THE PRIMARY STRUCTURE OF RIBOSOMAL PROTEIN eL12/eL12-*P* FROM *ARTEMIA SALINA* 80 S RIBOSOMES

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1. Introduction

Protein eL12/eL12-*P* from *Artemia salina* 80 S ribosomes plays a role in the elongation step of protein synthetic reactions [1–3]. It is an acidic protein which was shown [2,3] to be partially phosphorylated on a single serine residue. Like the homologous proteins from procaryotic organisms, eL12/eL12-*P* from *A. salina* occurs in more than one copy per large subunit [3]; the isolated protein dimerizes in aqueous solutions [3].

We reported on the amino-terminal part of this protein [4] and on a peculiar, alanine-rich segment, located in the carboxy-terminal half of the protein chain [5]. Here, we wish to report the complete primary structure of eL12/eL12-*P* from *A. salina*. This structure is compared with the published primary structure of protein HL20 from *Halobacterium cutirubrum* [6]. Some possible, evolutionary relationships with its procaryotic L7/L12 counterpart are discussed.

2. Materials and methods

Protein eL12/eL12-*P* was isolated from cysts of *A. salina* as in [1,3]. Its purity was checked by sodium dodecylsulfate gel electrophoresis and isoelectric focussing. Normally, a mixture of the phosphorylated and non-phosphorylated form was used for sequence analysis. The protein was enzymatically digested with trypsin treated with 1-chloro-4-phenyl-3-tosylamidobutan-2-one (Serva; Worthington), in 0.2 M ammonium carbonate (pH 8.5) for 2 h at 37°C; the protein to enzyme weight ratio was about 50:1. Digestions with *Staphylococcus aureus* protease (Miles) were performed in 0.2 M ammonium carbonate pH 8.0 for 16 h at 37°C; the protein to enzyme weight ratio was about 25:1; microbial growth was prevented by adding a small quantity of toluene to the mixture. Digestions with pepsine (Worthington) were performed in 0.05 M HCl for 1 h at 37°C; the protein to enzyme weight ratio was about 50:1. The peptides were separated on thin-layer cellulose plates (Merck; Macherey und Nagel) by conventional methods of peptide mapping [5], and detected with 0.001% (w/v) fluorescamine [5]. Elution was performed with 20% (v/v) pyridine + 50 mM ammonia, or with 50% acetic acid. BrCN-treatment was performed in 80% formic acid and 0.05 M 2-mercaptoethanol for 48–72 h at room temperature, using a reagent to protein weight ratio of 20. After lyophilization, BrCN-peptides were separated by paper chromatography and chromatography on phenyl–Sepharose (Pharmacia), as will be reported in detail [7].

Amino acid analysis was performed on a Beckman Multichrom M amino acid analyzer. Liquid phase and solid phase sequencing of the amino-terminal part of the protein was done as in [4].

Most tryptic peptides were sequenced on the solid phase apparatus as in [5]. The PTH amino acids were identified by thin-layer chromatography, gas chromatography and by amino acid analysis after hydrolysis in HI [8] or 6 N HCl containing 0.1% (w/v) $SnCl_2$ [9]. Manual Edman degradation of the other peptides was based on published methods (cf. [10,11]); PTH amino acids obtained in this way were analyzed both by thin-layer chromatography, and on the amino acid analyzer after back-hydrolysis.

3. Results

The amino acid sequence of protein eL12/eL12-*P* from *A. salina* ribosomes is depicted in fig.1. The single polypeptide chain consists of 111 amino acids and starts with an unmodified methionine residue at its amino-terminal end. This situation is different from that of *E. coli* L7/L12, where the chain length is 120 residues, and where the amino-terminal serine residue is partially acetylated [12]. Serine residue 98,

