COMPARISON OF PEPTIDE PATTERNS FROM ISOLATED 30 S AND 50 S RIBOSOMAL PROTEINS OF *ESCHERICHIA COLI* BY COLUMN CHROMATOGRAPHY

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1. Introduction

E. coli ribosomes contain a large number of ribosomal proteins whose structural homology has not been established. Information about such homology can be obtained by comparison of the peptide patterns from isolated ribosomal proteins. The present paper describes the tryptic digestion of 43 pure ribosomal proteins isolated from 30 S and 50 S subunits of *E. coli* and the separation of the tryptic peptides by column chromatography. An abstract of this work has appeared elsewhere [1]

2. Methods and materials

Ribosomal proteins were purified as described previously [2, 3] and numbered according to [4, 5]. Many of the lyophilized proteins were difficult to dissolve in ammonium-bicarbonate buffer, pH 8, or in H_2O. The following general procedure was therefore adopted: about 1 mg of protein was dissolved in 0.2 ml freshly prepared 8 M urea. The solution was diluted with water to 1 M urea at which most proteins remained soluble and digestion with TPCK-trypsin (Worthington, Freehold, N.J.) was carried out in the pH-stat at pH 8.3 at 25° for 8 hr. The final enzyme to substrate ratio (w/w) was 1:50. Enzyme was added in two portions, at the start and after 4 hr. Digested solutions were immediately frozen and lyophilized. With several proteins (S2, S7, S9, S10, S12, S21, L1, L18 and L19) this digestion procedure gave an unsatisfactory yield and/or number of peptides. In these cases digestion periods were lengthened up to 20 hr, and final enzyme to substrate ratios of 1:20 were used. Control experiments showed that the contribution of enzyme derived peptides to the total pattern was still negligible, even with 1:10 ratios and up to 48 hr digestion periods.

Column chromatography of the tryptic peptides followed by automatic detection with ninhydrin was carried out with volatile buffer gradients at 50° [6]. The procedure was adapted for the Bio-Cal BC-200 amino acid analyzer equipped with a column of 0.9 X 27 cm Aminex A-5 resin [7]. A linear gradient was used consisting of 250 ml 0.2 M pyridine-acetate, pH 3.1, and 250 ml 2.0 M pyridine-acetate, pH 5.0. After this gradient the column was washed with 150 ml limiting buffer of 2.0 M pyridine acetate, pH 5.6. The column was then re-equilibrated with the first buffer of the gradient. The pumping speed for buffer was 60 ml/hr and ninhydrin was delivered at the same speed. The sample was dissolved in 0.5 ml 0.2 M pyridine-HCl, pH 2.2, and applied quantitatively to the column without prior removal of the urea. As a marker, 40 mM of cysteic acid were added to the sample. All reagants were of reagent grade purity.

3. Results and discussion

As is evident from fig. 1, the presence of urea

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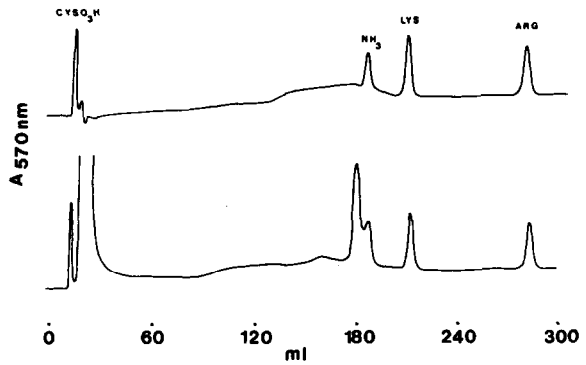

Fig. 1. Upper graph: Standard elution pattern of the marker amino acids:cysteic acid, lysine and arginine and of ammonia. Lower graph: Elution pattern of the same markers after carrying them through a digestion with TPCK-trypsin in the presence of urea.

