A COMPARISON OF THE ALANINE-RICH SEQUENCES OF THE L7/L12-RIBOSOMAL PROTEINS FROM RAT LIVER, *ARTEMIA SALINA* AND *ESCHERICHIA COLI*, WITH THE AMINO-TERMINAL REGION OF THE ALKALI LIGHT CHAIN A_1 FROM RABBIT MYOSIN

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1. Introduction

Bacterial ribosomes contain multiple copies of L7/L12, a 12 000 mol. wt acidic protein, which is involved in elongation factor-dependent GTP hydrolysis [1]. A similar protein has been detected in the ribosomes of a number of eucaryotic organisms [2], and primary structure studies of the proteins of procaryotes and eucaryotes have been initiated [3–5]. Recently, it was found that the amino-terminal region of L7/L12-protein from *Artemia salina* and *Saccharomyces cerevisiae* shows a sequence homology with the corresponding region of the protein from *Halobacterium cutirubrum* [6]. During our work on *A. salina* L7/L12, we found a large and alanine-rich tryptic peptide which is derived from the carboxy-terminal part of the molecule. Moreover, a homologous peptide was isolated from rat-liver L7/L12, and the two peptides were sequenced by solid-phase techniques. A substantial conservation of this part of the primary structure was observed.

Because of possible structural similarities between protein L7/L12 and certain motile proteins [7], we also compared the sequence of these alanine-rich peptides with known sequences of muscle proteins.

In this report, we present evidence of a remarkable sequence similarity between the alanine-rich regions of *Escherichia coli*, *A. salina* and rat-liver L7/L12 protein, and the amino-terminal region of the alkali-light chain A_1 of rabbit myosin.

2. Materials and methods

80 S ribosomes from *A. salina* cysts were prepar as in [8]. The preparation of 80 S ribosomes from rat-liver was essentially as in [9], after homogeniza tion of the liver tissue in a Potter homogenizer. Th L7/L12-type proteins were extracted from the ribo somes by the ethanol/ammonium chloride method and purified by column chromatography on CM- ar DEAE-cellulose, using some modifications of [8] t improve the yield.

Mapping of tryptic peptides according to standa techniques was performed either on paper (Whatm 3 MM), or on prewashed cellulose thin-layer plates (Merck). Peptides used for sequencing were detecte by staining the peptides as lightly as possible with 0.05% (w/v) ninhydrin in acetone, or with 0.001% (w/v) fluorescamine in acetone [10] after spraying with 2% (v/v) puridine in acetone. Identification of the alanine-rich peptides was facilitated by their characteristic shape on thin-layer plates (fig.1). Peptides were eluted with 25% (v/v) pyridine. The peptides, coupled to *p*-phenylenediisothiocyanate-treated aminopropyl glass (cf. [11]) were subjectec to Edman degradation [12] on a Sequemat solid-phase peptide sequencer. Phenylthiohydantoin ami acids were identified by thin-layer chromatography and, semi-quantitatively, by amino acid analysis aft back hydrolysis [13]. Residues which are unequivc cally determined by both identification methods ar

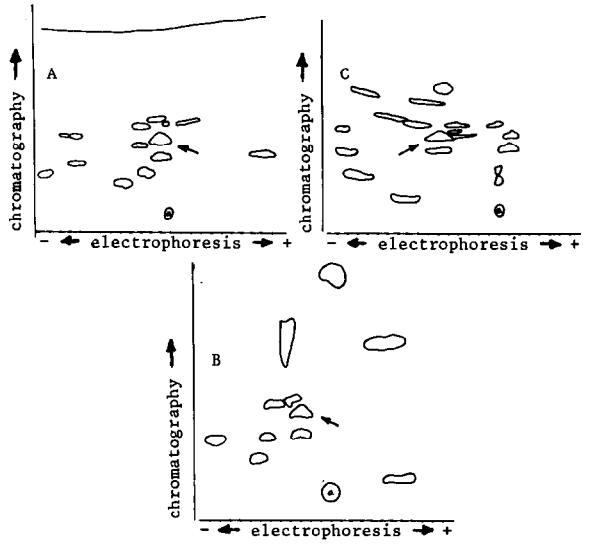

Fig.1. Peptide maps of *A. salina* and rat-liver L7/L12 ribosomal proteins. (A) *A. salina* L7/L12 digested with trypsin. (B) *A. salina* L7/L12 digested with *Staphylococcus aureus* protease. (C) Rat-liver L7/L12 digested with trypsin. The alanine-rich peptide in the maps is indicated by an arrow.

underlined. Less than 100 nmol coupled peptide was used in the degradation procedure. Yet, it was possible to identify the residues easily beyond the 20th degradation cycle, because of the very high repetitive yield (>95%), which appeared to be related to the nature of the peptide under investigation. The analysis of the peptide from *A. salina* L7/L12 was performed twice; the sequence starting at the 5th residue was confirmed by a BrCN peptide derived from this part of the polypeptide chain. The sequence of the peptide from rat-liver L7/L12 was also determined twice.

