AMINO ACID SEQUENCE OF A 50 S RIBOSOMAL PROTEIN INVOLVED IN BOTH EFG AND EFT DEPENDENT GTP-HYDROLYSIS

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1. Introduction

The only proteins in the 50 S ribosomal subunits of *E. coli* with a defined function are the two acidic proteins A_1 and A_2 (L_7 and L_{12}). Recently, much evidence has been accumulated that these two proteins of low molecular weight play an important role in the GTP-hydrolysis, associated with the elongation factors EFG and EFT [1–6].

Determination of the stoichiometry of ribosomal proteins indicates that 50 S ribosomes from logarithmic growing cells contain on the average at least one copy each of A_1- and A_2-protein per particle [7, 8]. However, in conditions of restricted bacterial growth, under which ribosomes show an intrinsically reduced activity in protein biosynthesis [9, 10], the number of copies of A_2-relative to A_1-protein per ribosome decreases drastically [7, 11]. In addition, particles reconstructed from "50 S cores", supplemented with an excess of A_2-protein, are significantly more active in promoting EFG- and EFT-factor mediated GTP-hydrolysis than similar particles constructed with the aid of A_1-protein [1, 6]. Since the only structural difference between the two proteins seems to be the state of acetylation of the aminogroup at the N-terminal serine residue [12], it has been suggested that acetylation provides a regulatory mechanism for translation [2, 7, 12]. However, a rigid test of the identity of A_1- and A_2-protein except from the acetyl group, requires a comparison of the complete primary structure of both proteins. The purpose of the present report is to present the complete primary structure of A_1- and A_2-protein and to draw attention to some remarkable features of this structure.

2. Experimental

The proteins A_1 and A_2 were purified from 70 S ribosomes from *E. coli* MRE 600 as described previously [7].

The primary structure of A_1 was determined on tryptic, chymotryptic and elastase peptides with the automatic solid phase Edman degradation technique [13, 14] and the Dansyl–Edman method [12].

The nonacetylated protein A_2 was sequenced up to 51 residues in the Beckman sequenator using a modification of the method of Edman and Begg [15], while the tryptic peptides of the remaining part (res.

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52–120) were sequenced with the solid phase method.

A full report of all the experimental details will appear elsewhere [16].

3. Results and discussion

The complete amino acid sequence of the two acidic proteins A_1 and A_2 is presented in fig. 1. On the basis of the proposed sequence the molecular weight of the isoelectric protein is 12,200.

A comparison of the primary structure of A_1- and A_2-protein demonstrates in agreement with previous studies [12], that both proteins are identical with exception of the acetyl-group at the N-terminus in A_1. This result proves that acetylation of a polypeptide chain (A-protein or L_7/L_{12} in the Berlin nomenclature) causes a structural change, which is responsible for the different behaviour of A_1 and A_2 in the *in vitro*

