# PROTEIN–CHEMICAL STUDIES ON *ESCHERICHIA COLI* MUTANTS WITH ALTERED RIBOSOMAL PROTEINS S6 AND S7

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## 1. Introduction

The isolation and characterization of mutants with altered ribosomal components can give a better insight into the structure, function, genetics and biogenesis of ribosomes. Since the early studies in this direction [1] many more mutants in which at least one ribosomal protein is altered have become available [2–5]. Mutants have been found with alterations in any one of almost all of the 53 proteins present in the *E. coli* ribosome.

Here, we describe protein–chemical investigations on two *E. coli* mutants in which protein S6 is altered and on one mutant with an altered protein S7. All 3 mutant proteins differ not only in their amino acid compositions but also in their lengths from the corresponding wild-type proteins.

## 2. Materials and methods

Ribosomes, their subunits and the altered proteins from mutants VT45 and N731 were isolated according to [6]. The isolation of the altered protein of mutant VT158 was done as in [7]. The purity of the isolated proteins was checked by two-dimensional gel electrophoresis according to [8].

Proteins were cleaved with TPCK-treated trypsin (from Merck) or *Staphylococcus aureus* protease (V-8 from Miles). Carboxypeptidases A and B (Worthington) were used for determination of the C-terminal amino acids. Peptides were separated by fingerprinting on thin-layer plates (Polygram CEL 300; Macharey and Nagel, Düren) as in [9]. They were sequenced by the dansyl-Edman method [10] in a modified form [11]. Amino acid analyses were done on a Durrum D-500 analyzer. The N-terminal regions of the proteins were sequenced in an improved Beckman sequenator as in [12]. More details of the protein–chemical methods are given in [13].

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## 3. Results

### 3.1. *Mutant VT45*

This mutant was isolated from the *E. coli* A19 strain VT which shows a novel type of streptomycin dependence. Streptomycin-independent isolates from the strain VT contain a great variety of altered ribosomal proteins as shown by two-dimensional gel electrophoresis [3]. One of these streptomycin-independent isolates was mutant VT45 which differed from its parent strain VT with respect to protein S6. As revealed on the electropherogram (fig.1) protein S6 from mutant VT45 was much less acidic than protein S6 from the parent strain VT since it migrated less to the anode in the first dimension. It also differed from S6 of strain VT with respect to the shape of the S6-spot on the electropherogram: the S6-spot of VT45 was round whereas that of VT is elongated. The reason for this difference will be discussed below.

Protein S6 of mutant VT45 was digested with trypsin. All tryptic peptides were isolated, and their amino acid compositions were determined. The only difference between protein S6 of mutant VT45 and that of the wild-type strain [7] was found to be in peptide T1 which is the C-terminal tryptic peptide of protein S6. The mutant peptide contained one lysine residue more and ~3 glutamic acid residues less than the corresponding wild-type peptide T1.

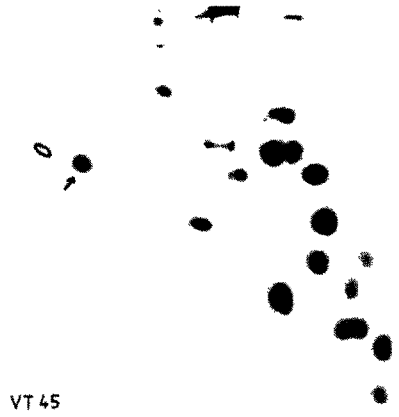


Fig.1. Two-dimensional gel electropherogram of 30 S ribosomal proteins of mutant VT45. Position of wild-type S6 is indicated by the outline and the spot of altered S6 is arrowed.

