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Evidence that Peptide Deformylase and Methionyl-tRNA$_f^{Met}$ Formyltransferase Are Encoded within the Same Operon in *Escherichia coli*

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Overexpression of the *fms* gene, the first translation unit of a dicistronic operon that also encodes methionyl-tRNA$_f^{Met}$ formyltransferase in *Escherichia coli*, sustains the overproduction of peptide deformylase activity in crude extracts. This suggests that the *fms* gene encodes the peptide deformylase. Moreover, the *fms* gene product has a motif characteristic of metalloproteases, an activity compatible with deformylase. The corresponding protein could be purified to homogeneity. However, its enzymatic activity could not be retained during the purification procedure. As could be expected from the occurrence in its amino acid sequence of a zinc-binding motif characteristic of metallopeptidases, the purified *fms* product displayed one tightly bound zinc atom.

Translation of mRNAs into proteins most often initiates on a methionine codon. This initiator codon corresponds to a specialized initiator methionine tRNA. In addition, in prokaryotes as well as in the chloroplasts and mitochondria of eukaryotes, a formyl group is added to the NH_2 of the methionine esterified to initiator methionine tRNA prior to the involvement of this tRNA in translation (10, 11, 19, 27) and formylmethionine instead of methionine is incorporated at the N terminus of nascent proteins (2, 5, 8, 31). However, examination of the N-terminal residue of the proteins synthesized in vivo in prokaryotes as well as in organelles reveals that the *N*-formylmethionine is not retained. Methionine, alanine, and serine were most frequently found as N-terminal residues (7, 15, 19, 30).

It was early proposed that the amino-terminal formylmethionine of a nascent protein could be removed posttranslationally (2, 9). In agreement with this hypothesis, two distinct enzymatic activities could be described: (i) a peptide deformylase capable of cleaving the formyl group from formylmethionine peptides (1, 18, 28, 32) and (ii) a methionine aminopeptidase which removes the N-terminal methionine from methionine peptides (1, 18, 23, 28, 32). The gene encoding this methionine aminopeptidase has been identified in *Escherichia coli* (4). Studies of the specificity of the corresponding protein both in vivo and in vitro indicate that removal of the initiator methionine is favored when the length of the side chain of the second amino acid is short enough (4, 14). In contrast to methionine aminopeptidase, the substrate specificity of peptide deformylase appears insensitive to the nature of the second residue following methionine (1). Actually, removal of the N-terminal formyl group from formylmethionine peptides occurs systematically. The coupled specificities of the two enzymes therefore explain why alanine, glycine, serine, or valine on the one hand and methionine on the other are usually found as N-terminal amino acids of proteins. However, peptide deformylase could not be further characterized because of its extreme instability (1, 18, 28). The lability of peptide deformylase activity might be the reason why a formylmethionine group is retained on nascent polypeptides synthesized in cell extracts from *E. coli* (1).

The *fmt* gene encoding the enzyme which catalyzes the formylation of Met-tRNA$_f^{Met}$ (methionyl-tRNA$_f^{Met}$ formyltransferase) has been recently cloned in our laboratory (13). The product of the *fmt* gene plays an essential role for optimal growth of *E. coli* cells (13), possibly by enabling Met-tRNA$_f^{Met}$ to participate in the initiation rather than in the elongation step of the translation process (12). *fmt* is the second cistron of an operon whose first cistron, which we called *fms*, encodes a putative 19-kDa polypeptide (12, 21). The translated amino acid sequence of *fms* shows a motif characteristic of neutral zinc aminopeptidases (16, 17, 25, 29) (Fig. 1). Since the enzyme function of *E. coli* peptide deformylase resembles that of an aminopeptidase, it is possible that the *fms* gene encodes peptide deformylase. To test this hypothesis, we cloned the *fms* gene and expressed it from an inducible promoter.

Peptide deformylase activity can be overexpressed from a plasmid carrying *fms*. The 821-bp *Xba*I-*Pst*I restriction fragment of the pBS936XB plasmid (13), which carries only *fms* as a full-length open reading frame, was ligated between the *Xba*I and *Pst*I restriction sites of pUC18, yielding the pUCdef plasmid in which the *fms* gene was placed under the control of the Lac promoter. JM101Tr cells (10 ml) carrying either the pUCdef or the pUC18 control plasmids were grown overnight in the presence of 0.3 mM isopropyl-β-D-galactopyranoside (IPTG) and 50 μg of ampicillin per ml. After centrifugation, cells were resuspended in 1 ml of 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [pH 7.0]) and sonicated. Cells debris were removed by centrifugation, and the total protein concentration in the cell extracts was measured (6). An aliquot (0.5 μl) of each of the two extracts was analyzed by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE [20% polyacrylamide gel; Phast-system; Pharmacia]). One protein band with an apparent molecular mass of 23 ± 1 kDa was specifically overexpressed in JM101Tr (pUCdef) extracts (Fig. 2). This band was absent from the gel with the JM101Tr (pUC18) cell extracts (Fig. 2).

