Volume 297, number 3, 226–228 FEBS 10685 February 1992

Proteolysis of *Bacillus stearothermophilus* IF2 and specific protection by fMet-tRNA

Manuela Severini[a], Theodora Choli[b*], Anna La Teana[a,b] and Claudio O. Gualerzi[a]

[a]*Laboratory of Genetics, Department of Biology, University of Camerino, 62032 Camerino (MC) Italy* and [b]*Max-Planck-Institut für Molekulare Genetik, DW-1000 Berlin 33, Germany*

Received 6 December 1991

Translation initiation factor IF2 from *Bacillus stearothermophilus* (741 amino acids, M_r 82,043) was subjected to trypsinolysis alone or in the presence of fMet-tRNA. The initiator tRNA was found to protect very efficiently the Arg^{308}-Ala^{309} bond within the GTP binding site of IF2 and, more weakly, three bonds (Lys^{146}-Gln^{147}, Lys^{154}-Glu^{155} and Arg^{519}-Ser^{520}). The first two are located at the border between the non-conserved, dispensable (for translation) N-terminal portion and the conserved G-domain of the protein, the third is located at the border between the G- and C-domains. Since IF2 is known to interact with fMet-tRNA through its protease-resistant C- (carboxyl terminus) domain, the observed protection suggests that, upon binding of fMet-tRNA, long-distance tertiary interactions between the IF2 domains may take place.

Translation initiation; Thermophilic initiation factor; Initiator tRNA

1. INTRODUCTION

Inititation factor IF2 is the largest of the three proteins involved in translational initiation in bacteria. The function and the genetics of this factor have recently been reviewed [1–3]. For its function IF2 interacts with the 30 S and 50 S ribosomal subunits as well as with GTP and fMet-tRNA. The binding site for fMet-tRNA has been localized in the 24.5 kDa carboxyl-terminal portion of the protein (the C-domain) which appears to be very compact and resistant to proteolytic attack; the 50 S binding site, the GTP/GDP binding site, the GTPase catalytic center and part of the 30 S binding site have been localized in the central domain of the protein, the G-domain of approximately 41 kDa [4,5]. The interaction of IF2 with GTP or GDP protects the G-domain against a proteolytic cleavage occurring at a specific site (Arg^{308}-Ala^{309}) localized within the GTP/GDP binding site [6,7]. The N-terminal portion of the protein, on the other hand, probably has a floppy structure which is very susceptible to proteolytic attack; this part of the protein displays a non-conserved sequence and does not seem to be implicated in any fundamental translational function [4,8,9] and its role remains obscure.

In the present paper, we have analyzed the pattern of trypsin digestion of *Bacillus stearothermophilus* IF2 in the presence and absence of fMet-tRNA and have identified four peptide bonds outside the main fMet-tRNA binding domain which are protected by this ligand. The main protection occurs at the same site previously found to be protected by GTP, while the other three sites are found at the border between the N-terminal portion and the G-domain and between the C- and G-domains of the molecule.

**Present address:* Aristotelian Univ. of Thessaloniki, School of Chemistry, Laboratory of Biochemistry, Thessaloniki, 54006 Greece.

Correspondence address: C.O. Gualerzi, Laboratory of Genetics, Dept. of Biology, University of Camerino, 62032 Camerino (MC) Italy. Fax: (39) (737) 40298.

2. MATERIALS AND METHODS

B. stearothermophilus IF2 was prepared from *E. coli* K12ΔH1Δtrp transformed with pPLc2833 carrying the *B. stearothermophilus infB* gene as previously described [10]. Purification of the factor followed essentially the published procedure [11].

Trypsinolysis was carried out at 37°C in 210 μl reaction mixtures containing 30 mM Tris-HCl, pH 7.1, 30 mM NH_4Cl, 1 mM 2 mercaptoethanol, 1.5% glycerol, 210 μg IF2 and 0.21 μg of trypsin (TPCK-treated). When indicated, the mixtures also contained 2100 pmol of fMet-tRNA. Aliquots containing ~30 μg of IF2 were removed at the indicated times and the proteolysis was analyzed on 15% SDS-PAGE. Electroblotting of peptides on PDVF membranes (Millipore) was carried out at 4°C for 1 h at 150 mA followed by 21 h at 350 mA as described [12]. Amino acid micro-sequencing was performed as previously described [13] in an Applied Biosystems pulse-liquid gas phase sequenator (model 477A).

