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Ribosomal Proteins, XII. Number of Proteins in Small and Large Ribosomal Subunits of *Escherichia coli* as Determined by Two-Dimensional Gel Electrophoresis*

E. Kaltschmidt and H. G. Wittmann

MAX-PLANCK-INSTITUT FÜR MOLEKULARE GENETIK, BERLIN-DAHLEM, GERMANY

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Abstract. Two-dimensional gel electrophoresis separates all of the component proteins of the ribosomal subunits of *Escherichia coli*. This method shows 21 proteins in the 30S, and 34 proteins in the 50S, subunit.

Waller[1] showed that the ribosomes of *Escherichia coli* contain a large number of protein components. Determination of the exact number of ribosomal proteins is important for precise knowledge of the structure and function of the ribosomes. Combinations of two or more conventional methods, such as disk electrophoresis on acrylamide gels under different conditions, ion exchange, and molecular sieve chromatography, indicate that the 30S and 50S ribosomal subunits contain 20 and 30–35 proteins, respectively.[2–6] We have recently developed a two-dimensional (2D) polyacrylamide gel electrophoresis procedure[7] by means of which all of the proteins of the ribosomal subunits can be rapidly and reproducibly separated in one operation. This paper describes the results of this procedure for the separation of proteins derived from 30S and 50S subunits, as well as 70S ribosomes, of *E. coli*. We find that the 30S subunits contain 21 proteins, and the 50S subunit as many as 34 proteins. A numbering system is proposed based on the position of the protein spots on the 2D electropherogram.

Materials and Methods. Electrophoresis: Two-dimensional electrophoresis was performed as described.[7] The acrylamide concentration in the first dimension was 8%, except in a few cases where it was 4%. Tris–EDTA–boric acid buffer, pH 8.6, and less frequently, pH 9.6, was used. The electrophoretic conditions in the second dimension were always 18% acrylamide gel in acetate buffer, pH 4.6.

Preparation of the proteins: The 70S ribosomes obtained by differential centrifugation were washed with 0.5 M NH_4Cl, 1 mM Mg^{++}. Subunits were separated by sucrose gradient centrifugation in a zonal rotor, B XV. Proteins were extracted from ribosomes by treatment with 67% acetic acid in the presence of 30 mM Mg^{++} (ref. 4). The RNA precipitate was washed with extracting solution for more quantitative extraction of the proteins. No further proteins were detected in the RNA precipitate after washing with 67% acetic acid in the presence of higher salt concentrations. The proteins were lyophilized and dissolved in the components of the sample gel. The solubility of the proteins improved when the extracted proteins, instead of being lyophilized, were directly dialyzed against the gel components. When mentioned in the text, the proteins were oxidized with performic acid or reduced and alkylated with iodoacetamide.[8]

Results and Discussion. The protein mixtures extracted from a large number of ribosome preparations were compared by two-dimensional electrophoresis.

The spots reproducibly appearing in the same position were numbered, separately for each subunit, along horizontal lines commencing at the top left (see Figs. 1 and 2). The spots on the electropherograms of the 30S, 50S, and 70S particles, which can be correlated with single proteins, are described below.

30S subunit: Electrophoresis of proteins from the 30S subunit reveals 21 spots (Fig. 1), marked S1 to S21. Four of these spots are positioned at the left