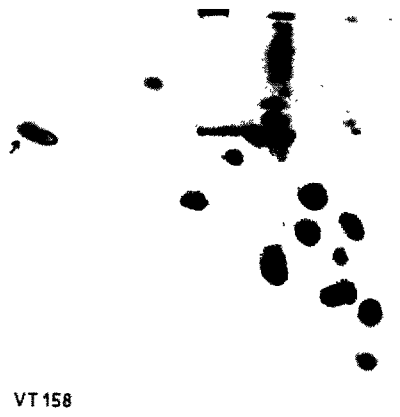


Fig.2. Two-dimensional gel electropherogram of 30 S ribosomal proteins of mutant VT158. Position of wild-type S6 is outlined and the spot of altered S6 is arrowed.

This finding indicated that the difference between the mutant and the wild-type protein was located at the C-terminus. In order to isolate a shorter C-terminal peptide than the tryptic peptide T1 for sequence analysis, protein S6 was digested with *Staph. aureus* protease (SP). After isolation of all SP-peptides their amino acid compositions were determined. The only peptide which was different between mutant and wild-type protein S6 was the C-terminal peptide SP9. This result confirmed the conclusion drawn from the analysis of the tryptic peptides: the difference between mutant and wild-type S6 was located at the C-terminus.

Peptide SP9 was sequenced manually by the micro-dansyl-Edman method giving the sequence: Ala–Gly–Asp–Ser–Lys–Glu. This differed from peptide SP9 of the wild-type protein S6 (see section 4):

(i) The mutant peptide contained a lysine instead of a glutamic acid residue present in the wild-type peptide in this position;

(ii) The mutant peptide was much shorter than the wild-type peptide SP9. It consisted of 6 amino acids only, whereas the wild-type peptide SP9 contains 7–10 amino acids due to the heterogeneity of wild-type protein S6 at its C-terminus [7].

The heterogeneity of protein S6 is the reason for the elongated shape of the S6-spot on the two-dimensional electropherogram (fig.1). Since the mutant protein S6 was homogeneous it showed a round spot, typical also of all other *E. coli* ribosomal proteins.

### 3.2. *Mutant VT158*

The origin of this mutant was similar to that of mutant VT45, being also derived from *E. coli* A19 strain VT as a streptomycin-independent mutant [5]. In contrast to VT45 whose ribosomal protein S6 migrates as a round spot more slowly to the anode than S6 of the parental strain, protein S6 of mutant VT158 showed an elongated spot and moved somewhat faster to the anode (fig.2). This indicated the mutant protein was more acidic than wild-type and had heterogeneity comparable to the wild-type protein S6.

After isolation of protein S6 from mutant VT158 it was digested with trypsin and all tryptic peptides were isolated. According to the fingerprint and the amino acid analyses the only difference between mutant and wild-type protein was the C-terminal tryptic peptide T1 which had at least one glutamic acid residue more in the mutant than in the wild-type protein.

Protein S6 from the mutant was digested with *Staph. aureus* protease. The resulting SP-peptides were separated and their amino acid compositions determined. The analyses confirmed that protein S6 of the mutant differed from that of the wild-type at the C-terminus. Three peptides (SP9–SP11) with

an increasing number of glutamic acid residues were found: SP9 corresponded to the sequence Ala–Gly–Asp–Ser–Glu (pos. 126–130); SP10 (pos. 126–134) had 4 Glu-residues more than SP9; and SP11 (pos. 126–136) had at least 2 Glu-residues more than SP10. Therefore peptide SP11 contained at least 7 Glu-residues at its C-terminus, i.e. the mutant protein was at least one glutamic acid residue longer than the wild-type protein (see section 4). This was confirmed by electrophoresis of ribosomal proteins of mutant VT158 and parental control VT in a two-dimensional gel system modified so that the several molecular species of protein S6 migrated to separate positions [14]. Using this system, not only was the S6 of mutant VT158 seen to be more acidic than wild-type, but the number of discrete spots was at least one more. This is what would be expected if protein S6 of mutant VT158 had more glutamic acid residues at the C-terminus than usual.

