PRIMARY STRUCTURE OF YEAST ACIDIC RIBOSOMAL PROTEIN YP A1

Takuzi ITOH

Department of Biochemistry and Biophysics, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima, Japan

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1. Introduction

The ribosomal acidic protein L7/L12 of *Escherichia coli* is important in the expression of the function of elongation factors [1]. Ribosomes from other bacterial species have proteins homologous to L7/L12. The ubiquity of these proteins as ribosomal constituents is indicated by a high degree of conservation in their N-terminal amino acid sequence in prokaryotic or in eukaryotic organisms [2–5]. Comparative primary structural analyses of these proteins would give some insights into the evolutionary changes of the ribosomes as well as the nature of the active sites within the molecules.

The complete primary structure of an L7/L12-equivalent from *Bacillus subtilis* and its comparison with those from other prokaryotes has been made [6,7]. The primary sequence of an acidic protein from the large subunit of a brine shrimp *Artemia salina* has been reported and its comparison with other sources presented [8]. We now report the complete primary structure of yeast acidic ribosomal protein, YP A1. This structure is compared with the published primary structures from other organisms such as *E. coli* [9], *B. subtilis* [6], *Halobacterium cutirubrum* [10] and *A. salina* [8]. The evolution and correlation of these acidic proteins are discussed.

2. Materials and methods

An acidic ribosomal protein YP A1 was isolated from *Saccharomyces cerevisiae* as in [11]. The identity and purity of the protein were checked by two-dimensional SDS–polyacrylamide gel electrophoresis [12] and two-dimensional urea–polyacrylamide gel electrophoresis [13]. Enzymatic digestions were performed with TPCK-trypsin for 3 h, or with chymotrypsin at 37°C for 1 h, in 0.1 M methylmorpholine–acetate buffer (pH 8.1). Thermolysin digestion was done at pH 8.1 for 2 h at 52°C and *Staphylococcus aureus* protease digestion was at 37°C (pH 8.1) for 20 h. Digestion with pepsin was in 0.05 M HCl at 37°C for 2 h, and with carboxypeptidase Y from yeast in 0.1 M pyridine–acetate buffer (pH 5.5) at 37°C for 15,30 and 60 min [14].

Peptides were isolated by the fingerprint technique on thin-layer plates (Cel 300 from Macherey and Nagel, Düren) at pH 6.5 in 10% pyridine-acetate or by gel filtration on Sephadex G-50 (superfine) column (120 × 0.7 cm) [7]. Elution was made with 15% acetic acid. Amino acid analyses were performed after hydrolysis in 5.7 N HCl–0.02% 2-mercaptoethanol with a Beckman 121M analyser. Determination of the amino acid sequences within the peptides was performed by the manual micro-Edman technique with dansylation [15] or with the double-coupling method with 4-*N*,*N*-dimethylaminoazobenzene 4′-isothiocyanate/phenylisothiocyanate [16].

3. Results and discussion

The primary structure of protein YP A1 was established by the required overlapping peptides produced by various enzyme digestions. The N-terminal region of the protein was determined by automatic Edman-degradation in a Beckman sequenator [5]. Treatment of the protein with trypsin gave 8 peptides which, with the exception of T8, were completely sequenced manually. The largest peptide T8, which

* This paper is 4th in the series, Yeast Ribosomal Proteins; the preceding paper is [11]