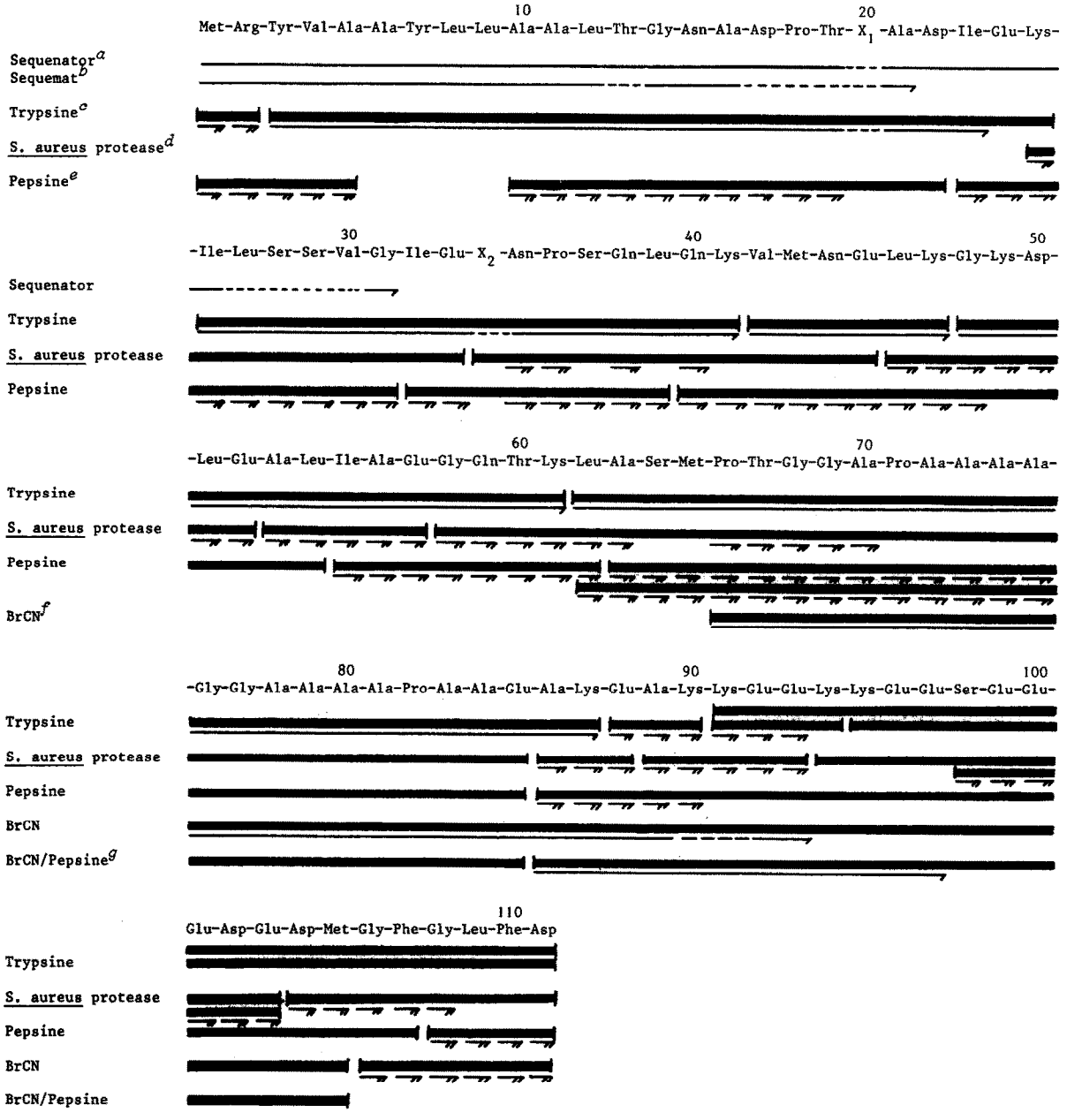

Fig.1

located in the carboxy-terminal acidic region of *A. salina* eL12, is the residue which is partially phosphorylated [2,3]. This modification is responsible for the occurrence of two forms having a slight difference in isoelectric point [2,3].

Residues 20 and 34 are not yet identified; on thin-layer chromatography, residue 20 gave a spot with the same mobility as that of PTH-Phe; gas chromatographic analysis after trimethyl sililation, however, revealed no peak. Residue 34 gave a spot with the mobility of PTH-Gln; amino acid analysis of a hydrolysate of this PTH amino acid revealed no clear peak. Amino acid analysis of peptides containing this residue showed a shoulder just before the elution of Gly.

It is worth noting that residue 24 is Glu instead of Gly (cf. [4]).

4. Discussion

If one compares the sequence of *A. salina* eL12/eL12-*P* with that of *E. coli* L7/L12, little or no homology is observable, perhaps with the exception of an alanine-rich stretch present in both proteins (fig.1; cf. [5]). This region comprises residue 70–86 in *A. salina* eL12(-*P*) and residue 34–48 in *E. coli* L7/L12. One could argue therefore whether any sequence similarity is not fortuitous.

