THE SUBUNIT STRUCTURE OF METHIONYL-tRNA SYNTHETASE FROM *ESCHERICHIA COLI*

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1. Introduction

Methionyl-tRNA synthetase from *E. coli* has been studied quite extensively and several structural features of the enzyme have been defined. The native enzyme is purported to be a tetramer of identical subunits with molecular weights about 44000 each, Lemoine et al. [1]. An active tryptic fragment of this enzyme, which can be crystallised in a form well suited to studies by X-ray diffraction [2] has also been isolated. This fragment was thought to be a dimer of identical subunits with molecular weights of about 33000 each, formed by the removal of a segment of molecular weight 11000 from the native protomer [3]. Another form of the enzyme has also been purified by Bruton and Hartley and shown to be a dimer of identical subunits with molecular weights of about 44000 [4].

The results described in this report show that the native enzyme is a homologous dimer, the protomers of which are actually about twice the molecular weight assigned previously and that the formation of the crystallisable fragment results from the removal of a segment of almost 200 residues from the C-terminal region of the native protomer.

2. Materials and methods

Methionyl-tRNA synthetase from *E. coli* EM 20031 was prepared as described previously [9]. The tryptic fragment of this enzyme was prepared according to the method of Cassio and Waller [3].

SDS-gel electrophoresis was carried out according to the method of Laemmli [5]. Ultracentrifugation in 6 M guanidine hydrochloride was carried out under the conditions described by Ullmann et al. [6].

Carboxypeptidase A digestion was carried out according to the method described by Fraenkel-Conrat et al. [7].

Automatic amino-terminal sequence determination [8] was carried out on a Beckman 890 Sequencer using Beckman chemicals. 150 nmoles of protein was subjected to about 25 cycles of degradation with the standard double-cleavage programme.

3. Results and discussion

During a study of the molecular weights of several aminoacyl-tRNA synthetases it was found that methionyl-tRNA synthetase from *E. coli* migrated on SDS-polyacrylamide gel electrophoresis with a mobility substantially lower than that expected on the basis of the accepted molecular weight of the protomers of this enzyme. The observed mobility corresponded to a molecular weight of about 85000 (fig. 1). Since this is about twice the molecular weight reported previously the possibility existed that the enzyme was not fully dissociated during electrophoresis. Therefore the experiments were repeated after boiling the enzyme in 2% SDS and 1% β-mercaptoethanol, reduction and carboxymethylation in 6 M guanidine hydrochloride or oxidation with performic acid. None of these drastic treatments effected any decrease in molecular weight of the enzyme on SDS-gel electrophoresis.

Previous estimates of the molecular weight of the

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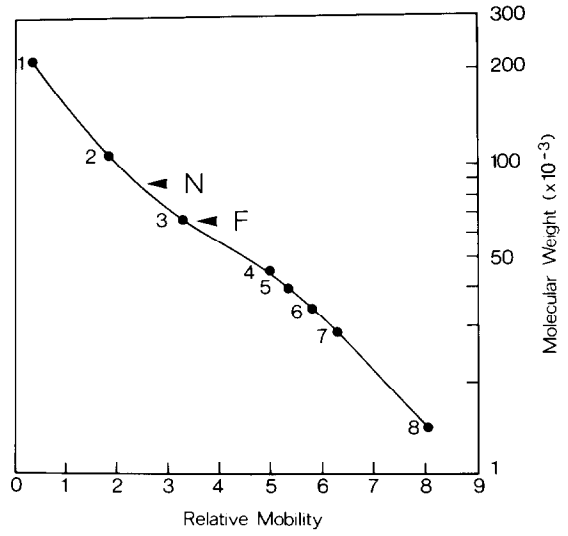

Fig. 1. Molecular weights of the protomers of methionyl-tRNA synthetase and its tryptic fragment 20 μg samples of each protein were oxidised with performic acid and subjected to SDS-gel electrophoresis, according to the method of Laemmli [5]. The mobilities were measured relative to that of the bromphenol blue marker. The proteins used (and their molecular weights) were: 1, myosin (220000); 2, oyster paramyosin (110000); 3, bovine serum albumin (66000); 4, muscle actin (48000); 5, aldolase (40000); 6, glyceraldehyde-3-phosphate dehydrogenase (35000); 7, carbonic anhydrase (29000); 8, lysozyme (14000). N, native methionyl-tRNA synthetase; F, tryptic fragment of methionyl-tRNA synthetase.

