AN ACIDIC, ALANINE-RICH 50 S RIBOSOMAL PROTEIN FROM *HALOBACTERIUM CUTIRUBRUM*: AMINO ACID SEQUENCE HOMOLOGY WITH *ESCHERICHIA COLI* PROTEINS L7 and L12

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1. Introduction

Halobacterium cutirubrum is a rather unique cell type in that it requires near-saturated, intra and extracellular salt concentrations for growth [1]. Moreover, the translation process in protein synthesis and the constituents thereof have adapted to these ionic conditions [2–4].

The 50 S ribosomal subunits from *H. cutirubrum* contain an alanine-rich, acidic protein L20 [2,5]. Its high alanine content (over 25%), its acidity and its lack of histidine, cysteine and tryptophan residues make this protein a possible equivalent to the only sequenced 50 S ribosomal protein L7/L12 of *E. coli*, which is known to be involved in elongation factor G dependent hydrolysis of GTP [6]. A large tryptic peptide (L20-T4), which belongs to the carboxyl-terminal half of the protein, has been isolated from *H. cutirubrum* L20. The amino-terminal regions of L20 and of the peptide L20-T4 have been sequenced. The results indicate a high degree of homology of the first 30 residues of L20 with the central region of *E. coli* L7/L12 and the first 15 residues of L20–T4 with the carboxyl-terminal region of *E. coli* L7/L12. Although homologies between ribosomal proteins from *E. coli* and *Bacillus stearothermophilus* were previously reported [7,8], this is the first definite evidence of amino acid sequence homology between the proteins from *E. coli* and *H. cutirubrum*.

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2. Materials and methods

The ribosomes were isolated from *H. cutirubrum* cells grown at 42°C [3]. Fractionation of the ribosomal proteins was by DEAE-cellulose column chromatography [5], with the isolated *H. cutirubrum* L20 being identified by two-dimensional electrophoresis [2] and amino acid composition [5].

Tryptic peptides of L20, prepared by incubation at 37°C for 3 hr with a 1% (weight enzyme: weight protein) trypsin solution, were fractionated by gel filtration chromatography on a Sephadex G-50 superfine column (1.5 × 270 cm) with distilled water as the eluant. The resulting peaks, which were monitored at 230 nm, were analysed for amino acid composition with a Durrum D-500 amino acid analyser after hydrolysis at 110°C for 18 hr with 6 N HCl in vacuo. Peptide L20-T4 was eluted soon after the void volume and was well separated from other smaller tryptic peptides.

The amino-terminal sequence of protein L20 (6 mg) and L20-T4 (2 mg) was determined by automatic Edman degradation [9] using a Beckman Model 890C sequencer with the quadral protein program. The thiazolinone derivatives, converted to their PTH-derivatives, were identified by thin-layer chromatography on silica gel plates as described by Wittmann-Liebold [10] The thiazolinone or PTH-derivatives were hydrolysed separately with 6 N HCl and H1 [11] at 130°C for 20 hr, and the amino acid formed was analysed with a Durrum D-500 amino acid analyser.

3. Results and discussion

The first thirty residues of the protein L20 has been established as:

$$\begin{array}{l} \overset{1}{} \qquad\qquad\qquad\qquad \overset{5}{} \\ NH_2\text{–Met–Glu–Tyr–Val–Tyr–Ala–Ala–Leu–} \\ \qquad\quad \overset{10}{} \qquad\qquad\qquad\qquad\quad \overset{15}{} \\ \text{Ile–Leu–Asn–Glu–Ala–Asp–Glu–Glu–Leu–} \\ \qquad\qquad\quad \overset{20}{} \qquad\qquad\qquad\qquad \overset{25}{} \\ \text{Thr–Glu–Asp–Asp–Ile–Thr–Gly–Val–Leu–} \\ \qquad\qquad\qquad\quad \overset{30}{} \\ \text{Glu–Ala–Ala–Gly} \end{array}$$

A repetitive yield of 92.8% is obtained.