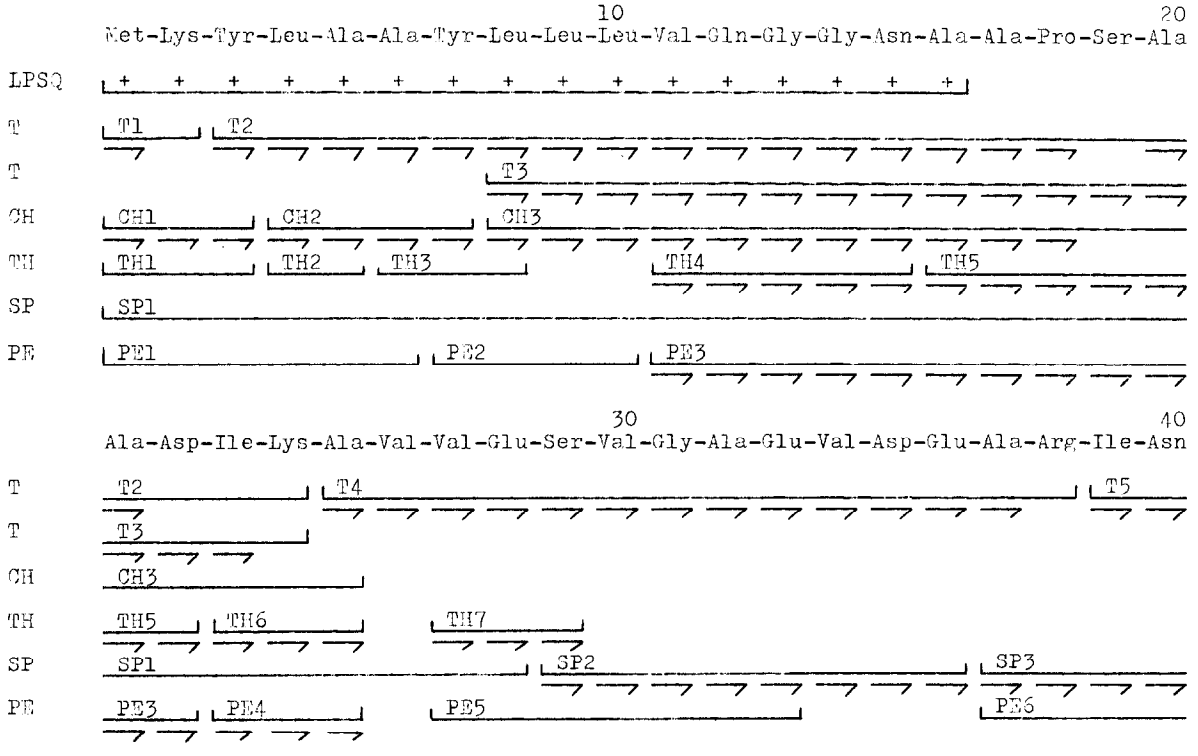
was purified through Sephadex G-50 in 15% acetic acid, was split into 3 peptides (T8-SP1, T8-SP2 and T8-SP3) by *S. aureus* protease digestion, or into 4 peptides (T8-TH1, T8-TH2, T8-TH3 and T8-TH4) by thermolysin. These peptides were sequenced manually. Digestion of the protein with thermolysin, chymotrypsin, *S. aureus* protease and pepsin resulted in 12,5,10 and 6 peptides, respectively. These peptides were sequenced manually. The long peptide SP7 was sequenced manually as far as possible, and was completed by treating it with other enzymes. Treatment of the protein with carboxypeptidase Y released the aspartic acid, phenylalanine and leucine from the C-terminal region. The combination of these results gave an alignment of all peptides and therefore the entire amino acid sequence as shown in fig.1.

The amino acid composition of YP A1 derived from its sequence is:

Asp_7, Asn_2, Thr_2, Ser_9, Glu_{15}, Gln_2, Pro_3, Gly_{15}, Ala_{21}, Val_6, Met_2, Ile_4, Leu_9, Tyr_2, Phe_3, Lys_7, Arg_1.

This is in good agreement with the data obtained from the complete hydrolysis of the protein. YP A1 consists of 110 amino acid residues and has mol. wt 11 020.

By comparing the N-terminal regions of the acidic proteins, it was found [5] that there exists a considerable sequence similarity among yeast (YP A1), *A. salina* and a halophilic bacterium, *H. cutirubrum*. Using the complete amino acid sequence of YP A1 presented here, the similarity was calculated to be 50% between *A. salina* [8] and yeast. In these two eukaryotic proteins, 4 highly conserved regions were found: two clusters of hydrophobic amino acids, one near the N-terminal:
Tyr–Leu–Ala–Ala–Tyr–Leu–Leu (residues 3–9)
and another near the C-terminal:
Asp–Met–Gly–Phe–Gly–Leu–Phe–Asp (residues 106–113);
and two sequences in the middle part (residues 56–60 and residues 67–71) (fig.2). In *H. cutirubrum* (a member of metabacteria [17]), the size of the ribosome and the number of ribosomal proteins are of the eubacterial type, and yet the acidic protein (A-protein; H20) from this bacterium showed a considerable sequence similarity to the YP A1 (31%) when the 58 residues from the N-terminal are compared (fig.2). On the other hand, there is very little apparent sequence similarity between the eukaryotic YP A1 and the eubacterial L12 either from *E. coli* [8] or from *B. subtilis* [6]. Despite this sequence dis-



