The amino acid sequence of eukaryotic translation initiation factor 1 and its similarity to yeast initiation factor SUI1

Marcelle A.M. Kasperaitis, Harry O. Voorma, Adri A.M. Thomas*

Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

Received 7 April 1995

Abstract Eukaryotic initiation factor eIF-1 was purified from rabbit reticulocytes. Amino acid sequence analysis revealed that the protein contained a blocked amino-terminus. After cleavage with the endoproteinase Asp-N, three peptides were sequenced. The obtained partial sequences were identical to sequences of SUI1ISO1, the human homologue of the yeast translation initiation factor SUI1. The SUI1 gene product was identified as a protein involved in the recognition of the protein synthesis initiation codon. A similar mode of action has been suggested for eIF-1.

Key words: Initiation; Translation; Eukaryote; eIF-1; SUI1

1. Introduction

Initiation of eukaryotic protein synthesis is mediated by at least 10 proteins, containing 26 peptide chains. The role of some initiation factors is still unclear. This is especially true for eIF-1 and eIF-3, two proteins whose amino acid sequence is unknown.

The single-polypeptide eIF-1 stimulated mRNA and Met-tRNA binding to the 40S ribosomal subunit, as well as to the 80S ribosome [1–4]. The dependence on eIF-1 in these processes varied in different laboratories, reason to suggest that eIF-1 may not be crucial for translation initiation. However, we could show that the binding of the second aminoacyl-tRNA complex to the ribosome was nearly completely dependent on eIF-1 [2]. The observations led us to assume that eIF-1 had a stabilizing effect on the binding of Met-tRNA, facilitating the codon-anticodon interaction [2].

Recently, a yeast protein was found to be involved in the interaction of the ribosome with the initiation codon, similar to the mode of action of eIF-2 [5]. The molecular mass of this SUI1 protein was 12,300 Da, suggesting that SUI1 may be one of the small initiation factors eIF-1, eIF-1A, or eIF-5A. The amino acid sequence of the latter two proteins was solved and not similar to that of SUI1 [5,6].

In this paper, we purified eIF-1 from rabbit reticulocyte, confirmed its ability to stimulate Met-tRNA binding, and sequenced the cleaved protein by Edman degradation. The resulting peptide sequences were found in the EMBL database, and corresponded to the mammalian homolog of SUI1.

2. Materials and methods

Reagents were purchased from the following suppliers: sodium dodecyl sulphate (electrophoresis grade) from Biorad; hydrogenated Triton-X100 (protein grade) from Calbiochem Corp., trifluoroacetic acid and acetonitrile (both HPLC grade) from J.T. Baker, and [^{35}S]Methionine (1300 Ci/mmol) from Amersham. Endoproteinase Asp-N was obtained from Boehringer Mannheim. All chemicals used in protein sequencing were purchased from Applied Biosystems. PVDF membranes (Immobilon-P) were obtained from Millipore Corp. All other chemicals were reagent grade quality. Milli Q water was used throughout.

2.1. Purification of rabbit reticulocyte eIF-1

Two sources of eIF-1 were used in this study. Ribosome-bound eIF-1 was purified from rabbit reticulocytes as described previously [7] and used as a reference protein. Due to the low amount of eIF-1 obtained, amino acid sequencing was performed on eIF-1 isolated from postribosomal supernatants. In this case a modified isolation procedure was used. After Sepharose 6B-heparin affinity chromatography [8] and 40–70% ammoniumsulphate precipitation, 414 mg of protein was subjected to Fast Protein Liquid Chromatography (FPLC). All FPLC columns, except for Q- Sepharose FF were equilibrated in 20 mM HEPES-KOH (pH 7.6), 10% (v/v) glycerol, 100 mM KCl, 0.1 mM EDTA and 7 mM 2-mercaptoethanol. Tris-HCl (20 mM, pH 7.6) was used instead of HEPES-KOH for the Q Sepharose column. Proteins were eluted from the ion-exchange columns with step elution gradients of KCl. eIF-1 did not bind to Q-Sepharose FF 16/10 and eluted between 200 and 400 mM KCl from SP Sepharose FF 16/10 columns. After 0–70% ammoniumsulphate precipitation and dialysis of the dissolved precipitate, 32 mg of protein was subjected to Superose 12 HR 10/30 gelfiltration, using 8 mg of protein per run. eIF-1 eluted between 14.5 and 16.5 ml, comparable to cytochrome C. Finally 0.46 mg of protein containing eIF-1 was concentrated by Mono S HR 5/5 chromotography and dialysed against 20 mM HEPES-KOH (pH 7.6), 10% (v/v) glycerol, 100 mM KAc, 0.1 mM EDTA and 1 mM DTT. Fractions were analyzed by Fast Green staining of SDS-polyacrylamide gels and by its ability to stimulate Met-tRNA binding to 40S initiation complexes. eIF-1 activity was measured by binding of [^{35}S]Met-tRNA to 40S initiation complexes [2] with 2.8 μg eIF-2/eIF-2B, 7 μg eIF-3, 0.15 μg eIF-1A, and 4 pmol of [^{35}S]methionyl-tRNA with a specific activity of 50,000 cpm/pmol. The assays were performed without the addition of mRNA or AUG.