The amino acid composition of the large peptide L20-T4 is shown in table 1. This peptide consists of 65 residues and contains only 9 different amino acids. The first 15 residues of its amino-terminal region is established as:

–Ala–Leu–Val–Ala–Ala–Leu–Glu–Asp–Val–
Asp–Ile–Glu–Glu–Ala–Val–

Fig. 1 shows the recovery of amino acids after 6 N HCl hydrolysis of each residue of peptide L20-T4. The repetitive yield is 91.5%. The thin-layer chromatography shows that all aspartic and glutamic acid residues are in acidic forms and not in their amide forms. The HI hydrolysates further confirms the sequence established above. This amino-terminal region contains most of the hydrophobic residues (Val, Ile and Leu) of L20–T4.

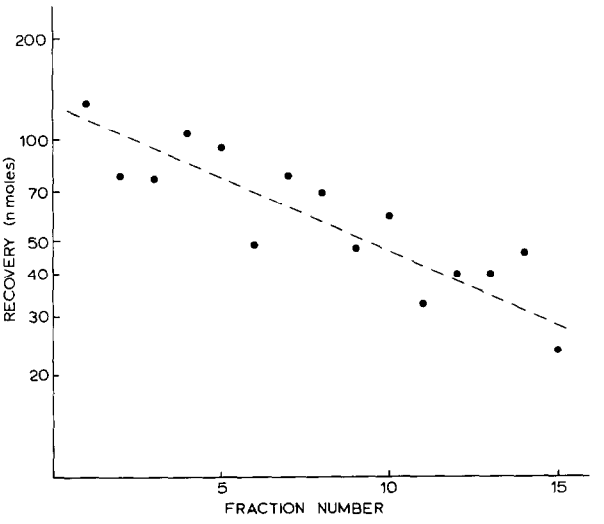

Fig. 1. Semi-log plot of amino acid recoveries against residue number of Peptide L20-T4. Yields were calculated from amino acid analysis after hydrolysis at 130°C, 20 hr with 6 N HCl.

Table 1
Amino acid composition of tryptic peptide L20-T4 of acidic, alanine-rich ribosomal protein L20 from *Halobacterium cutirubrum*

	Molar ratio*	Proposed residues
Asx	14.00	14
Ser	2.82	3
Glx	12.16	12
Pro	2.65	3
Gly	5.97	6
Ala	19.83	20
Val	3.04	3
Ile	1.11	1
Leu	3.09	3
Total	64.67	65

* Each value is adjusted so that the value for aspartic acid is 14.00. The following corrections for incomplete hydrolysis or decomposition were applied: serine 10%, valine 5%, isoleucine 10%.

This amino acid sequence of this peptide does not correspond to the known sequence of the amino-terminal of the protein L20 (above) of *H. cutirubrum*. Since all tryptic peptides which belong to the amino-terminal half of this protein are isolated, L20-T4 must belong to the carboxyl-terminal half of this protein.

When the amino-terminal region L20-T4 of *H. cutirubrum* is compared with the amino acid sequence [6] of *E. coli* L7 and L12, it becomes evident that this portion is homologous to the carboxyl-terminal region of the *E. coli* proteins (fig. 2). Seven out of the 15 residues are identical to each other: there are two pairs of alanine, leucine and glutamic acid and a pair of valine. In addition, 6 other residues can be formed by a single nucleotide change in the codons. The apparent base change per codon for the 15 residues is only 0.67, thus this homology is highly significant.

In contrast, this high degree of homology is not evident in the amino-terminal region of the proteins: the first 15 residues of *H. cutirubrum* L20 have no ho-