However, if one compares the sequence of *A. salina* eL12 with that of another procaryotic protein, namely HL20 of *H. cutirubrum* [6], a different picture arises, see fig.2. Protein HL20 is the most acidic ribosomal protein of this extreme halophile organism. Although data on the function of this protein are lacking, its published primary structure shows a remarkable similarity with *A. salina* eL12, especially at the beginning and the end of the polypeptide chains. Moreover, both proteins contain a large, very acidic region in the carboxy-terminal part, preceded by a region, very rich in Ala (cf. [5]). In fact, if one accepts the deletions, indicated in fig.2, 25% of eL12 from *A. salina* and HL20 from *H. cutirubrum* are homologous. Interestingly, yeast ribosomes contain a similar protein as judged from the amino-terminal sequence, which illustrates the conservative character of at least this part of eucaryotic eL12 [4].

According to [13], HL20 from *H. cutirubrum* and L7/L12 from *E. coli* also show significant sequence homologies with respect to each other. If one lines up the first residue of *H. cutirubrum* HL20 with residue 35 of *E. coli* L7/L12, 13 out of the first 30 residues are in identical positions. Moreover, the region comprising residues 40–54 of *H. cutirubrum* HL20 show 50% sequence homology with the carboxy-terminal 15 residue segment of *E. coli* L7/L12 [6,13]. Apparently, *H. cutirubrum* HL20 and *E. coli* L7/L12 are related possibly by duplication of an ancestor of the *E. coli* L7/L12 gene as depicted in fig.3. From this scheme it follows that *H. cutirubrum* belongs to a group of procaryotes, which may bridge other procaryotic to eucaryotic organisms. Indeed, evidence has accumulated that *H. cutirubrum* has a number of biochemical properties common to eucaryotic cells [14]. However, it was suggested [15]

Fig.1. The primary structure of protein eL12/eL12-*P* from *Artemia salina* ribosomes. The peptides obtained by digestion of the intact protein with proteolytic enzymes and with BrCN, are indicated by the thick lines. The long arrows (——›) comprise regions determined by automatic Edman degradation; the small arrows (——›) represent manual Edman degradation, each arrow indicating an identified amino acid residue. Schematic outline of sequence determination:

[a] The intact protein was subjected to automated liquid phase Edman degradation [4]

[b] The intact protein was subjected to automated solid phase Edman degradation [4]

[c] Tryptic peptides were sequenced mostly by the solid phase technique [4]

[d] Peptides, obtained with *S. aureus* protease were often only partially sequenced due to the rather low amount of peptide material, obtained with this enzyme. The peptides derived from the amino-terminal part of the protein stick at the origin during peptide mapping, and were not used for sequence analysis

[e] Peptides, obtained with pepsine were sequenced manually as indicated

[f] BrCN peptide 66–105 was sequenced by solid phase methods; the first 28 residues could be identified. BrCN peptide 106–111 was sequenced manually. The BrCN peptides 1–43 and 44–65 were often isolated as one peptide. They were not used for sequence analysis

[g] BrCN peptide 66–105 was digested with pepsine, and the first 12 residues of the C-terminal half (86–105) were identified using solid phase methods