2.2. Tricine-SDS-PAGE and electroblotting

20 μg of eIF-1, purified as described above, was subjected to discontinuous SDS-polyacrylamide gelelectrophoresis [9]. The gel was preelectrophoresed with 0.5 M Tris-HCl (pH 8.45), 0.1% (w/v) SDS, and 1 mM thioglycolic acid for 16 h at 100 V and 4°C. During electrophoresis and blotting onto PVDF membranes 1 mM thioglycolic acid was present in all buffers. The blot was extensively washed with water and stained with 0.2% (w/v) Ponceau S in 10% (v/v) acetic acid. The eIF-1 band and a similar sized membrane piece without protein were excised, destained for one minute with 0.2 mM NaOH, 20% (v/v) acetonitrile, rinsed extensively with water and then cut into squares of approximately 1 mm^2 prior to elution.

2.3. Preparative elution of eIF-1

Membrane-bound eIF-1 was eluted by incubating the PVDF pieces in 25 μl of 50 mM Tris-HCl (pH 8.8), containing 2% (w/v) SDS and 1% (v/v) hydrogenated Triton X-100 for 16 h at room temperature [10,11]. After centrifugation, the supernatant was transferred to another vial and pooled with consecutive washes of the membrane pieces with 100 mM Tris-HCl (pH 7.6), 1% (v/v) hydrogenated Triton X-100 to a final volume of 200 μl. Most of the SDS was removed by repeated extraction,

*Corresponding author. Fax: (31) (30) 51 36 55.
E-mail: a.a.m.thomas@pobox.ruu.nl

SSDI 0014-5793(95)00427-0

without acidification of the sample with 400 μl heptane/isoamyl alcohol 4:1 (v/v), saturated with 100 mM Tris-HCl (pH 8.0) [12]. The PVDF membrane pieces without protein were treated similarly.

2.4. Enzymatic digestion of eIF-1

Endoproteinase Asp-N was added at an enzyme-substrate ratio of 1/20 (w/w), followed by incubation at 37°C for 24 h. The sample containing no protein was treated identically. To remove residual SDS prior to reversed phase chromatography, the digested samples were acidified to 5% (v/v) trifluoroacetic acid and extracted with 400 μl of heptane/isoamyl alcohol, saturated with 5% trifluoroacetic acid [12]. This step was repeated three times. The samples were then lyophilized and dissolved in 50 μl of 0.1% (v/v) trifluoroacetic acid.

2.5. Purification of peptides by reversed phase chromatography

Peptide isolation was performed with the SMART™ system (Pharmacia LKB). Digested samples (50 μl) were applied to a reversed phase C2/C18 precision column 3.2/3 (0.24 ml gel volume) equilibrated with 0.1% (v/v) trifluoroacetic acid, using a flow rate of 300 μl/min at room temperature. A linear gradient of acetonitrile (0–50% in 24 min) containing 0.1% (v/v) trifluoroacetic acid was used to elute the peptides. The column eluants were monitored for absorbance at 214 nm, fractions of 75 μl were collected and aliquots from individual fractions of interest were analyzed for amino acid sequence.