3. Results and discussion

There is a striking homology in the alanine-rich regions of L7/L12 protein from rat-liver and *A. salina* (fig.2). Moreover, there is also a substantial analogy with the alanine-rich sequence of *E. coli* L7/L12 protein (fig.2). A large part of the differences between the three sequences can be explained by single-point mutations.

Consideration of the complete sequence of procaryotic and eucaryotic L7/L12 shows the following: In *E. coli* L7/L12, the alanine-rich region is situated in the amino-terminal part of the polypeptide chain, whereas in the *A. salina* and rat-liver protein these regions are located in the carboxy-terminal part (in preparation). Simultaneously, the amino-terminal region of eucaryotic and *H. cutirubrum* L7/L12 protein shows a sequence relationship with a restricted portion of the alanine-rich sequence of *E. coli* L7/L12, the alignment starting at position 35 of the latter chain [6].

In our opinion, the universal occurrence of an alanine-rich section in a distinct protein of the large subunit of ribosomes supports the idea of a special function in protein biosynthesis.

A possible hint towards this function may be obtained from a comparison with sequences of motile proteins [7]. Indeed, in the amino-terminal region of the alkali light chain A_1, a sequence rich in alanine has been found [14]. If we align this chain with the two

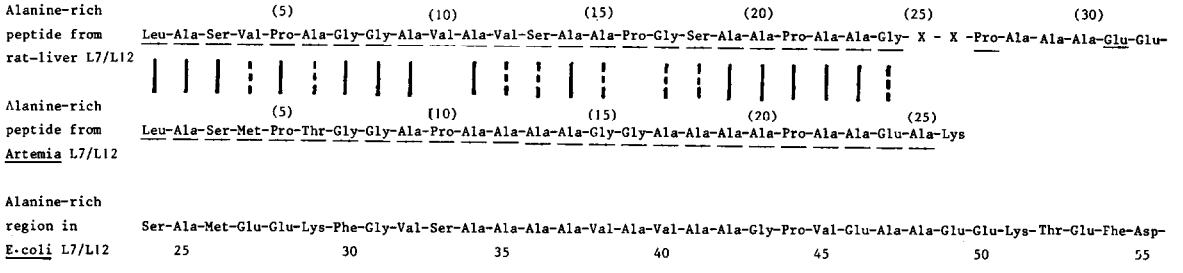

Fig.2. Comparison of the alanine-rich tryptic peptides from L7/L12 protein from *A. salina* and rat-liver, with the alanine-rich region in L7/L12 protein from *E. coli*. Residues which were unequivocally determined by both thin-layer chromatography and amino acid analysis are underlined. Equal residues are indicated by solid lines; residues which may be related by single point mutations are indicated by broken lines.

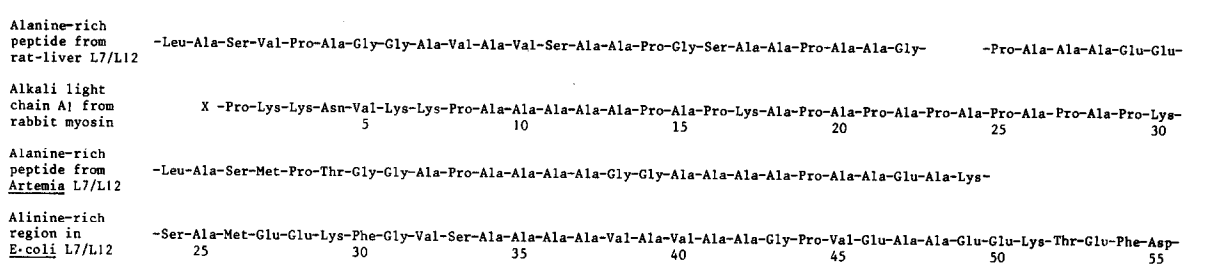

Fig.3. Comparison of the alanine-rich sequences of the L7/L12 proteins from rat-liver, *A. salina* and *E. coli*, with the amino-terminal region of the alkali light chain A_1 from rabbit myosin.

eucaryotic L7/L12 proteins in a manner as depicted in fig.3, within this 30-residues region a sequence similarity of 10 amino acid residues in the case of rat-liver L7/L12, and 9 in that of *A. salina* L7/L12, is observed. It should be emphasized that a comparable sequence similarity is obtained when the position of the alkali light chain is shifted with respect to the L7/L12 chains a few residues in either direction. Undoubtedly this recurrence in overlap is intrinsic to the high content of one amino acid (alanine) and the paucity of variation of the other amino acids in the regions compared. As far as we know, this type of clustering of alanine residues has not been observed in other proteins so far sequenced, and may therefore reflect a property shared by these two classes of proteins. It is interesting to note that the amino-terminal sequence of the A_1 moiety of myosin has been correlated with strengthening the interaction between myosin and actin [15,16], while the function of the L7/L12 proteins is believed to be related with a transient binding site for the elongation factors [1]. Moreover, there are indications that actin and elongation factor Tu show a hitherto unknown structural relationship ([17] cf. [18]). The analogy between muscle contraction and factor-dependent translocation requiring nucleotide hydrolysis has been noted [19].

The evaluation of the observed sequence similarity is presently difficult and should await perhaps a complete three-dimensional structure determination of both kinds of proteins.

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