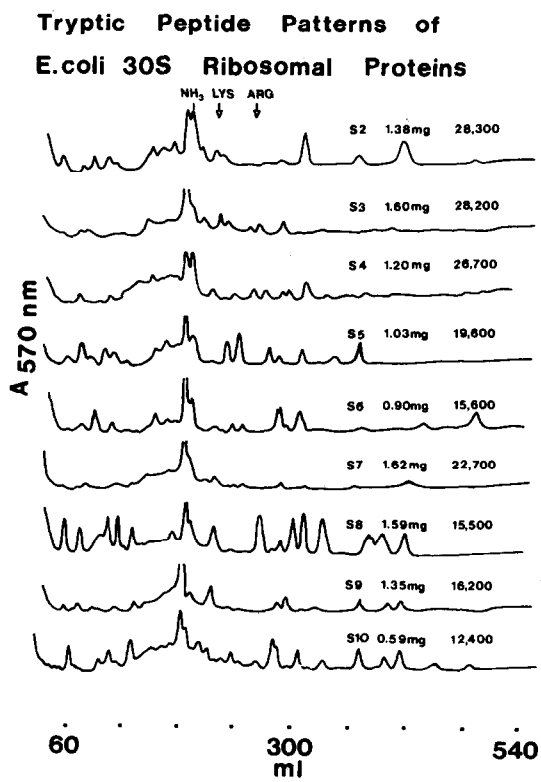

Fig. 2. Elution patterns of the tryptic peptides of 30 S ribosomal proteins S2 to S10. In this and all following figures, the urea peak has been omitted from the patterns. Each protein is numbered [4], and the amount digested and chromatographed, and the molecular weight are indicated [16]. The absorbancy scale on the coordinate is indicated by the cut off point of the unidentified peak preceding NH_3, being approximately 1.0.

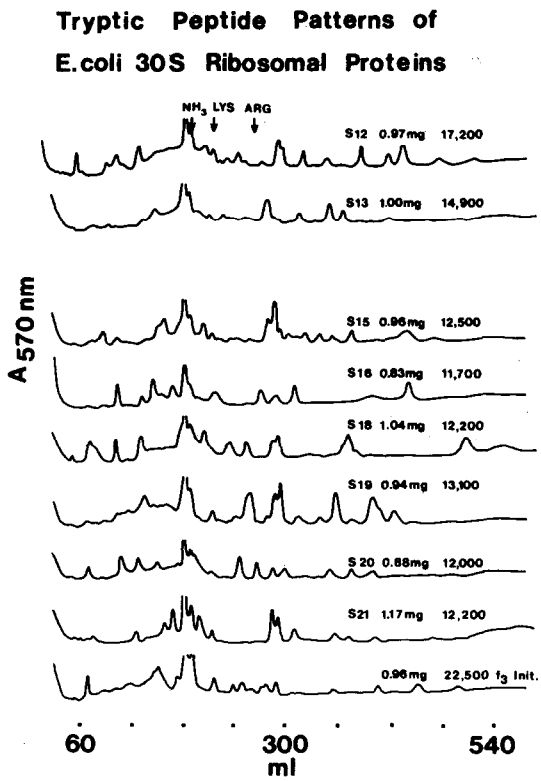

Fig. 3. Elution patterns of the tryptic peptides of 30 S ribosomal proteins S12 to S21 and of initiation factor F_3.

during chromatography does not interfere with the elution of peptides, except for the very acidic ones, of which there are rather few in the predominantly basic ribosomal proteins. Urea is eluted as a sharp zone without tailing, immediately following the elution of the cysteic acid marker. Fig. 1 shows that there was no significant carbamylation of free amino groups during the digestion by cyanate resulting from urea breakdown. This is evident from experiments where cysteic acid, arginine and lysine were incubated with TPCK-trypsin with and without urea. A non-identified decomposition product of urea eluted immediately in front of the ammonia peak. The resulting doublet was a useful reference marker to align the graphs. The elution position of the marker peaks varied between experiments by only a few ml, out of a total elution volume of 650 ml.

Figs. 2 and 3 show the tryptic peptide patterns obtained with 30 S ribosomal proteins. The elution patterns of the various proteins show pronounced differences. This result is in agreement with earlier

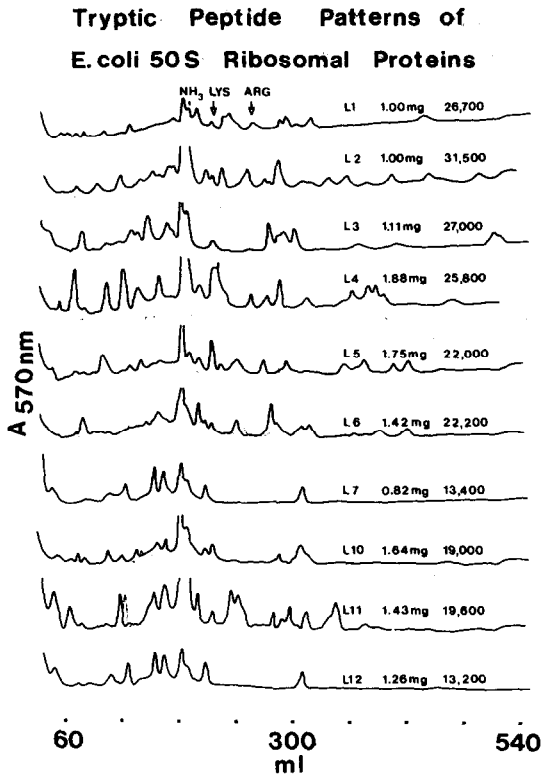

Fig. 4. Elution patterns of the tryptic peptides of 50 S ribosomal proteins L1 to L12.

studies on fingerprints [8–10]. The tryptic peptide pattern of purified initiation factor F3 [11] which is included in fig. 3 for comparison is similar to the profiles from 30 S ribosomal proteins.