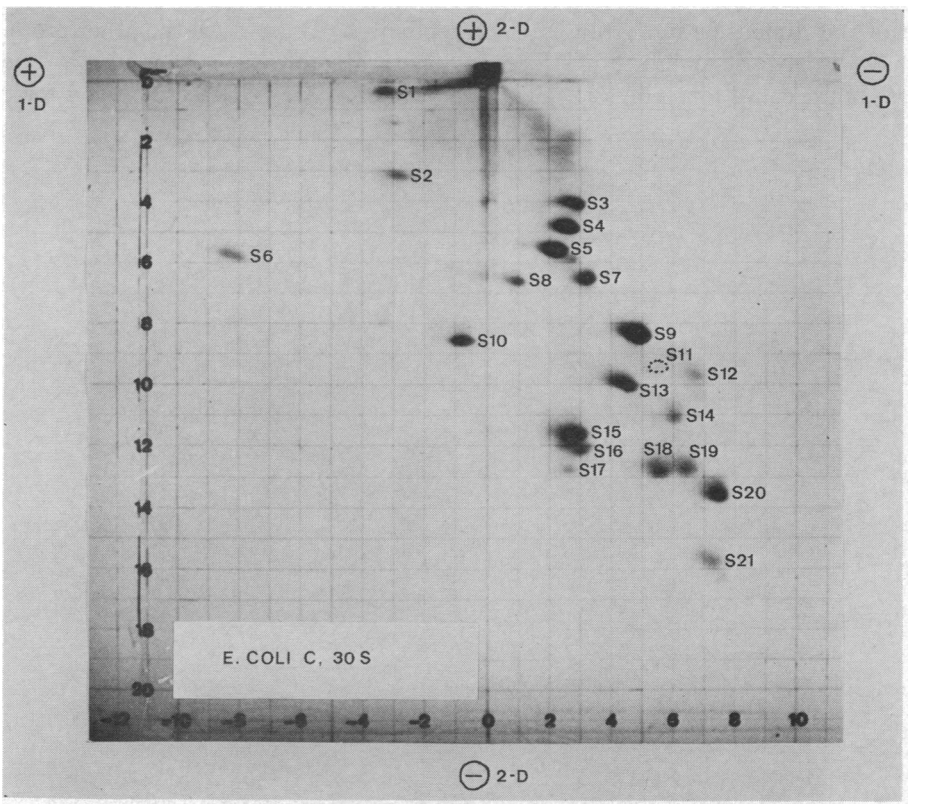

FIG. 1. Two-dimensional (2D) electropherogram of 30S ribosomal proteins of *E. coli* C. Electrophoresis in the first dimension: 8% acrylamide, pH 8.6; in the second dimension: 18% acrylamide, pH 4.6. Electrodes set as marked, in the first dimension, the anode at left; in the second dimension, the anode at the top.

of the starting-point indicating isoelectric points <pH 8.6. The other 30S proteins possess isoelectric points >pH 8.6. The positions of the spots can be reproduced very precisely. Their intensities are always the same, with one exception (S11). The intensity of S11 varies slightly depending on the treatment of the starting material. This spot, corresponding to the encircled position in Fig. 1, is only faintly visible; however, it can sometimes be seen very clearly (see Figs. 4 and 5). The possibility cannot be excluded that this spot is derived in variable amounts from another protein.

To verify that no spot contained more than one protein, the acrylamide concentration in the first dimension was varied from 4–12%, and the pH of the

buffers between pH 4.9 and 12.0. It is very unlikely that proteins with different molecular weights and/or charges migrate together in one spot under all the conditions attempted. Examination of the electrophoretic fingerprinting patterns of the 30S subunit did not reveal any further proteins. Therefore, it is clear that the number of proteins does not exceed 21.

50S subunit: 34 spots were detected in the electropherograms of the 50S subunit. These spots are marked L1–L34. Seven of them migrate to the anode in the first dimension indicating isoelectric points <pH 8.6. All the other proteins have isoelectric points >pH 8.6. 31 spots are always clearly visible under standard conditions (Fig. 2). Three more (L8, L31, L34) are included for the

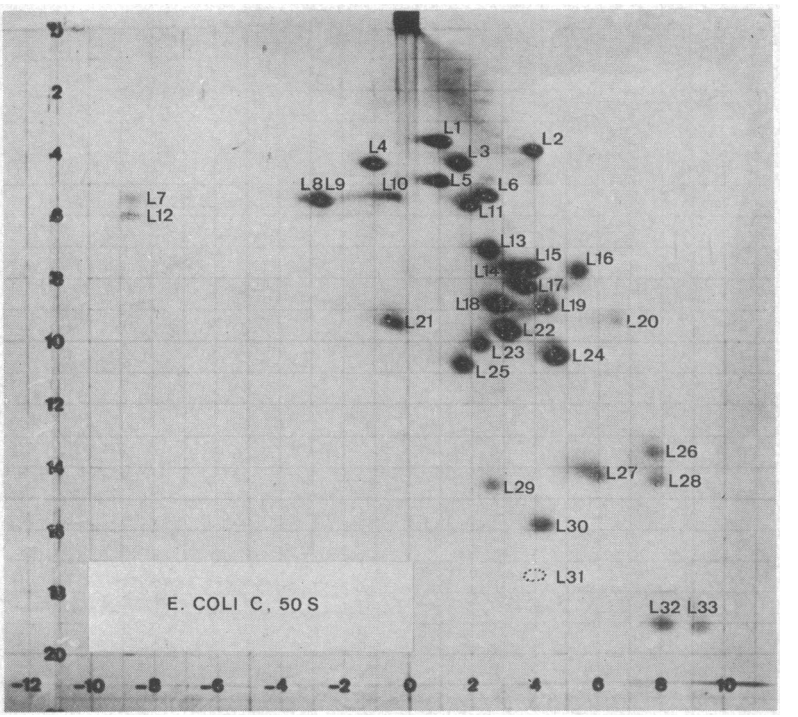

FIG. 2. 2D-electrophoretogram of 50S proteins of *E. coli* C. Conditions as in Fig. 1.