2.6. Peptide sequencing

Samples to be subjected to automatic amino acid sequencing were lyophilized, dissolved in 15 μl 1% (v/v) trifluoroacetic acid and spotted onto Biobrene treated glass fiber filters. The samples were analyzed using a Model 476A gas-phase protein microsequencer with an on line 120A PTH analyzer (Applied Biosystems, Inc.). Chemicals for sequencing and analysis of data were as described by the manufacturer.

3. Results and discussion

eIF-1 isolated from ribosomal washes was over 90% pure. The eIF-1 preparation isolated with FPLC techniques from

Fig. 1. Gel analysis of purified eIF-1. Ribosomal eIF-1 (0.3 μg, lane 1) and eIF-1 purified from post-ribosomal supernatant (2 μg, lane 2) were analyzed by 13.5% SDS-polyacrylamide gelelectrophoresis and Fast Green staining. The molecular mass of marker proteins is indicated on the left. The 12,327 Da marker protein is bovine heart cytochrome *c*, co-migrating with eIF-1.

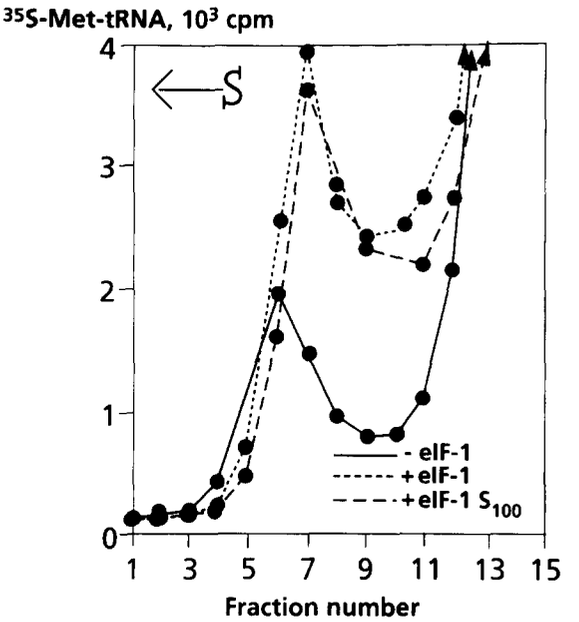

Fig. 2. Met-tRNA binding to the 40S initiation complex. Binding of [^{35}S]Met-tRNA to 40S subunits was done as described in Methods. Sedimentation was from right to left. Minus eIF-1: ——, with 30 ng of ribosomal eIF-1: ---, with about 70 ng of eIF-1, purified as described in the text: – – –.

postribosomal supernatants was contaminated to 40% with other proteins, as can be seen from the stained SDS-polyacrylamide gel (Fig. 1). Both preparations promoted the binding of ^{35}S-methionyl-tRNA to 40 S initiation complexes to a similar extent (Fig. 2). After blotting, the membrane piece containing pure eIF-1 was used for sequencing.

Precautions were taken during electrophoresis and blotting against inadvertently blocking of the N-terminus of the protein [13]. Nevertheless, in spite of these precautions initial sequence efforts performed on eIF-1 preparations were unsuccessful, indicative of a blocked N-terminus.

In order to cleave eIF-1 with endoproteinases, the protein had to be eluted from the PVDF membrane. The procedure described by Fernandez et al. [10] had to be modified by the addition of 2% SDS, which was subsequently removed prior to proteolytical scission.

Endoproteinase Glu-C only attained cleavage of 40% of eIF-1, while endoproteinase Arg-C did not digest eIF-1 at all. The low activity of both enzymes was probably due to residual amounts of SDS, present in the eIF-1 sample. However, the endoproteinase Asp-N cleaved more than 90% of the starting material. The Asp-N protease digest of eIF-1 was used for sequencing. This endoproteinase cleaves N-terminally of aspartic and cysteic acid but not of reduced cysteine.