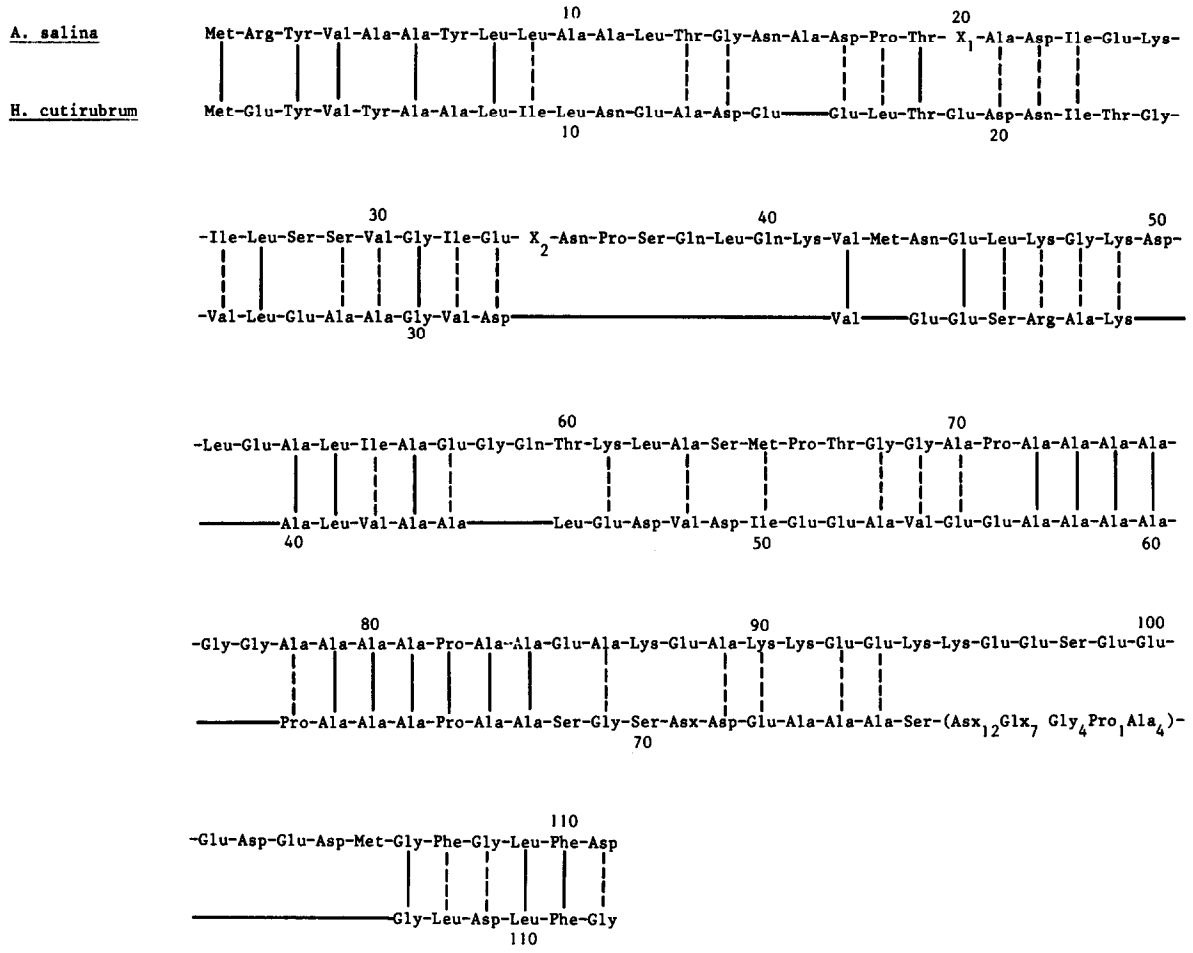

Fig.2. Comparison of the amino acid sequence of eL12/cL12-*P* from *Artemia salina* with that of HL20 from *Halobacterium cutirubrum*. Equal residues are indicated by solid lines between the two peptide chains; residues which may be related by single point mutations are indicated by broken lines.

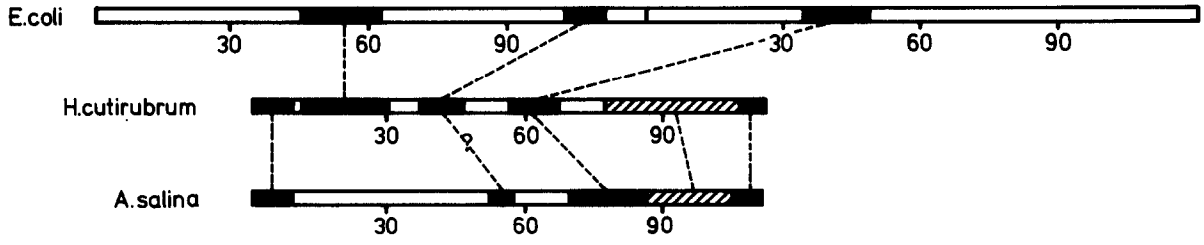

Fig.3. Model for the evolutionary relationship between *E. coli* L7/L12, *H. cutirubrum* HL20, and *A. salina* eL12/eL12-*P*. Homologous areas are connected with dashed lines. Hatched areas at the C-terminal part of *H. cutirubrum* HL20 and *A. salina* eL12/eL12-*P* represent the corresponding acidic regions; the homology between these regions is not known, due to incomplete sequence information about the *H. cutirubrum* protein.

that there are three separate lines of descent, one leading to the eubacteria (e.g., *E. coli*), a second to the archaebacteria to which *Halobacterium* supposedly belongs, and a third to the eucaryotes. Sequence analysis of L7/L12-type ribosomal proteins from the methanobacteria [6] may help to decide whether archaebacteria are indeed a separate class in itself.

Finally, it is remarkable to note that tubulin also contains a carboxy-terminal polypeptide stretch, which is very rich in Glu and possesses also a serine residue, which is partially phosphorylated [16]. Whether this analogy reflects a common structural principle, remains to be seen. A full account of the sequence work on *A. salina* eL12/eL12-*P* will be published [7].

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