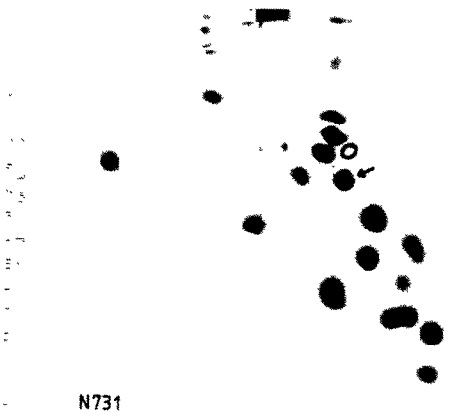


Fig.3. Two-dimensional gel electropherogram of 30 S ribosomal proteins of mutant N731. Position of wild-type S7 is outlined and the spot of altered S7 is arrowed. Protein S6 is also altered in this mutant.

### 3.3. *Mutant N731*

This mutant was derived from *E. coli* K strain N729, isolated as a *nek*-mutant with resistance to neomycin and kanamycin [15]. N729 differed from its parental strain with respect to protein S6 as shown by polyacrylamide gel and cellulose acetate electrophoresis [16].

Protein-chemical analysis has shown that protein S6 from the mutant N729 terminates at pos. 131 whereas the wild-type protein chain continues beyond this position [7]. During the cultivation of mutant N729 in our laboratory it gave rise to a new mutant, designated N731, which differed from N729 by a drastically altered protein S7 (H. G. Wittmann, unpublished): the position of the altered protein S7 on the two-dimensional electropherogram was indistinguishable from that of protein S7 from the B-strain of *E. coli* although N729 is a K-strain (fig. 3). As described [17] *E. coli* B- and K-strains differ in proteins S5 and S7. The difference in protein S5 is caused by a replacement of only one amino acid [18,19] whereas that in protein S7 is much more drastic: protein S7 from *E. coli* B is 24 amino acids shorter than S7 from *E. coli* K [20].

Since protein S7 of the *E. coli* K mutant N731 was indistinguishable from S7 of *E. coli* B on the electropherogram the question arose whether the sequences of both S7-proteins were identical. Therefore, protein S7 from mutant N731 was purified and digested with trypsin. The tryptic peptides were isolated and their amino acid compositions were determined. All peptides of S7 from N731 were identical to those of S7 from *E. coli* B whereas no peptides were found which corresponded to the region of 24 amino acids by which S7K is longer than S7B [20]. Furthermore, amino acid analyses and treatment with carboxypeptidases of the S7-protein isolated from mutant N731 and S7B gave indistinguishable values within the experimental error. These results show that the mutation leading to the difference in S7 between the wild-type strains K and B can also occur spontaneously in the laboratory. This spontaneous mutation from a long to a short form of the protein S7 has been observed also in other mutants than N731 ([21]; E.R.D., K. Isono, unpublished). In this context it is interesting that ribosomal protein S7 from other species of Enterobacteriaceae, e.g., *Salmonella, Shigella, Serratia* and *Erwinia* resembles B-type S7 rather than K-type S7 [22], so these mutants represent a reversion to the generally prevailing form of S7.

## 4. Discussion

The appearance of *E. coli* wild-type protein S6 as an elongated and not (as in the case of the other proteins) a round spot on the two-dimensional gel

electropherogram indicated a heterogeneity of the S6-protein chains. This heterogeneity was directly demonstrated by protein-chemical analysis which revealed a variable number of glutamic acid residues at the C-termini of the S6-protein chains [7]. The heterogeneity is caused by an enzymatic and post-translational addition of glutamic acids at the C-terminus of protein S6 [23].