The generated eIF-1 peptides were separated by reversed phase chromatography (Fig. 3). No detectable amounts of material eluted after a retention time of 12 min, even after doubling the percentage of acetonitrile. The asterisk-tagged peaks contained non-proteineous material. The eluates of three well-resolved peptides were lyophilized and subjected to Edman degradation employing a gas-phase protein microsequencer. The sequences of these three peptides were:

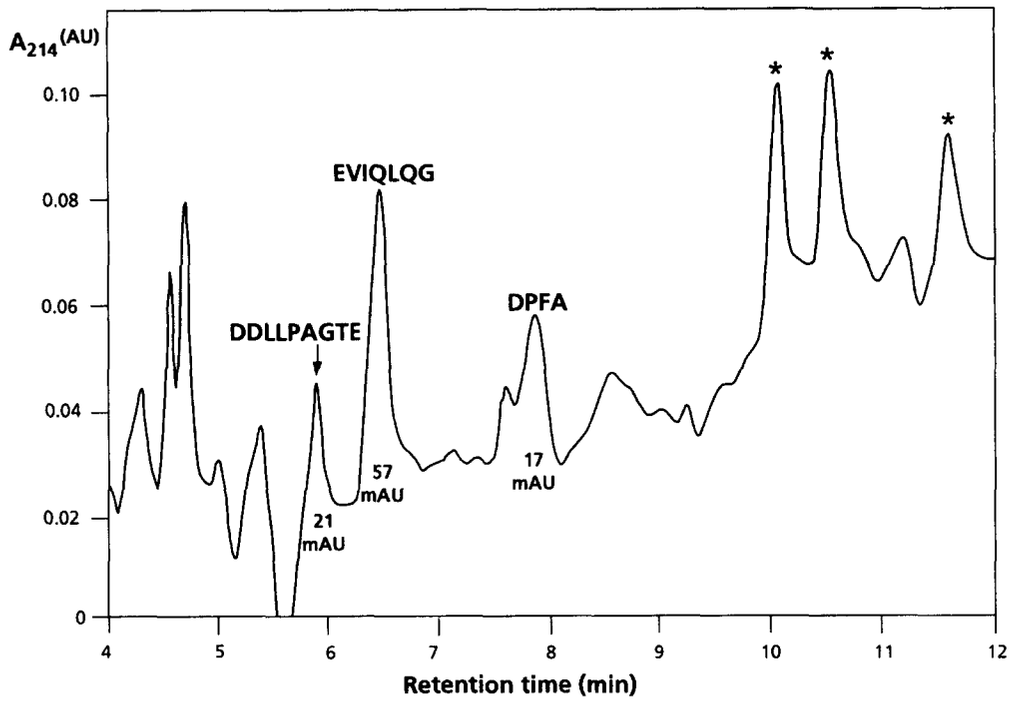

Fig. 3. Peptide analysis of protease-cleaved eIF-1. Separation of peptides, obtained after Asp-N protease cleavage of eIF-1, was done with the SMART system (LKB/Pharmacia). The amino acid sequence of the peptides and the absorbance units corrected for background signal of membrane without protein, is indicated. Protein sequence analysis of the asterisk-tagged peaks did not give any results.

(1) Asp-Asp-Leu-Leu-Pro-Ala-Gly-Thr-Glu (DDLLPAGTE),
(2) Glu-Val-Ile-Gln-Leu-Gln-Gly (EVIQLQG) and
(3) Asp-Pro-Phe-Ala (DPFA).

Peptides 2 and 3 started with an Asp-residue as expected, and peptide 1 with a Glu-residue, which was quite surprising, but not without precedent when using large amounts of protease or long incubation times [14]. The sequence of peptides 2 and 3 were present in the EMBL database and corresponded to amino acid sequences 76 to 82 and 10 to 13 respectively, of the SUI1 gene product of the yeast *Saccharomyces cerevisiae* [5]. The valine residue (position 77 in SUI1) in the sequence EVIQLQG is an isoleucine in SUI1. The amino acid sequence of peptide 1 was identical to the positions 20 to 28 of the mammalian isolog of the SUI1 gene product as identified by assembling expressed sequence tags [15]. According to the authors the human SUI1ISO1 gene product is highly similar to proteins from yeast, rice, mosquito and *Methanococcus*, suggesting that they all function as initiation factors [15].

Yoon and Donahue recognized the SUI1 protein as a translation initiation factor and reasoned it to be the putative yeast equivalent of mammalian eIF-1 or eIF-1A, or an initiation factor not yet identified by biochemical studies [5]. Later the sequence of eIF-1A was solved and found to be dissimilar to SUI1 [6].

