 7.7   | 6.0   |

Structure determination

| Resolution | SeMet sites | Figure of merit (overall) | Phasing power (overall) |
|------------|-------------|----------------------------|-------------------------|
| 30–2.1 Å   | 2           | 0.46                       | 3.0                     |

Refinement statistics

| Bond length | Bond angle | Backbone | Side chain |
|-------------|------------|----------|------------|
| 0.011 Å     | 1.19°      | 3.9 Å^2  | 7.8 Å^2    |

*Rsym = |I – <I>|/|I|, where (I) = average intensity obtained from multiple observations of symmetry-related reflections.

Phasing power = [Fobs^2/Fcalc^2 – Fobs^2/Fcalc^2]^1/2.

Root-mean-square deviation from ideal geometry and root-mean-square variation in the B-factors of bonded atoms.

The crystal structure of the N-terminal segment of human eIF2α was solved by MAD phasing and refined using SQUEEZE (25) and sharpened using SHARP (26). Density modification to 2.1 Å achieved an electron density map in which most of the protein residues could be modeled unambiguously with the program O (Fig. 1) (28). Native data from 30 to 1.9 Å were used for structure refinement. Successive rounds of rebuilding, interspersed with torsion angle simulated annealing (CNS (29)) and positional and individual B-factor refinement (REFMAC5 (30)) were carried out to generate a model with Rfactor = 0.21 and Rfree = 0.23, respectively (Table 1). The model contains 180 amino acid residues, 4 zinc ions, and 96 solvent molecules. The average B-factor for the entire model is 36.6 Å^2. According to PROCHECK (31), 92.2% of the residues lie on the most favored regions, 7.1% on additional allowed regions, and 0.6% on generously allowed regions. No residues were found in not allowed regions.

Relatively few baculovirus-expressed proteins have had sufficiently high SeMet incorporation to allow successful MAD phasing (19). It is worth mentioning that the N-terminal piece found in the crystal structure contains three methionine residues, but only SeMet-20 and SeMet-44 are located in rigid parts of the secondary structure, SeMet-29 is part of a more mobile loop connecting residues 3 and 4. The structure was determined using x-ray crystallography at 1.9 Å resolution. The crystal structure contains residues 3–182 with a disordered loop from residues 51 to 68 (Fig. 2). The final model, with approximate dimensions of 57 × 36 × 35 Å, is divided into two major domains: the OB domain and the helical domain.

**RESULTS AND DISCUSSION**

The structure of an ~21.4-kDa N-terminal fragment of human eIF2α was determined using x-ray crystallography at 1.9 Å resolution. The crystal structure contains residues 3–182 with a disordered loop from residues 51 to 68 (Fig. 2). The final model, with approximate dimensions of 57 × 36 × 35 Å, is divided into two major domains: the OB domain and the helical domain.

**OB Domain**—The first 87 N-terminal residues have an oligonucleotide-binding fold (OB fold) (32), with a five-stranded antiparallel β-barrel arranged with a Greek key topology capped by a turn of 310 α-helix located between β3 and β4 (Fig. 2). A β-hairpin connects β1 and β2; β2 and β3 are linked by a four-residue loop and β4 by a three-residue loop. The loop connecting β3 and β4 is longer by 17 residues, and it was not completely modeled due to a lack of interpretable electron density. The visible part, residues 48–50, begins with a turn of 310 helix that is stabilized through a hydrogen bond between the carbonyl group of Leu-46 and nitrogen of Gln-49. Serine 51

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*Found on the Web at www.solve.lanl.gov.*
The α1 helix comes just after this 3_10 helix (Fig. 2). Residues 63 and 64 at the end of the loop are also visible with a hydrogen bond between the carbonyl group of Arg-63 and the main chain nitrogen of Arg-66. β1 contains a β-bulge at residue Val-23, and a left-handed Gly-65 (φ and ψ have the same absolute values as the right-handed Gly but with opposite signs) is present at the beginning of β4; both are standard features of the OB fold.

The eIF2α N-terminal domain is the latest example of the large oligonucleotide/oligosaccharide-binding fold. The structural classification of the protein data base (SCOP (34)) counts at present 61 different structures containing an OB fold, divided into seven superfamilies. Using the DALI server software (35), the OB domain of human eIF2α was identified as a member of the nucleic acid-binding superfamily. Examples of members of this family are: S1 RNA-binding domain from the Escherichia coli polynucleotide phosphorylase (33), cold shock proteins A and B (36, 37), domain II of eukaryotic translation initiation factor 5A (38, 39), translational initiation factor IF1 (40), and the N-terminal domain of aspartyl-tRNA synthetase (41), among others.

Although eIF2α shows very little, if any, sequence homology with members of this family, their structures can easily be superimposed, and the root mean square deviation along the coordinates of all Cα atoms is around 2.0 Å. The main differences are restricted to loop regions, especially the length of the loop connecting β3 and β4 containing the phosphorylation site. Among the eIF2α sequences, the β3 to β4 residues are highly conserved (Fig. 3); in human eIF2α, this region contains a turn of a 3_10 helix (Fig. 2) as observed in the NMR structure of E. coli polynucleotide phosphorylase (33) and the crystal structure of translation initiation factor 5A from Pyrococcus aerophilum (38). Other family members have different structures in this connecting region. Translational initiation factor IF1 (40) and the N terminus of aspartyl-tRNA synthetase (41) contain an α-helix, and cold shock proteins A and B have a long loop lacking a defined secondary structure (36, 37).