3. RESULTS AND DISCUSSION

The time course of trypsin digestion of IF2 with and without fMet-tRNA was analyzed electrophoretically. The digestion patterns obtained were characteristic, reproducible, and clearly different, depending on the presence or absence of the ligand during the digestion (Fig. 1). In the absence of fMet-tRNA, the digestion pattern is virtually identical to that previously reported [7] as also confirmed by the peptide sequences determined (see below). In the presence of the initiator tRNA, on the other hand, the proteolysis yields some characteristic peptides of higher molecular weight which remain un-

 Published by Elsevier Science Publishers B.V.

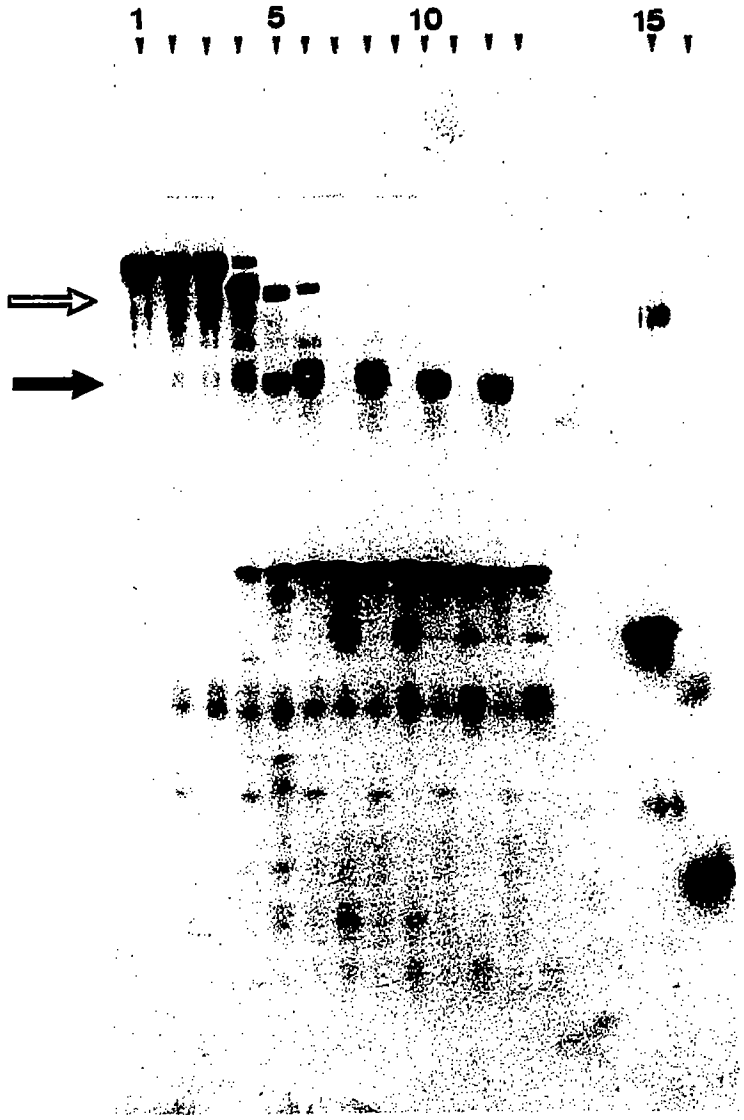

Fig. 1. Time course of trypsinization of *B. stearothermophilus* IF2 in the presence and absence of fMet-tRNA. The experimental conditions are described in section 2. The lanes contained *B. stearothermophilus* IF2 digested with trypsin for the indicated time: lane 1, undigested; lanes 2 and 3, 0 min; lanes 4 and 5, 8 min; lanes 6 and 7, 30 min; lanes 8 and 9, 60 min; lanes 10 and 11, 120 min; lanes 12 and 13, 150 min. The samples in the even-numbered lanes were incubated in the presence of $fMet\text{-}tRNA^{fMet}$, those in the odd-numbered lanes in the absence of $fMet\text{-}tRNA^{fMet}$. Lanes 15 and 16, molecular weight markers, bovine serum albumin (68,000), initiation factor IF3 (21,000), β-lactoglobulin (18,000), lysozyme (14,300), initiation factor IF1 (8,000).

digested for a long time, while other fragments of lower molecular weight are completely absent or appear later in lower amounts (Fig. 1), a clear indication that some peptide bonds which are accessible to trypsin in the free molecule become inaccessible in the IF2–fMet-tRNA complex (Fig. 1). It is noteworthy that uncharged $tRNA^{fMet}$, which does not interact with IF2, does not affect in any way the time course and the products of the trypsinolysis (not shown).