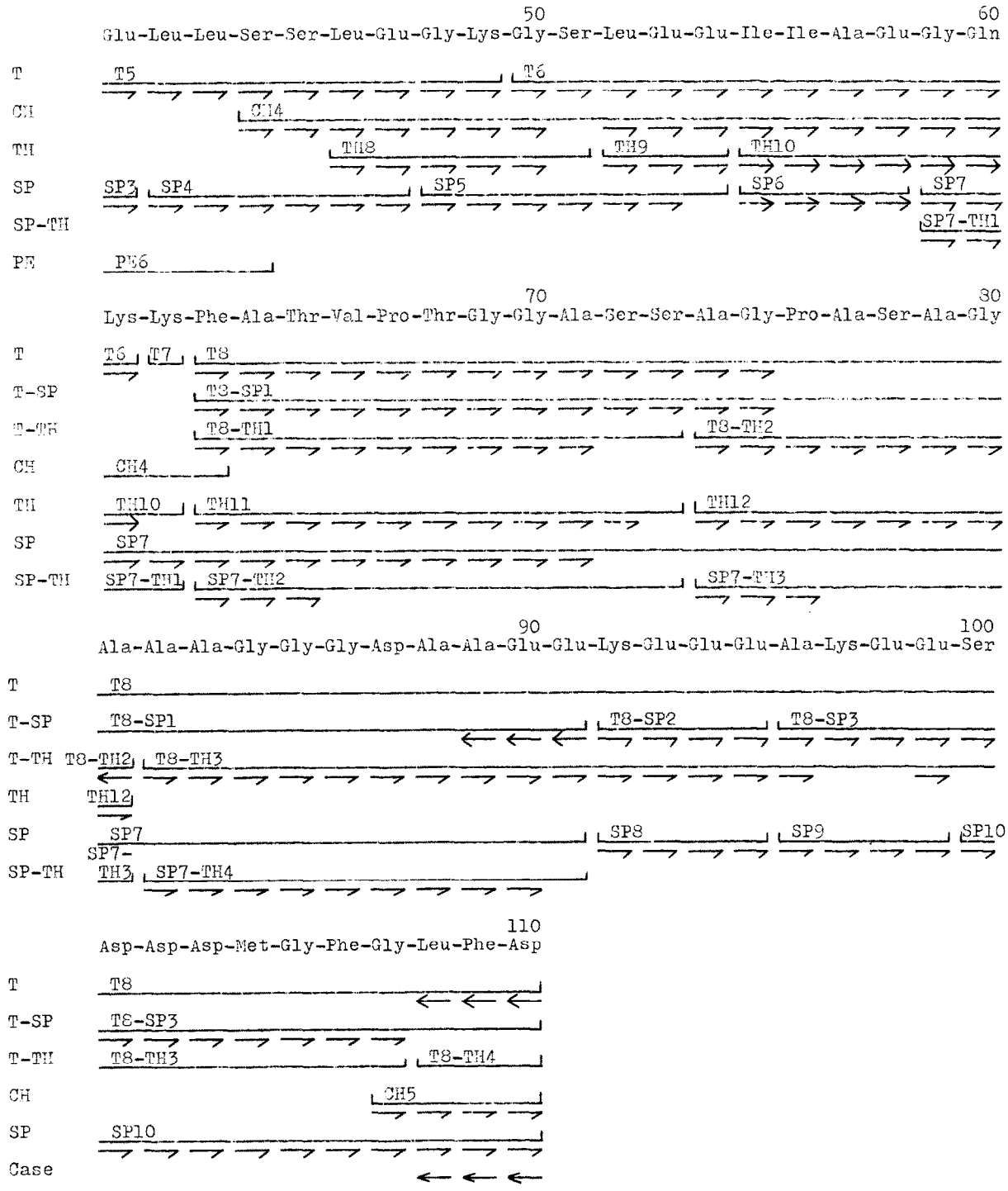


Fig.1. Primary structure of protein YP A1 from *Saccharomyces cerevisiae*. *Abbreviations:* LPSQ, liquid-phase sequenator; T, tryptic digestion; CH, chymotryptic digestion; SP, digestion with *Staphylococcus aureus* protease; TH, digestion with thermolysin; PE, digestion with pepsin; SP-TH, thermolytic digestion of SP-peptide; T-TH, thermolytic digestion of tryptic peptide T8; T-SP, *S. aureus* protease digestion of tryptic peptide T8; case Y, carboxypeptidase digestion.

The following symbols are used for the methods by which the individual peptides were sequenced: (+), identification by automatic liquid phase sequenator; (→), identification as the dansylated amino acids with combined dansyl-Edman degradation technique; (—→), identification as the DABTH-amino acids to use the double-coupling method with 4-*N*,*N*-dimethylaminoazobenzene-4'-isothiocyanate/phenylisothiocyanate; (←), identification as amino acids released after carboxypeptidase Y digestion.