Figs. 4, 5 and 6 give the tryptic peptide patterns for the 50 S ribosomal proteins. These show no close similarity except between proteins L7 and L12, which have practically identical tryptic patterns (fig. 7). Other studies on these two proteins, indicate correspondence in amino acid composition [12, 13], α-helix content [12, 14], migration in two-dimensional polyacrylamide gel electrophoresis [4] and immunological properties [15]. It can be concluded from these results that the proteins L7 and L12 have identical or almost identical structures and that a repeat of structural elements exists in the 50 S subunit.

The maximum theoretical number of tryptic peptides was calculated from the amino acid composition of the 30 S and 50 S proteins [13]. In tables 1 and 2 this number is compared to the number of observed

Table 1

Protein	Number of			
	Lys res.	Arg. res.	Calculated peptides	Observed peaks
S2	18	13	32	17
S3	27	19	47	18
S4	21	25	47	25
S5	12	10	23	21
S6	7	12	19	22
S7	14	20	35	14
S8	12	8	21	18
S9	11	19	31	23
S10	6	9	16	15
S12	13	18	32	20
S13	11	16	28	24
S15	8	14	23	28
S16	7	12	20	16
S18	9	12	22	20
S19	14	9	24	26
S20	15	11	27	17
S21	14	22	37	18

Comparison of the number of observed peaks with the maximum theoretical number of tryptic peptides of single 30 S proteins. Data for amino acid compositions and molecular weights are taken from [13, 16].

peaks. Small peaks as well as large ones were counted because staining with ninhydrin without previous alkaline hydrolysis gives unequal color yields for different peptides. For the major of proteins the observed number of peaks comes rather close to the theoretical figure but for some there is a discrepancy.

There are several reasons why a close agreement between the calculated number of tryptic peptides and the actually observed number of peaks cannot be expected: (1) The high percentage (up to 34 mole %) of basic amino acids in ribosomal proteins implies a high probability that two basic amino acid residues are adjacent in the protein chain. Depending on the degree to which one or other or both of the basic amino acids are cleaved, the number of peptides found will vary. (2) Single lysine or arginine residues will enlarge the peaks for these amino acids, but the number of resulting peptides will be lower than calculated. (3) Individual peaks can contain more than one peptide, especially when many peptides are separated. (4) Some peptides, especially those containing tryptophan or other aromatic amino acids, are strongly adsorbed to the standard resin, i.e. polystyrene sulfonic acid, and

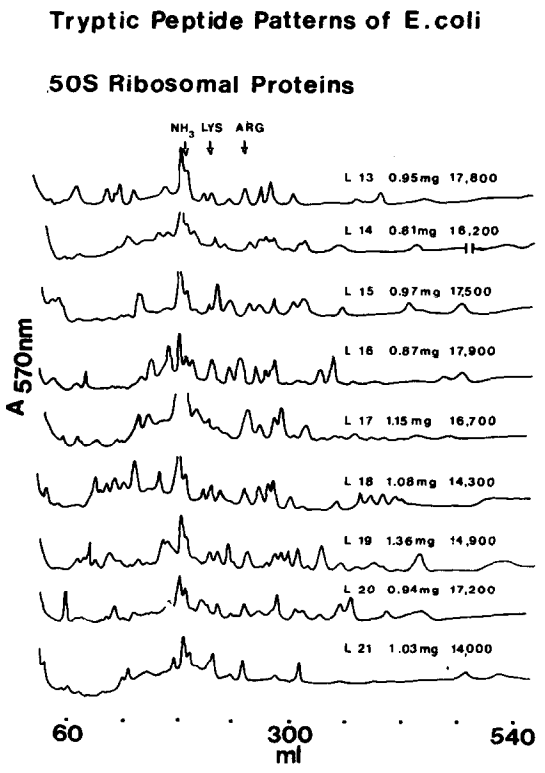

Fig. 5. Elution patterns of the tryptic peptides of 50 S ribosomal proteins L13 to L21.