The peptide bonds protected by fMet-tRNA were identified from the comparison of the N-terminal sequences of the electroblotted proteolytic fragments obtained in the presence and absence of fMet-tRNA and taking into account their size and the primary structure of *B. stearothermophilus* IF2 [10]. Thus, it was established that, in the presence of fMet-tRNA, the retarded appearance of the ~65 kDa fragment (compare lane 4 and 5) and of the 40 kDa fragment (compare lane 4 with 5 and lane 6 with 7) is due to a reduced rate of cleavage at positions 'd'/'e' and 'g', respectively, while the prolonged stability of the ~40 kDa fragment (indicated by the black arrowhead in Fig. 1) results from a strong protection at position 'f' of peptides already cleaved at positions 'd' (or 'e') and 'g'. Thus, the fMet-tRNA provides a weak protection of the bonds Lys^{146}-Gln^{147}, Lys^{154}-Glu^{155} and Arg^{519}-Ser^{520} and a strong protection of the Arg^{308}-Ala^{309} bond.

During the course of trypsinolysis, the N-terminal por-

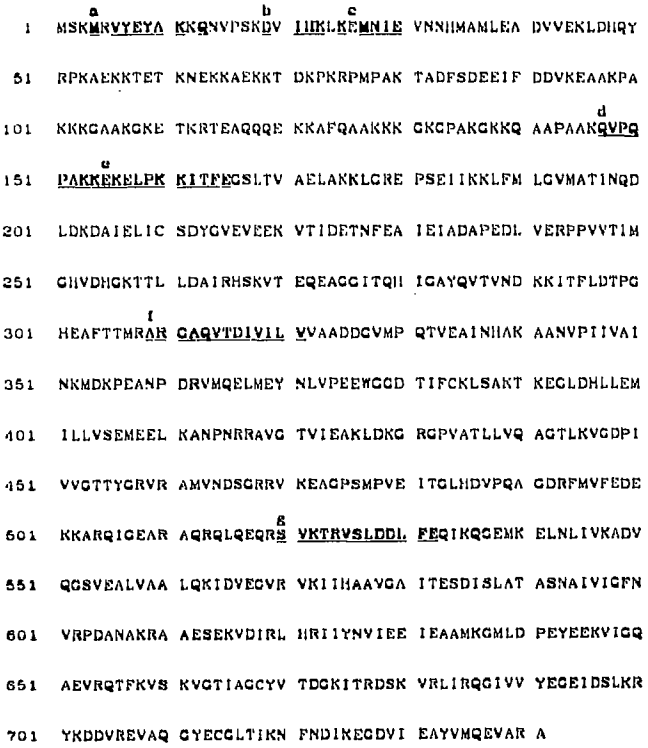

Fig. 2. Identification of the trypsin cleavage sites in the *B. stearothermophilus* IF2 molecule. To identify the peptide bonds cleaved by trypsin hydrolysis and those which are protected by fMet-tRNA, the electrophoretically separated proteolytic fragments were transferred by electroblotting onto membranes and subjected to automatic sequencing (see section 2). The figure shows the complete primary structure of *B. stearothermophilus* IF2 [8]. The sequence determined by automated amino acid sequencing are indicated in bold letters and underlined. The individual cleavage sites identified are indicated by the lower case letters above the N-terminal amino acid of the corresponding peptide.

tion (i.e. upstream of the cleavage site 'e' and 'd') of IF2 is rapidly degraded yielding small and discrete peptides of $M \leq 5000$. This part of the molecule displays a very low degree of conservation among the four IF2 sequences known so far and removal of this portion of the molecule by proteolysis [9] or by genetic manipulations [4,8] yields IF2 fragments which are still substantially active in all IF2 translational functions tested in vitro.