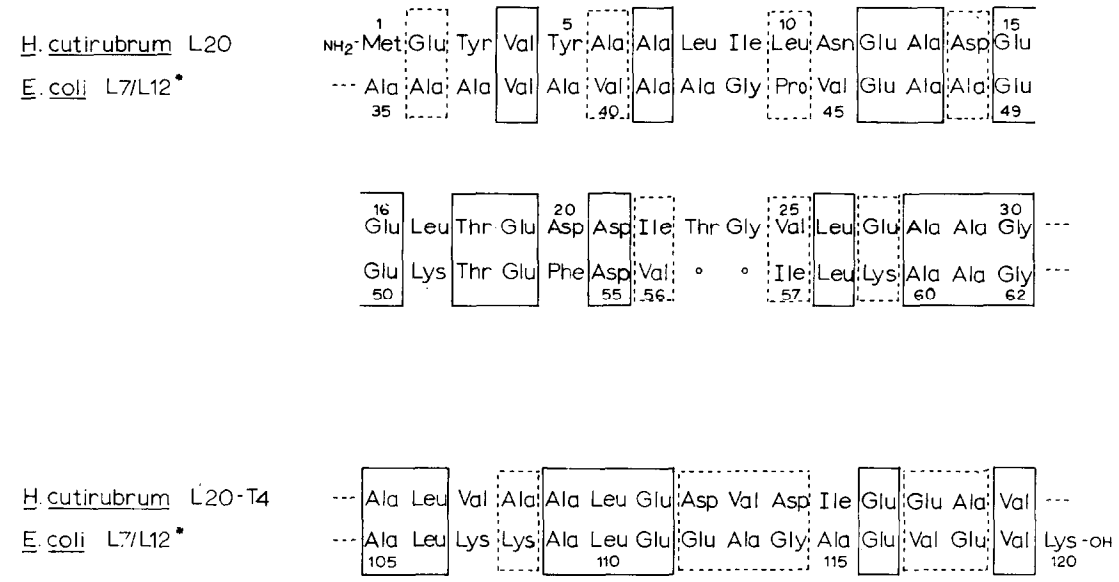

Fig. 2. Comparison of homologous sequence between *H. cutirubrum* L20 and *E. coli* L7/L12* (L7 and L12 of *E. coli* are identical except that L7 possesses an amino-terminal acetyl group (ref. [6])): Residues shown within solid boxes are identical, and those within dotted boxes are related by single point mutation.

mology with either *E. coli* L7/L12 or *B. stearothermophilis* A_2 (fig. 3). However, if *H. cutirubrum* L20 is aligned with *E. coli* L7/L42 as in fig. 2 homology is found. These apparent structural homologies are striking as the L20 protein from *H. cutirubrum* shows no immunological cross-reactivity with the antisera raised to either *E. coli* L7/L12 or *B. stearothermophilus* A_2 [5]. The data confirms our view that homologies between distantly related ribosomal proteins are best shown by direct sequence analysis as other less rigorous techniques are often misleading.

H. cutirubrum requires nearly saturated solutions of NaCl for growth [1], and its ribosomal proteins are largely acidic, unlike those for other cell types [3,4]. Despite these physiological differences and apparent structural dichotomies, the fact that regions of *E. coli* and their corresponding region of *H. cutirubrum* L20 are highly conserved during the evolution of these dinstinct procaryotic cell types suggest these portions of these molecules must have some real importance in protein synthesis.

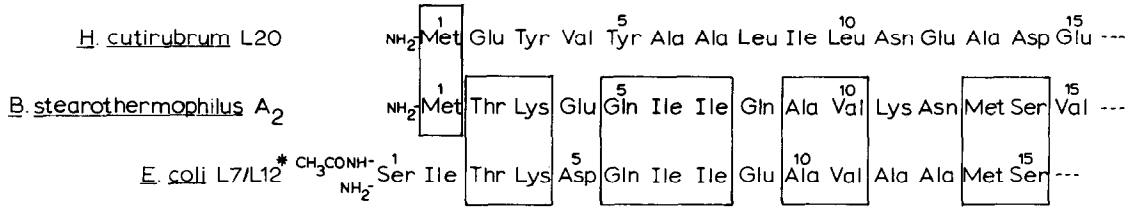

Fig. 3. Comparison of the amino-terminal amino acid sequence of protein 120 from *H. cutirubrum* with the amino-terminal regions from *E. coli* (ref. [6]), and *B. stearothermophilus* A_2 (ref. [8]).* L7 and L12 of *E. coli* are identical except L7 has an amino-terminal acetyl group. Residues shown within solid boxes are identical.

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