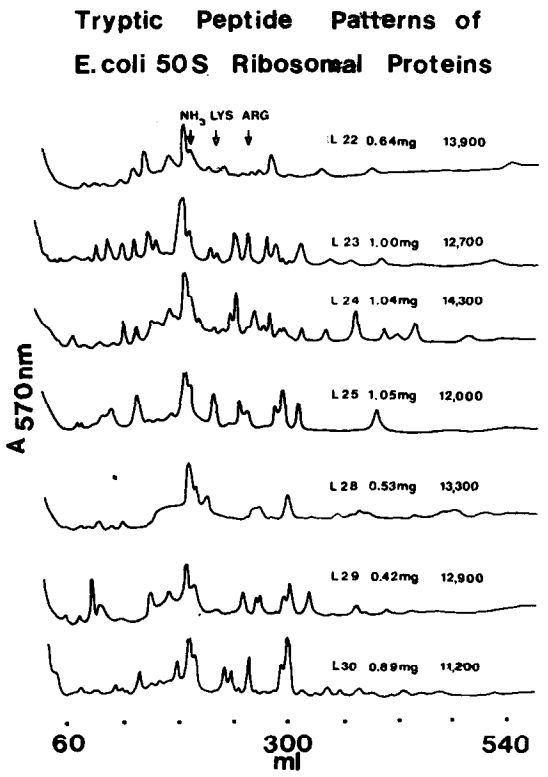

Fig. 6. Elution patterns of the tryptic peptides of 50 S ribosomal proteins L22 to L30.

so are many peptides longer than appoximately twenty amino acids. (5) Peptides with glutamine at the *N*-terminus are easily converted to pyroglutamyl peptides which are not detected by the standard ninhydrine procedure without additional alkaline hydrolysis. (6) Peptides containing chemically modified labile amino acids, e.g. oxidised cysteine or methionine, may be eluted in more than one peak containing the reduced and oxidized forms; this phenomenon has been demonstrated for ribosomal proteins [17]. Despite treatment of trypsin with TPCK, peptide bonds other than those involving basic amino acids can be split producing a larger number of peptides than calculated. In view of these facts there is reasonable agreement between the observed and expected number of peaks in tables 1 and 2.

As mentioned above, there are two proteins (L7 and L12) with almost identical structures in 50 S subunits. Whether weaker homologies exist between other ribosomal proteins, can be decided by preparative

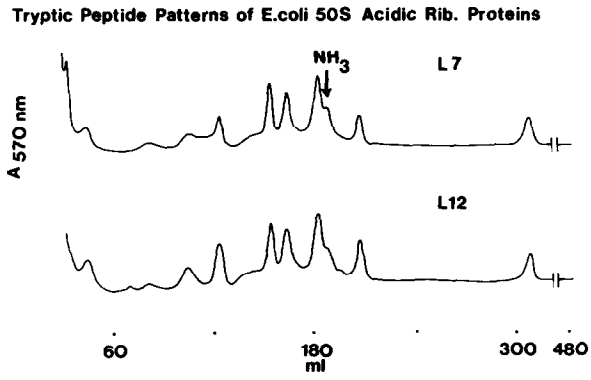

Fig. 7. Comparison of the tryptic peptides of the acidic 50 S ribosomal proteins L7 and L12.

Table 2

Protein	Number of			
	Lys res.	Arg. res.	Calculated peptides	Observed peaks
L1	21	11	33	18
L2	24	29	54	26
L3	20	13	34	23
L4	17	15	33	22–25
L5	16	15	32	23
L6	15	11	27	24–26
L7	11	1	13	9
L10	11	11	23	19
L11	13	10	24	26
L12	11	1	13	10
L13	11	13	25	21
L14	13	13	27	23
L15	13	14	28	22–25
L16	17	15	33	26–28
L17	10	19	30	26–28
L18	8	12	21	24
L19	12	13	26	30
L20	17	20	38	25–28
L21	11	10	22	19–20
L22	15	12	28	19
L23	15	9	25	21–24
L24	16	8	25	28
L25	12	7	20	13
L28	9	14	24	20
L29	10	9	20	15
L30	9	9	19	23

Comparison of the number of observed peaks with the maximum theoretical number of tryptic peptides of single 50 S proteins. Data for amino acid compositions and molecular weights are taken from [13, 16].

isolation of peptides and determination of their amino acid composition. This has so far been done for 18 proteins [17, 18] and in these no peptides longer than three amino acids had identical or similar compositions. This finding is in good agreement with immunological studies on 45 isolated ribosomal proteins of *E. coli* [15, 19] in which no cross-reaction (with one exception) has been found between these proteins. Only proteins L7 and L12 have complete immunological identity. Therefore, it can be concluded from chemical and immunological studies that most of the proteins in *E. coli* ribosomes have quite distinct primary structures.

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