Peptide deformylase activity in each extract was measured with a method similar to that previously described by Adams

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His	Glu	Met	Asp	His	*fms* gene product
		Leu			
His	**Glu**	**Met**	Ser	**His**	thermolysin family
		Phe	Thr		
		Ile			
His	**Glu**	Leu	Gly	**His**	matrix metalloproteinases family
		Phe			
		Ile	Arg		
		Leu	**Asp**		
		Met	Glu		PROSITE pattern
His	**Glu**	Phe	His	**His**	for neutral aminopeptidases
		Trp	Lys		
		Tyr	Pro		
		Val			

FIG. 1. Alignment of a portion of the amino acid sequence of the *fms* gene product with the zinc motif of zinc aminopeptidases of the thermolysin family. Aligned from top to bottom are the amino acids from His-132 to His-136 of the *fms* gene product (13), the zinc motif of the thermolysin family (see references 17 and 29 and the references quoted therein), the consensus sequences of matrix metalloproteinases (see references cited in reference 25) and the zinc-binding region signature of neutral zinc aminopeptidases as it is defined in the PROSITE library (3, 17). Residues matching those of the *fms* gene product are indicated in boldface. Note that a conserved glutamic acid, at a distance of 20 to 25 residues towards the C-terminal part of this motif, was proposed to be a third ligand of the zinc atom in the thermolysin family (29). Such a residue could be also found in the case of the *fms* gene product (Glu-159).

(1). A fraction of the cellular extract was first incubated at 37°C during various times (0.5 to 5 min) in the presence of 10 mM *N*-formyl-Met-Ala (Serva), 50 mM HEPES (pH 7.0), and 250 mM KCl. Removal of the formyl group was monitored as a function of time by assaying the unmasking of the primary amine of Met-Ala with the ninhydrin procedure (24). Under these conditions, an activity value of 0.75 μmol of unmasked primary amine per s and per mg of total protein was measured in the case of JM101Tr (pUCdef) extracts, whereas peptide deformylase activity remained at <0.004 $\mu mol \cdot s^{-1} \cdot mg^{-1}$ in the crude extract corresponding to control JM101Tr (pUC18) cells. We found that this peptide deformylase activity (overproduced at least 200-fold) disappeared upon any attempts at cellular fractionation (molecular sieving, anion exchange, etc.) or strongly decreased upon addition of various agents such as 2-mercaptoethanol, EDTA, or ammonium sulfate. Such an extreme lability of the enzyme activity has been observed previously (1, 18) and until now has prevented the purification of peptide deformylase (1, 18). However, we observed that, although peptide deformylase activity was lost, the amount and size of the overproduced 23-kDa protein remained. Consequently, purification could be undertaken by monitoring the polypeptide by SDS-PAGE.

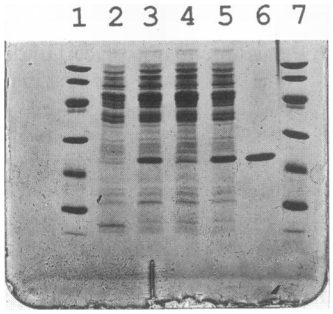

FIG. 2. Overproduction and purification of the *fms* gene product. Samples (3.5 μg) of a crude extract from JM101Tr cells carrying either control plasmid pUC18 (lanes 2) or plasmid pUCdef in the presence (lanes 3 and 5) or in the absence (lane 4) of 0.3 mM IPTG were analyzed by SDS-PAGE (20% polyacrylamide gel) together with 1 μg of the protein resulting from the purification procedure described in the text (lane 6) and the low-molecular-mass markers from Pharmacia (94, 67, 30, 20, and 14.4 kDa [lanes 1 and 7]).

Purification of inactive peptide deformylase to homogeneity. JM101Tr cells carrying the pUCdef plasmid were used to inoculate a flask of 2× tryptone-yeast extract medium (0.5 liter) containing 50 μg of ampicillin per ml and 0.3 mM IPTG. Cultures were grown overnight at 37°C, harvested by centrifugation and resuspended in 40 ml of 50 mM HEPES (pH 7.0). The sample was sonicated, and cell debris was removed by centrifugation. Streptomycin sulfate (0.3% [wt/vol]) was added to the supernatant. After centrifugation, the supernatant was submitted to an $(NH_4)_2SO_4$ precipitation (80% saturation) and centrifuged. From this step on, the peptide deformylase activity was no longer detectable. The pellet was redissolved in 6 ml of 20 mM KH_2PO_4 (pH 7.0) plus 50 mM KCl, dialyzed against the same buffer to remove ammonium sulfate, and finally applied to a gel filtration column (1.6 by 50 cm [Superose 6; Pharmacia]) equilibrated in the same buffer. The column was eluted at 0.2 ml/min. An aliquot of each fraction was analyzed by SDS-PAGE. Fractions containing the 23-kDa protein were pooled (14 ml) and applied to an anion exchanger (1.6 by 10 cm [Q-Hiload; Pharmacia]) equilibrated in 20 mM KH_2PO_4 (pH 7.0). A linear KCl gradient (0.2 M/h; 2.5 ml/min) was used. The fractions containing the 23-kDa protein were pooled and submitted to precipitation with $(NH_4)_2SO_4$ (80% saturation). The pellet was redissolved in 7 ml of 20 mM KH_2PO_4 (pH 7.0) plus 1.2 M ammonium sulfate and loaded on a hydrophobic interaction column (1.0 by 10 cm [Bakerbond HiPropyl]) equilibrated in 20 mM KH_2PO_4 (pH 7.0) plus 1.7 M ammonium sulfate. Elution was achieved by using a reverse ammonium sulfate gradient (1.1 M/h; 0.8 ml/min). The fractions containing the homogeneous 23-kDa protein were pooled (11 mg in 7 ml), dialyzed, and stored at −30°C after extensive dialysis against 20 mM KH_2PO_4 (pH 7.0) plus 55% glycerol. A molar extinction coefficient at 280 nm of 3,100 ± 400 $M^{-1} \cdot cm^{-1}$ could be measured from the protein content by using bovine serum albumin as the standard (6, 22). This value is comparable to that directly calculated from the amino acid sequence (2,360 $M^{-1} \cdot cm^{-1}$).

