A POSSIBLE ROLE IN PROTEIN SYNTHESIS FOR THE RIBOSOMAL-BOUND AMINOPEPTIDASE IN *ESCHERICHIA COLI* B *

A.T.MATHESON and A.J.DICK**
Biochemistry Laboratory National Research Council of Canada
Ottawa, Canada

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1. Introduction

Protein synthesis in *Escherichia coli* is initiated by *N*-formyl methionine [1, 2]. Some time after the formation of the first peptide bond the *N*-formyl methionyl residue is removed to expose the *N*-terminal group found in the native protein. The removal of this residue is thought to involve two enzymes; peptide deformylase [3, 4] which removes the formyl group and a specific aminopeptidase which removes the methionyl group. Although several groups [4, 5, 6] have reported methionyl-splitting activity in crude bacterial preparations the nature of this peptidase is still unknown.

During an extensive study on the substrate specificity of a ribosomal-bound aminopeptidase in *E. coli* B it became apparent that the peptidase showed a rather narrow substrate specificity of such a nature to suggest it might be involved in the selective removal of the methionyl group from newly synthesized proteins. Some of the pertinent data is summarized in this communication.

2. Methods

E. coli B (NRCC No. 745) was grown at 30°C with vigorous aeration in a 150 l fermenter in a nutrient broth-salt-glucose medium [7], harvested in early log phase growth and stored at –40°C.

The ribosomes were isolated and washed five times by differential centrifugation as previously described [8]. The final traces of the soluble peptidases were removed by centrifugation through a discontinuous sucrose gradient.

The large scale purification of the ribosomal peptidase (r-peptidase) will be reported elsewhere [9]. A small scale purification of this enzyme has previously been described [10].

The peptidase activity was measured from the initial velocity using either the ninhydrin method [11] or the pH stat method [12]. Protein was measured by the method of Lowry et al. [13] using bovine serum albumin as standard. The substrates were obtained from Mann Research Laboratories or Fox Chemical Co.

3. Results

The substrate specificity of the r-peptidase to certain peptides is shown in table 1. In this experiment the enzyme was still attached to the sucrose-washed ribosomes. Similar results were obtained with the purified enzyme. The enzyme showed strong preference to leucyl and methionyl dipeptides but little or no activity to alanyl and seryl dipeptides except when the *C*-terminal amino acid was leucine or methionine.

As the size of the leucyl or methionyl peptide increased there was a substantial increase in the rate of hydrolysis of the *N*-terminal amino acid. For example, in the series methionyl-glycine, methionyl-glycyl-methionine and methionyl-glycyl-methi-

* N.R.C.C. No. 11027.
** N.R.C.C. Postdoctorate Fellow, 1967–1968; present address: Department of Biochemistry, University of Alberta, Edmonton, Alberta.

Table 1
Substrate specificity of r-peptidase.

C-terminal residue	N-terminal residue			
	L-leucyl-	L-methionyl-	L-alanyl	L-seryl-
-L-leucine	100	96	30	14
-L-methionine	86	80	18	10
-L-phenylalanine	38	25	7	5
-glycine	33	25	0	1
-L-valine	44	13	0	3
-L-serine	34	13	0	–
-L-alanine	30	11	0	0
-glycyl-glycine	79	87	0	0
-L-alanyl-L-serine		88		
-glycyl-L-methionine		155		
-glycyl-L-methionyl-L-methionine		165		

Reaction mixture contained 12.5 μM tris, pH 7.8, 0.25 μM Mn^{2+} and 0.5 mg washed ribosomes. These components were incubated at 30°C for 30 min, 2 μM substrate was added to give a total volume of 250 μl and 25 μl aliquots were removed at various times and assayed by the ninhydrin method [11] to obtain the initial velocity of the reaction. The rates of hydrolysis are shown as a percentage of the rate of hydrolysis of L-leucyl-L-leucine (0.026 μmoles/min/mg protein).

Table 2
K_m values for r-peptidase.