The lack of cleavage by CNBr [16] or BNPS-skatol [17] (results not shown) is explained by the lack of methionine and tryptophan residues. The NH_2-terminal methionine is probably cleaved from the protein, as serine is the second residue. Methionine removal and blockade of the serine residue is common [18]. The number of amino acid residues in human eIF-1, excluding the aminoterminal methionine is 112, making a calculated molecular mass of 12,603.

The sequence around the initiation codon is conserved between rice, yeast, and human: CGT ATG T. In spite of the absence of the essential A or G at −3, and the G at +1 [19], the rice homolog GOS2 is expressed at high levels [20]. Apparently, expression is mainly regulated at the transcriptional level.

The amino acid sequences of the three isolated peptides, spaced over a major part of the protein, irrevocably prove the yeast SUI1 function to be identical to that of rabbit reticulocyte eIF-1 and to human SUI1ISO1 gene products.

Acknowledgements: We thank Rina van Geelkerken (Dept. of nephrology and hypertension, University Hospital, Utrecht) and Jos van Bommel (LKB, Pharmacia) for their help with the purification of the peptides, Fridolin van der Lecq (Sequence Centre, Institute for biomembranes, Utrecht) for his expertise on protein sequence analysis, and Cor van der Mast for his enthousiastic assistence during preparation of the manuscript.

References

[1] Trachsel, H., Erni, B., Schreier, M.H. and Staehelin, T. (1977) J. Mol. Biol. 116, 755–767.
[2] Thomas, A., Spaan, W., van Steeg, H., Voorma, H.O. and Benne, R. (1980) FEBS Lett. 116, 67–71.
[3] Benne, R. and Hershey, J.W.B. (1978) J. Biol. Chem. 253, 3078–3087.
[4] Schreier, M.H., Erni, B. and Staehelin, T. (1975) J. Mol. Biol. 116, 727–753.
[5] Yoon, H. and Donahue, T.F. (1992) Mol. Cell. Biol. 12, 248–260.
[6] Dever, T.E., Wei, C.-L., Benkowski, L.A., Browning, K., Merrick, W.C. and Hershey, J.W.B., J. Biol. Chem. 269, 3212–3218.
[7] Benne, R., Brown-Luedi, M.L. and Hershey, J.W.B. (1978) J. Biol. Chem. 253, 3070–3077.
[8] Van der Mast, C.A., Thomas, A., Goumans, H., Amesz, H., and Voorma, H.O. (1977) Eur. J. Biochem. 75, 455–464.
[9] Schägger, H. and Von Jagow, G. (1987) Anal. Biochem. 166, 368–379.

[10] Fernandez, J., DeMott, M., Atherton, D. and Mische, S.M. (1992) Anal. Biochem, 201, 255–264.
[11] Szewczyk, B. and Summers, D.F. (1988) Anal. Biochem. 168, 48–53.
[12] Bosserhof, A., Wallach, J. and Frank, R.W. (1989) J. Chromatogr. 473, 71–77.
[13] Moos Jr., M., Nguyen, N.Y. and Liu, T.-Y. (1988) J. Biol. Chem. 263, 6005–6008.
[14] Ingrosso, D., Fowler, A.V., Bleibaum, J. and Clarke, S. (1989) Biochem. Biophys. Res. Comm. 162, 1528–1534.
[15] Fields, C. and Adams, M.D. (1994) Biochem. Biophys. Res. Comm. 198, 288–291.
[16] Scott, M.G., Crimmins, D.L., McCourt, D.W., Tarrand, J.J., Eyerman, M.C. and Nahm, M.H. (1988) Biochem. Biophys. Res. Comm. 155, 1353–1359.
[17] Crimmins, D.L., McCourt, D.W., Thoma, R.S., Scott, M.G., Macke, K. and Schwartz, B.D. (1990) Anal. Biochem. 87, 27–38.
[18] Arfin, S.M. and Bradshaw, R.A. (1988) Biochemistry 27, 7979–7984.
[19] Kozak, M. (1989) J. Cell Biol. 108, 229–241.
[20] De Pater, B.S., Van der Mark, F., Rueb, S., Katagiri, F., Chua, N.-H., Schilperoort, R.A. and Hensgens, L.A.M. (1992) Plant J. 2, 837–844.