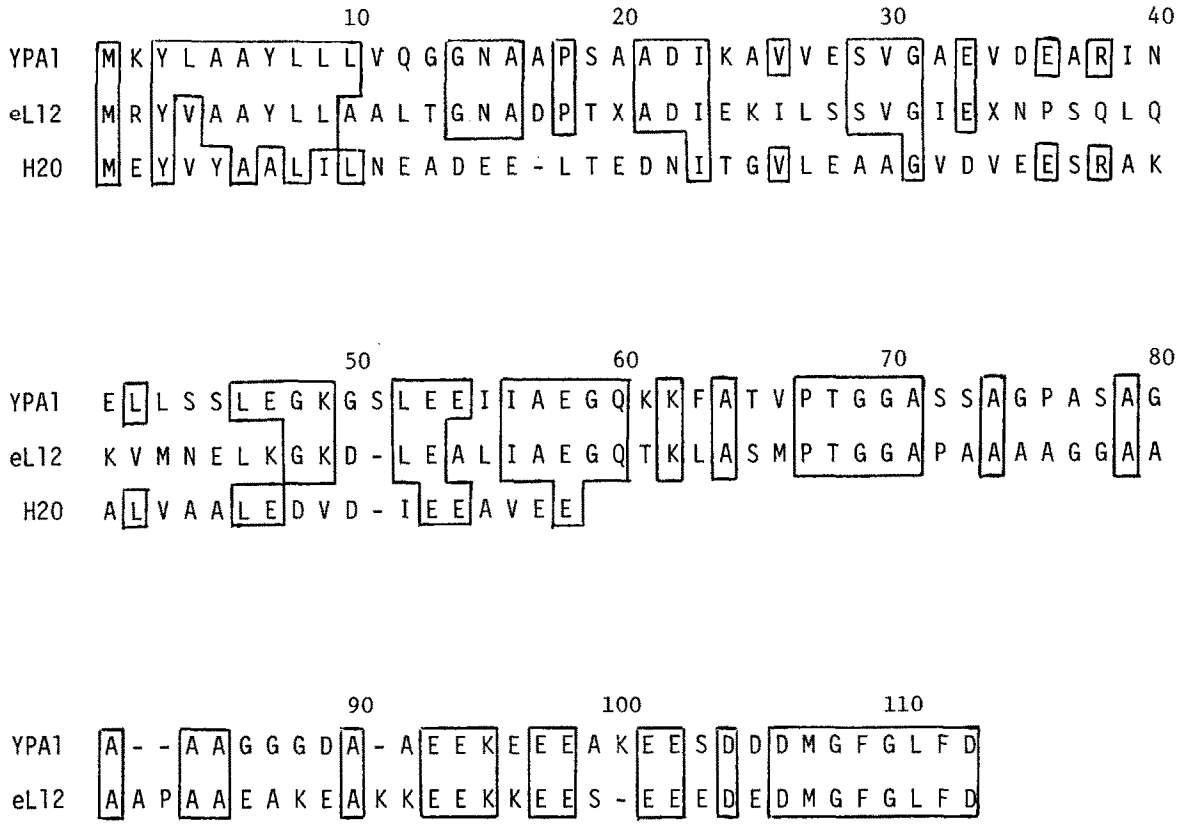


Fig.2. Similarity of amino acid sequences of acidic ribosomal proteins among yeast, *Artemia salina* and *Halobacterium cutirubrum*. *Abbreviations:* YP A1, yeast acidic protein; eL12, *A. salina* acidic protein; HL20, acidic protein from *H. cutirubrum*. The regions identical to YP A1 are boxed.

similarity, all of these acidic proteins reveal several common structural features throughout eubacteria (*E. coli* and *B. subtilis*) and eukaryotes (yeast and *A. salina*) [18]. These are the presence of:

(i) The alanine-rich region in the C-terminal half in the eukaryotic protein and the N-terminal half in the prokaryotic protein;

(ii) The acidic amino acid-clustering region in the carboxy-terminal region both in the eukaryotic and the prokaryotic proteins;

(iii) Unique secondary structure of β-turn or bend structure [19] in one of the highly conserved regions, Pro–Thr–Gly–Gly–Ala (residues 67–71) in the eukaryotic, and Thr–Gly–Leu–Gly–Leu–Lys–Glu–Ala–Lys (residues 76–84) in the prokaryotic proteins.

There are two possibilities for the evolutionary relationships among eukaryotic, metabacterial and eubacterial acidic proteins:

(1) These proteins are homologous, sharing a common ancestor. If we assume that the eubacterial branching occurred first among the 3, the sequence similarities suggest that metabacteria diverged next and finally eukaryotes. These relationships were also recognized in the 5 S RNA phylogenic tree [17,20]. Some extensive changes, such as insertion, deletion, translocation or duplication [8], might have occurred in the acidic protein somewhere between the branching point of eubacteria and that of metabacteria (supposing the eubacterial-type as the prototype).

(2) That these proteins have different ancestors; several common features in these proteins discussed above might be brought about by what we may call convergence, to attain the similar function.

At present we do not know which possibility is correct.

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