Substrate	Relative specific activity	K_m (mM)
L-methionyl-L-leucine	96	2.5
L-leucyl-L-leucine	100	9.5
L-alanyl-L-leucine	30	11.0

Conditions: Similar to table 1 except purified enzyme used (about 300 fold purification). The specific activity (μmoles substrate hydrolysed/min/mg protein) was set at 100 for L-leucyl-L-leucine.

onyl-methionine, the rate of hydrolysis of the *N*-terminal methionyl group increased over 6 fold as we went from the di- to the tetrapeptide. It should also be noted that the alanyl and seryl tripeptides were not hydrolysed by the r-peptidase.

Further evidence that the r-peptidase shows a preference for methionyl peptides was obtained from preliminary studies on the K_m values for various peptides. Table 2 shows the K_m values for the 3 peptides methionyl-leucine, leucyl-leucine and alanyl-leucine. Although the rates of hydrolysis of methionyl-leucine and leucyl-leucine were about equal, the K_m value for methionyl-leucine was 4 times lower than that for leucyl-leucine and alanyl-leucine and may indicate a tighter binding of the enzyme to methionyl peptides.

4. Discussion

Certain conditions must be met by any enzyme considered for the role of selectively removing methionine from the *N*-terminal position of newly synthesized proteins. Waller [14] observed that the *N*-terminal groups in *E. coli* proteins were not random but consisted mainly of methionine (45%), alanine (30%) and serine (15%). Capecchi [15] obtained data which suggest that *N*-terminal alanine and serine result from the hydrolysis of methionyl-alanine and methionyl-serine bonds. Our enzyme shows a strong preference for methionyl bonds as indicated by its

substrate specificity (table 1) and lower K_m value (table 2). It will readily hydrolyse methionyl-alanine and methionyl-serine bonds thereby exposing the *N*-terminal alanine and serine groups.

Capeechi [15] also concluded that *N*-terminal methionine groups in the native protein arise from the methionine moiety of the *N*-formyl-methionine initiator. This would indicate that certain methionyl bonds are not hydrolysed by the aminopeptidase. Of the 11 methionyl dipeptides tested so far (methionyl-histidine, methionyl-threonine, methionyl-glutamate, and methionyl-aspartate, in addition to those listed in table 1) only methionyl-aspartate was not hydrolysed to some extent by our enzyme. Only a small number of bacterial proteins containing *N*-terminal methionine have been sequenced, but in almost all these proteins the *N*-terminal methionine group is followed by either asparagine [16, 17] or glutamine [18, 19]. Therefore, as suggested earlier by Adams [4], when methionine is followed by asparagine or glutamine no hydrolysis should take place. We are at present making peptides containing methionyl-asparagine or methionyl-glutamine in the *N*-terminal position.

N-terminal alanyl or seryl bonds should be immune to further hydrolysis by the aminopeptidase. Of the alanyl and seryl dipeptides tested only those containing leucine or methionine in the *C*-terminal position are hydrolysed to any extent by our enzyme. These bonds, however, have so far not been found in the *N*-terminal position of *E. coli* proteins. Only a limited number of *E. coli* proteins containing *N*-terminal alanine or serine have been sequenced and in these cases the *N*-terminal group is followed by amino acids such as glycine (e.g. T4D phage head protein [20] with a *N*-terminal sequence of alanyl-glycyl-valyl —) and serine (e.g. R-17 phage coat protein [21] with a sequence of alanyl-seryl-asparagyl —). These bonds were not hydrolysed by our enzyme.

These initial results indicate a good correlation between the substrate specificity of our enzyme and the known *N*-terminal sequences of *E. coli* proteins. Further work is in progress to determine whether this enzyme is involved in the removal of the methionyl group during protein synthesis.

Recently, Vogt [22] has crystallized an aminopeptidase from the soluble fraction of *E. coli* K-12 which appears to have similar physical properties to the enzyme we have purified from the ribosomal fraction of *E. coli* B.

A full account of the substrate specificity and general properties of our enzyme will be reported elsewhere.

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