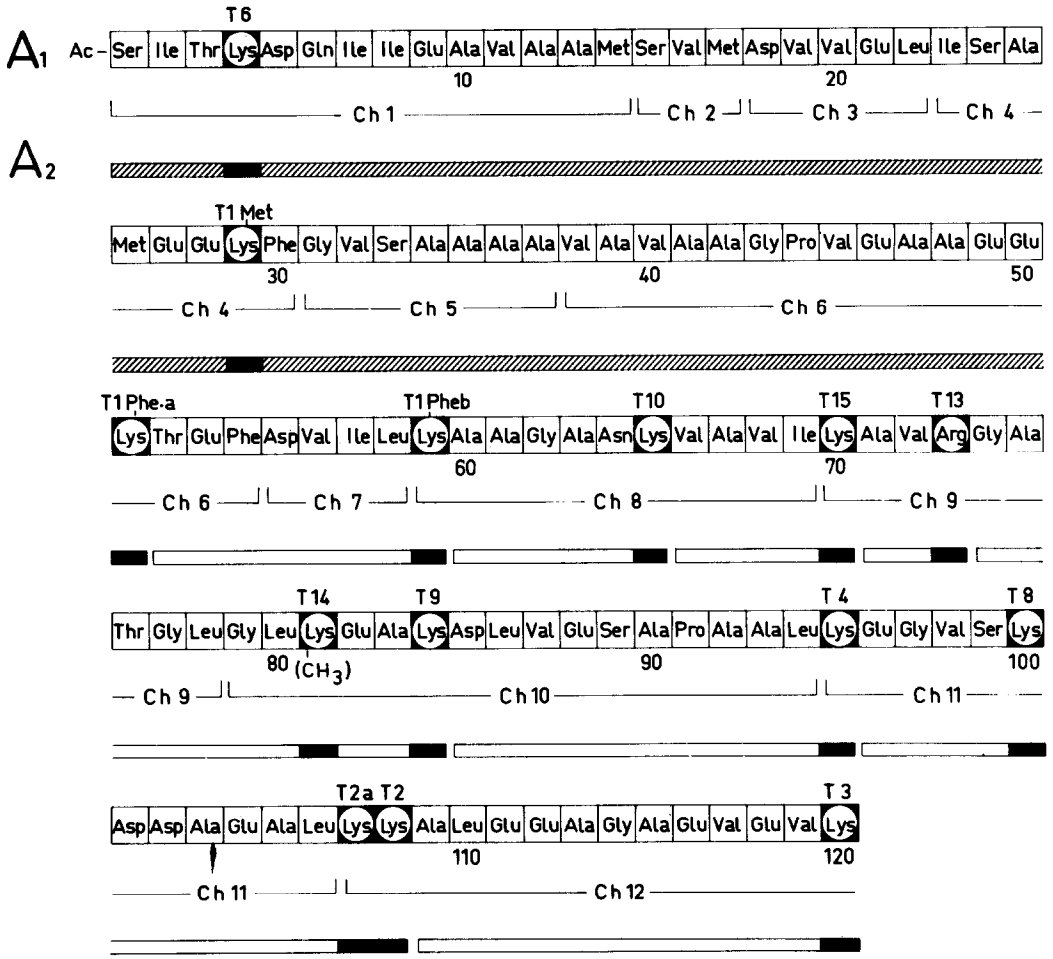

Fig. 1. The primary structure of A_1- and A_2-protein. A-protein consists of 120 amino acids, A_1 starting with N-acetyl-serine and A_2-protein starting with serine at the N-terminal end. The tryptic peptides are indicated with T and the number placed above the lysine residues determines the C-terminal ends of each peptide. T_1Met is a core peptide which contains the three methionine residues of the protein; T_1Phe is a core peptide which contains the two phenylalanine residues of A-protein. T_1Phe splits partially (20%) in T_1Phe a and T_1Phe b, each of which of the latter peptides contain one phenylalanine residue. Ch_1, Ch_2, etc. indicate the chymotryptic peptides of the protein; the numbering is consecutive from the N-terminus. The shaded bar, which extends till residue 51, represents the part of A_2-protein sequenced with the Beckman sequencer, a complete identity in sequence with A_1-protein for this stretch was found. The tryptic peptides of the remaining part of A_2 (res. 52–120) were sequenced with the aid of the automatic solid phase Edman degradation technique (open bars) and found to be identical to the corresponding ones of A_1.

Table 1
Distribution of residues in A-protein.

	Residues 1–55	Residues 56–81	Residues 80–120
[1]Basic residues A_1	3	5	6
A_2	4	5	6
[2]Acidic residues	11	0	12
[3]Net charge A_1	−8	+5	−6
A_2	−7	+5	−6
[4]Hydrophobic residues	18	9	8
Aromatic residues	2	0	0
Prolyl residues	1	0	1
Longest sequence lacking a basic residue	24 residues (5–28)	7 residues (74–80)	11 residues (109–119)

1) Includes lysine, arginine and α-aminogroups.
2) Includes aspartic acid, glutamic acid and α-COOH-groups.
3) Assumes that all basic and acidic groups are in charged form.
4) Includes only leucine, isoleucine, valine, methionine and phenylalanine.

GTP-ase assay [1, 6]. That specific modification of the α-amino-group of an enzyme can profoundly change its catalytic activity is not a novel finding, since this has also been demonstrated for other proteins such as trypsin and chymotrypsin [17, 18]. In addition the multimeric occurrence of A-protein in the large ribosomal subunit supports possible hypotheses concerning the function of this protein both in the movement of the ribosome along the messenger and in the *in vivo* regulation of translocation [2, 7, 12].

A number of additional structural features became apparent on analysis of this sequence. As shown in table 1 there is a fairly unequal distribution of hydrophobic and hydrophilic residues along the polypeptide chain. The hydrophobic residues amino acids (Val, Met, Ile, Leu, Phe) cluster in the amino-terminal part of the molecule. A strong clustering of alanine residues to the extent as found at the positions 34 to 42 has to our knowledge not been reported [19]. Concerning the distribution of positive and negative charges along the polypeptide chain, three separate regions can be distinguished (table 1). Starting from the amino-terminus, the protein contains a negatively charged region (res. 1–55), followed by a positively charged region (res. 56–81) and ending in a negatively charged C-terminal region (res. 82–120). The significance of this positively charged central section is not clear, but it may serve a function in an electrostatic interaction, which is not obvious from the overall charge of the protein.

It is worth emphasizing that both A_1 and A_2 are about 50% methylated at the ε-aminogroup of lysine 82 (ε-N-monomethyl-lysine is also found in bacterial flagelline [20–21]. Whether this means that A_1 and A_2 are equally exposed in situ to a methylating enzyme is unknown.

Concerning the α-helicity of this protein, circular dichroism measurements of Möller et al. [23] have given an estimate of 60% for a mixture of A_1 and A_2 at neutral pH, while Dzionara [24] obtained 55% for L_7 and 48% for L_{12}, both measured at pH 3. Theoretical estimates based on the proficiency of certain amino acid sequences to occur in an α-helical state predict an even higher percentage of α-helix in this protein [25, 26]. A check on the validity of this estimate should await more information on the 3-dimensional structure of A_1- and A_2-protein.

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