A comparison of the C-termini of proteins S6 from the *E. coli* wild-type and from mutants analyzed here and in [7] gave the following results:

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          126              130                  135
W.T.:   -Ala-Gly-Asp-Ser-Glu-Glu-(Glu-Glu-Glu-Glu)
VT158:  -Ala-Gly-Asp-Ser-Glu-Glu-(Glu-Glu-Glu-Glu-Glu)
N729:   -Ala-Gly-Asp-Ser-Glu-Glu
VT45:   -Ala-Gly-Asp-Ser-Lys-Glu
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It has been shown [24] with several S6 mutants, including strain N729, that there is a mutation in the gene for the enzyme which post-translationally adds glutamic acids to the C-terminus of protein S6. This mutation inactivates the enzyme, resulting in a lack of Glu-residues beyond pos. 131 of S6. It is interesting that this enzyme can apparently add more Glu-residues in mutant VT158 than in the wild-type. However, it cannot add Glu-residues to the C-terminus of mutant VT45, where there is a lysine instead of a glutamic acid residue in pos. 130, the penultimate residue. Obviously, more work on the three-dimensional structure of protein S6, is necessary to understand these observations in more detail.

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**References**

[1] Wittmann, H. G. and Wittmann-Liebold, B. (1974) in: Ribosomes (Nomura, M. et al. eds) pp. 115–140, Cold Spring Harbor Lab. Press, Long Island NY.
[2] Isono, K., Krauss, J. and Hirota, Y. (1976) Mol. Gen. Genet. 149, 297–802.
[3] Dabbs, E. R. and Wittmann, H. G. (1976) Mol. Gen. Genet. 149, 303–309.
[4] Isono, K., Cumberlidge, A. G., Isono, S. and Hirota, Y. (1977) Mol. Gen. Genet. 152, 239–243.
[5] Dabbs, E. R. (1978) Mol. Gen. Genet. 165, 73–78.
[6] Hindennach, I., Stöffler, G. and Wittmann, H. G. (1970) Eur. J. Biochem. 23, 7–11.
[7] Hitz, H., Schäfer, D. and Wittmann-Liebold, B. (1977) Eur. J. Biochem. 75, 497–512.
[8] Kaltschmidt, E. and Wittmann, H. G. (1971) Anal. Biochem. 36, 401–412.
[9] Wittmann-Liebold, B. and Lehmann, A. (1980) in: Methods in Peptide and Protein Sequence Analysis (Birr, C. ed) pp. 49–72, Elsevier/North-Holland, Amsterdam, New York.
[10] Gray, W. R. and Hartley, B. S. (1963) Biochem. J. 89, 379–390.
[11] Chen, R. (1976) Hoppe-Seyler's Z. Physiol. Chem. 375, 873–886.
[12] Wittmann-Liebold, B. (1980) in: Polypeptide Hormones (Beers, R. F. et al. eds) pp. 87–120, Raven, New York.
[13] Wittmann-Liebold, B., Brauer, D. and Dognin, J. M. (1977) in: Solid-phase Methods in Protein Sequence Analysis (Previero, A. and Coletti-Previero M. A. eds) pp. 219–232, Elsevier/North-Holland, Amsterdam, New York.
[14] Subramanian, A. R. (1980) J. Biol. Chem. 255, 6941–6946.
[15] Apirion, D. and Schlessinger, D. (1969) Proc. Natl. Acad. Sci. USA 63, 794–799.
[16] Wittmann, H. G., Stöffler, G. and Apirion, D. (1973) unpublished.
[17] Kaltschmidt, E., Stöffler, G., Dzionara, M. and Wittmann, H. G. (1970) Mol. Gen. Genet. 109, 303–305.
[18] Wittmann-Liebold, B. and Wittmann, H. G. (1971) Biochim. Biophys. Acta 251, 44–53.
[19] Wittmann-Liebold, B. and Greuer, B. (1978) FEBS Lett. 95, 91–98.
[20] Reinbolt, J., Tritsch, D. and Wittmann-Liebold, B. (1978) FEBS Lett. 91, 297–301.
[21] Schmitt, S., Hayes, F. and Reinbolt, J. (1980) Eur. J. Biochem. 107, 87–94.
[22] Geisser, M., Tischendorf, G. W., Stöffler, G. and Wittmann, H. G. (1973) Mol. Gen. Genet. 127, 111–128.
[23] Reeh, S. and Pedersen, S. (1979) Mol. Gen. Genet. 173, 183–187.
[24] Icho, T. and Isono, K. (1980) in preparation.