The site proposed as the RNA binding site is found in nearly the same position in all members of the nucleic acid-binding superfamily; this is the α-barrel region, where three loops connecting β1 and β2, β3 and β4, and β4 and β5 come together (Fig. 2). In most cases RNA interaction seems to involve solvent-exposed aromatic residues and positively charged residues on the surface of the protein. The only structure available for this group of proteins bound to nucleic acids is the complex of the N-terminal fragment of aspartyl-tRNA synthetase with the anti-codon loop of its cognate tRNA (41) where there is a π-stacking interaction between one of the bases and a conserved Phe residue. In eIF2α the corresponding region contains two tyrosine residues, Tyr-32 and Tyr-81, clustered with nonpolar residues Met-44, Leu-46, Ile-82, and Gly-43 and two residues, Glu-42 and Asp-83, with negatively charged side chains (Fig. 2).

The OB domain of human eIF2α does not have any of the clustered positive charges that are observed for the other members of this family. This structural feature is consistent with the observation that, unlike other family members in which biochemical and genetic experiments and limited structural data support the idea of direct interaction with nucleic acids (36–37, 40–41), there is little or no evidence that eIF2α binds RNA (18). Most cross-linking and genetic experiments suggest that β- and γ-subunits of eIF2 are the subunits involved in initiator tRNA and ribosomal RNA binding (8, 42).

**Helical Domain**—The helical domain comprises residues 88–182. In contrast to the highly conserved architecture of the OB domain, the helical domain adopts a previously unreported fold consisting of a 28-residue-long α-helix (α1), a series of small α-helices (α2, α3, α4, and α6), and one 3_10 helix folded into a very compact domain (Fig. 2). The OB and helical domains are linked by a disulfide bridge between Cys-69 in β4 and Cys-97 in α1 (Fig. 2). Alignment of eIF2α sequences suggests that this disulfide bridge is only possible in mammalian eIF2α (Fig. 3). The last residue in our model, Arg-182, is solvent-exposed, and some uninterpretable electron density can be observed around this region, suggesting that proteolytic attack may not be specific.

**Groove between the OB and Helical Domains**—A negatively charged cavity is formed where the OB and helical domains come together (Fig. 4). The residues defining this groove come...
from several secondary structural elements: Tyr-8, located at the beginning of the OB domain; Asp-17 at \( \beta_1 \); Asp-37 and Tyr-38 in the loop connecting \( \beta_2 \) and \( \beta_3 \); and Asp-138 on \( \alpha_3 \). Interestingly, all of the residues found in this region are highly conserved among the species (Fig. 4). The \( \alpha_1 \)-subunit of eukaryotic translation initiation factor 2 (eIF2\( \alpha \)) contains a polylysine motif. The negatively charged groove in eIF2\( \alpha \) might serve as the direct interaction site for the polylysine repeat.

**Phosphorylation Site**—eIF2\( \alpha \) regulates protein synthesis through phosphorylation/dephosphorylation of Ser-51. Phosphorylation of eIF2\( \alpha \) converts eIF2 into a competitive inhibitor for the guanine exchange factor eIF2B. The eIF2 binding experiments demonstrate that eIF2B binds to phosphorylated eIF2 with a higher affinity than to unphosphorylated eIF2.

It has been suggested that eIF2\( \alpha \) is required for the interaction between eIF2 and eIF2B (8). The high affinity interaction between eIF2\( \alpha \) and the regulatory domain composed of \( \alpha_1 \)- and \( \delta \)-subunits of eIF2B is required for the proper binding of eIF2 to the catalytic domain of eIF2B, allowing normal rates of nucleotide exchange (43). The phosphorylation site of eIF2\( \alpha \) lies on the long loop connecting \( \beta_3 \) and \( \beta_4 \) of the OB domain, and it is close to the nominal RNA-binding site (Fig. 2). The lack of electron density suggests that this region is very mobile. Not surprisingly, the NMR structure of the translational initiation factor IF1 from *E. coli* shows considerable flexibility for the corresponding loop (40).

The simplest way for eIF2B to detect and respond to phosphorylation at Ser-51 would be for eIF2B to make direct contact with eIF2 in the Ser-51-containing loop region. An interesting feature of the highly conserved loop sequence is the predominance of positively charged residues (Arg-52, Arg-53, Arg-54, Lys-60, Arg-63) following Ser-51 (Fig. 3). This proximity and conservation, combined with the recent results that eIF2\( \alpha \) and the regulatory domain eIF2B interact independently of phosphorylation, suggest a model in which the positively charged residues participate in an interaction that is enhanced by phosphorylation.

In summary, the present study provides the first structural analysis of any member of the eIF2 heterotrimeric complex and provides a framework for further genetic, biochemical, and structural analyses of protein-protein interactions involving eIF2\( \alpha \).

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