Cleavage at the Arg^{519}-Ser^{520} bond, on the other hand, yields a ~24-25 kDa fragment corresponding to the entire (or virtually entire) C-terminal fragment of the protein; this represents a structurally compact domain which resists even long digestion periods with trypsin or with other proteolytic enzymes [4,7]; it has been shown that IF2 binds fMet-tRNA via this C-domain. Since this domain is already very resistant to proteolysis [4,7], it is not surprising that no additional protection of this part of the molecule by fMet-tRNA is found. It is remarkable, on the other hand, that fMet-tRNA protects very efficiently the Arg^{308}-Ala^{309} bond, a site which is also very efficiently protected from proteolysis by guanosine nucleotides [4,7]; these residues lie within the GTP/GDP binding site of IF2, in the middle of the structural elements conserved among G-proteins [14,15] but no binding of fMet-tRNA with this part of the molecule has ever been observed and under no circumstance has it been found that the binding of GTP or GDP and fMet-tRNA to IF2 may influence each other [6,16]. Thus, it is unlikely that the protection of this site by the initiator tRNA might be due to a direct interaction. More likely, C-domain-bound fMet-tRNA may occupy a 'pocket' constituted by the C- and G-domains thus shielding the GTP/GDP binding site from the proteolytic enzyme. Similar explanations could provide a rationale for the (weak) protection observed at the sites situated at the borders between the N-terminal portion of IF2 and the G-domain and between the G-domain and the C-domain. An alternative explanation for the observed effects could be the occurrence of a long range ligand-induced conformational rearrangement of the IF2 domains.

Acknowledgements: Supported in part by the Italian MURST and by the National Research Council (CNR) PF 'Biotecnologia e Biostrumentazione' and PF 'Ingegneria Genetica' to C.O.G.

REFERENCES

[1] Hershey, J.W.B., in: *Escherichia coli* and *Salmonella typhimurium*–Cellular and Molecular Biology (F.C. Neidhardt, J.L. Ingraham, K.B. Low, B. Magasanik, M. Schaechter and H.E. Umbarger, Eds.) American Society for Microbiology, Washington, DC, 1987 pp. 613–647.
[2] Gualerzi, C.O. and Pon, C.L. (1990) Biochemistry 29, 5881–5889.
[3] Gualerzi, C.O., La Teana, A., Spurio, R., Canonaco, M.A., Severini, M. and Pon, C.L., in: The structure, function and evolution of ribosomes (W.E. Hill, A. Dahlberg, R.A. Garrett, P.B. Moore, D. Schlessinger and J. Warner, Eds.) American Society for Microbiology, Washington, DC, 1990, pp. 281–291.
[4] Gualerzi, C.O., Severini, M., Spurio, R., La Teana, A. and Pon, C.L. (1991) J. Biol. Chem. 266, 16356–16362.
[5] Severini, M., Spurio, R., La Teana, A., Pon, C.L. and Gualerzi, C.O. (1991) J. Biol. Chem. 266, in press.
[6] Pon, C.L., Paci, M., Pawlik, R.T. and Gualerzi, C. (1985) J. Biol. Chem. 260, 8918–8924.
[7] Severini, M., Choli, T., La Teana, A. and Gualerzi, C.O. (1990) FEBS 276, 14–16.
[8] Pon, C.L. and Gualerzi, C.O., in: Gene expression and regulation: the legacy of Luigi Gorini (M. Bissell, G. Dehò, G. Sironi and A. Torriani, Eds.) Elsevier, Amsterdam, 1988, pp. 137–150.
[9] Cenatiempo, Y., Deville, F., Dondon, J., Grunberg-Manago, M., Sacerdot, C., Hershey, J.W.B., Hansen, H.F., Petersen, H.U., Clark, B.F.C., Kjelgaard, M., la Cour, T.F.M., Mortensen, K.K. and Nyborg, J. (1987) Biochemistry 26, 5070–5076.
[10] Brombach, M., Gualerzi, C., Nakamura, Y. and Pon, C. (1986) Mol. Gen. Genet. 205, 97–102.
[11] Pawlik, R.T., Littlechild, J., Pon, C. and Gualerzi, C. (1981) Biochem. Int. 2, 421–428.
[12] Hunkapiller, H.W., Lujan, E., Ostrandem, E. and Hood, L.E. (1983) Methods Enzymol. 91, 227–247.
[13] Graack, H.R., Grohmann, L. and Choli, T. (1988) FEBS Lett. 242, 4–8.
[14] Jurnak, F. (1985) Science. 230, 32–36.
[15] March, P.E. and Inouye, M. (1985) Proc. Natl. Acad. Sci. USA 82, 7500–7504.
[16] Petersen, H.U., Roll, T., Grunberg-Manago, M. and Clark, B.F.C. (1979) Biochem. Biophys. Res. Commun. 91, 1068–1074.