Biochemical characterization of the purified peptide deformylase. The N-terminal amino acid sequence of the protein purified above was determined (SerValLeuGlnValLeu) and was found to be identical to that of the translated gene product (13), taking into account the removal of the N-terminal methionine (4, 14). A molecular mass of 19,175 ± 50 Da was deduced from matrix-assisted laser desorption mass spectroscopy analysis of the purified protein, in perfect agreement with the molecular mass calculated from the amino acid sequence translated from *fms* (19,207 Da). This observation excludes the possibility that the loss of activity of the enzyme during the purification procedure was caused by proteolytic attack. Upon molecular sieving on a Superdex 75 column (1.6 by 60 cm [Pharmacia]) calibrated with bovine serum albumin (67 kDa), glutathione-*S*-transferase (53 kDa), ovalbumin (43 kDa), methionyl-$tRNA_f^{Met}$ formyltransferase (34 kDa) and *E. coli* thioredoxin (11.7 kDa), the *fms* product appeared to elute with an apparent molecular mass of 23 ± 2 kDa. This value is identical to that observed under denaturing SDS-PAGE (23 ± 1 kDa [Fig. 2]) and is close to that directly deduced from the amino acid sequence (19.2 kDa) or obtained by mass spec-

trometry measurement. This suggests that the inactive protein behaves as a monomeric species.

The *fms* product tightly binds a zinc atom. To measure the zinc content of the protein, the *fms* product was first dialyzed overnight against a buffer containing 20 mM Tris (pH 7.5)–0.1 M KCl with or without 0.1 mM EDTA. Atomic $A_{213.9}$ in the peak height mode during 5 s after 0.1-ml injections was monitored with a Varian AA775 spectrophotometer equipped with an air-acetylene burner (20). Zinc concentrations in serial dilutions of the two enzyme samples were calculated by comparison with solutions of known zinc concentrations (1 to 20 μM) prepared by diluting a standard 15.3 mM $ZnCl_2$ solution (Merck). Taking into account a molar extinction coefficient of 2,800 ± 700 $M^{-1} \cdot s^{-1}$ at 280 nm, a value of 0.8 ± 0.2 mol of zinc per mol of polypeptide chain was found. This value was not changed by prior treatment of the enzyme with EDTA. It is likely that the bound zinc atom corresponds to the zinc motif characteristic of zinc aminopeptidases (Fig. 1). This hypothesis is currently being tested by measuring the zinc content in mutant proteins with single substitutions at the level of the residues which are expected to chelate the metal. These residues (His-132, His-136, and the remote Glu-159) were deduced from comparisons with the neutral aminopeptidases of the thermolysin family, whose zinc-chelating side chains have already been established (see reference 29 and references therein).

Concluding remarks. This brief note establishes that the *fms* gene encodes a zinc-containing protein showing homologies with zinc aminopeptidases. Moreover, a plasmid overexpressing the *fms* gene causes a strong increase of peptide deformylase activity in cell extracts. We conclude that the *fms* and *fmt* genes encoding peptide deformylase and methionyl-$tRNA_f^{Met}$ formyltransferase, respectively, are very likely to be associated within a common operon in *E. coli*. Further evidence in favor of the identification of *fms* as the gene encoding peptide deformylase will obviously require the recovery of the enzymatic activity after purification of the protein. Attempts to support this perspective are currently under way. At this time, it is not clear why peptide deformylase activity is lost during purification. Clearly, the loss of enzymatic activity from the first step of the purification cannot be explained by the removal of the zinc atom. In fact, it has been suggested that peptide deformylase is associated with the ribosome (18, 26, 28). At this stage, an involvement of some ribosomal components as a cofactor of the reaction catalyzed by the *fms* product cannot be ruled out.

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ADDENDUM IN PROOF

Using a genetic approach, Mazel et al. (EMBO J., in press) have cloned a gene that they call *def*, which encodes *E. coli* peptide deformylase. They show that this gene actually corresponds to the *fms* locus and that it is essential for cell growth.

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