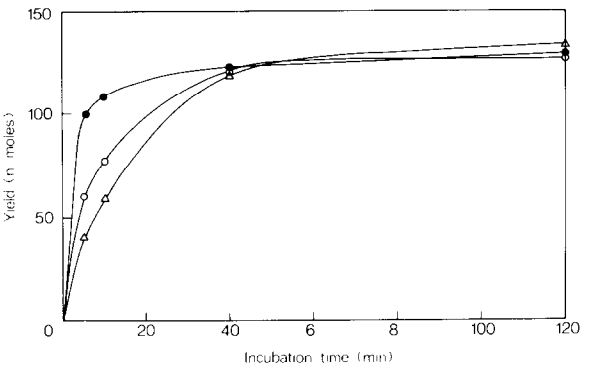

Fig. 2. Carboxypeptidase digestion of native methionyl-tRNA synthetase. 80 nmoles of enzyme were reduced and carboxymethylated in 6 M guanidine hydrochloride and digested with 400 μg carboxypeptidase A in 0.2 M *N*-ethylmorpholine acetate pH 8.5. Aliquots were removed at intervals, acidified with glacial acetic acid, centrifuged and lyophilised. Norleucine was added as the internal standard; the recoveries estimated from the amino acid analysis were corrected using the internal standard. The amino acids released were (•) alanine, (○) threonine, (△) valine.

protomers of this enzyme have relied substantially on ultracentrifugation in 6 M guanidine hydrochloride. In an attempt to reconcile my results with those obtained previously we have studied the enzyme by this method under the conditions used in previous studies. However, the sedimentation behaviour of the enzyme manifested considerably non-ideality which complicated the analysis of the data and the study was not pursued.

Strong evidence for the revised molecular weight of the protomer was obtained from quantitative analysis of carboxypeptidase digests of the enzyme. A measured amount of the carboxymethylated protein was digested with carboxypeptidase A and the release of amino acids monitored by amino acid analysis. The kinetics of the release of amino acids during digestion (fig. 2) indicated the following C-terminal sequence:

- - - VAL–THR–ALA–COOH

In the experiment shown 80 nmoles of enzyme (mol. wt 170000) were used and the yield of amino acids in the C-terminal sequence was about 130 nmoles showing that the enzyme contains two protomers and not four.

It has also been shown that native methionyl-tRNA synthetase can be converted into an active, crystallisable fragment by low concentrations of trypsin. The fragment was purported to be a dimer with two very similar protomeric units with molecular weights of 33000 each [3]. We have studied the structure of this fragment by SDS gel electrophoresis and found that it does not dissociate under the conditions used for electrophoresis and migrates with a mobility corresponding to a molecular weight of about 65000 (fig. 1). The implication of this result is that trypsin cleavage causes the removal of a segment with a molecular weight of about 20000 from the native protomer. In order to determine the region which trypsin removes during the formation of the fragment the N-terminal sequences of the native enzyme and the tryptic fragment were determined on the Automatic Sequencer. It was clear that both the native protomer and the fragment contained the following N-terminal sequence:

Ala–Gln–Val–Ala–Glu–Lys–Ile–Lue–Val–
Thr–Cys–Ala–Leu–Pro–Tyr–Ala- - - -

It follows that trypsin removes a region with a molecular weight of about 20000 from the C-terminus of the native protomer.

These observations show that the methionyl-tRNA synthetase from *E. coli* is a dimeric protein containing two very similar protomers with molecular weights of about 85000 each. The tryptic fragment which has been crystallised is a monomer generated by the removal of about 200 residues from the C-terminus of each protomer followed by dissociation of the modified enzyme. However, it is also clear that proteolysis of the enzyme can occur in a different way since Bruton and Hartley [4] have isolated another form of the enzyme which contains two very similar protomers with molecular weights about half that of the native protomers.

Presumably that form of the enzyme was generated by a split in the middle of the protomer. Some evidence is available which suggests that trypsin can also effect such a split in the middle of the native protomer albeit to a lesser extent than that which generates the 66K fragment (unpublished observation).

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