following reasons: *L8.* When the acrylamide concentration in the first dimension is varied, L8 migrates out of the complex L8/L9 as a clearly discernible spot (Fig. 3). This separation of L8/L9 into two components can also be observed with 70S proteins (Fig. 4) when the electrophoresis is performed in 4% acrylamide gel in the first dimension. If the electrophoresis in the first dimension is conducted at pH 9.6, the complex L8/L9 can likewise be separated into two components. Further spots were not discovered by variation of the acrylamide concentration and pH. *L31.* This spot, which is hardly, or not at all, visible at normal protein concentrations in the starting gel (Fig. 2), becomes distinctly discernible with larger amounts of protein (Figs. 4 and 5). *L34.* This spot can

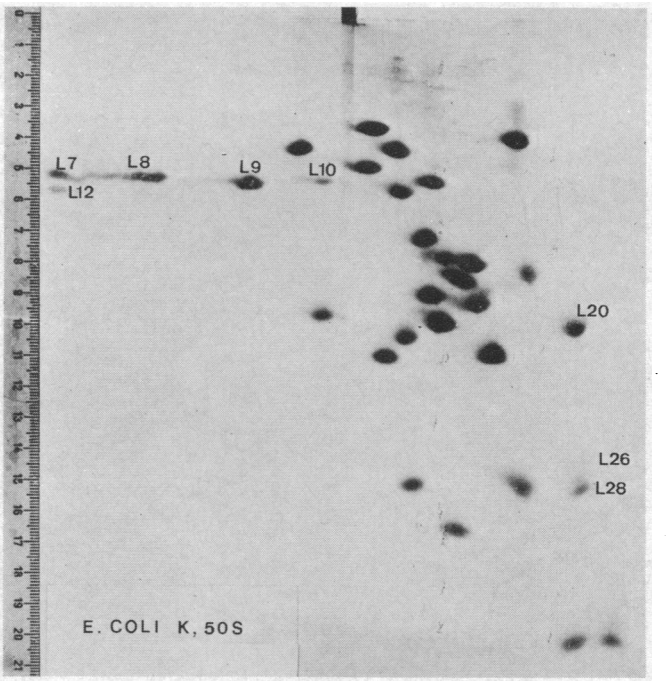

FIG. 3. 2D-electrophoretogram of 50S proteins of *E. coli* K. First dimension, 4% acrylamide, pH 8.6. Second dimension as in Fig. 1.

be detected by shortening the electrophoresis running time in the first dimension (Fig. 5). Under standard conditions, the spot migrates out of the gel to the cathodic buffer vessel.

Three of the spots, namely L12, L20, and L26, vary considerably in intensity. *L12.* This spot is only faintly visible in Figs. 2 and 3; however, in Fig. 4 (higher protein load) it stands out far more clearly. After isolating the acidic proteins of the 50S subunit this spot appears with almost the same intensity as L7 (Fig. 6). The proteins L7 and L12 could be isolated as single components. Both proteins appear to be very similar (ref. 9, and in preparation). If the 50S proteins are oxidized, both spots are recovered in the same position as before oxidation. Further investigation should clarify the reasons for the electrophoretic separation into two spots. *L20.* The intensity of this spot varies in different preparations of the ribosomal proteins. This may be due to varying degrees of solubility of this protein in the sample gel for different preparations. This assumption is supported by the fact that varying amounts of proteins are held back at the origin upon electrophoresis in the first dimension. If the starting protein mixture, instead of being lyophilized, is directly dialyzed against the sample gel considerably less material is left behind at the starting point. *L26.* The variable intensity of L26 is apparent on comparing, for example, Figs. 2 and 3. It can not be excluded that L26 is derived from L28. This requires further investigation. In the meantime, we assign a separate number to this spot.

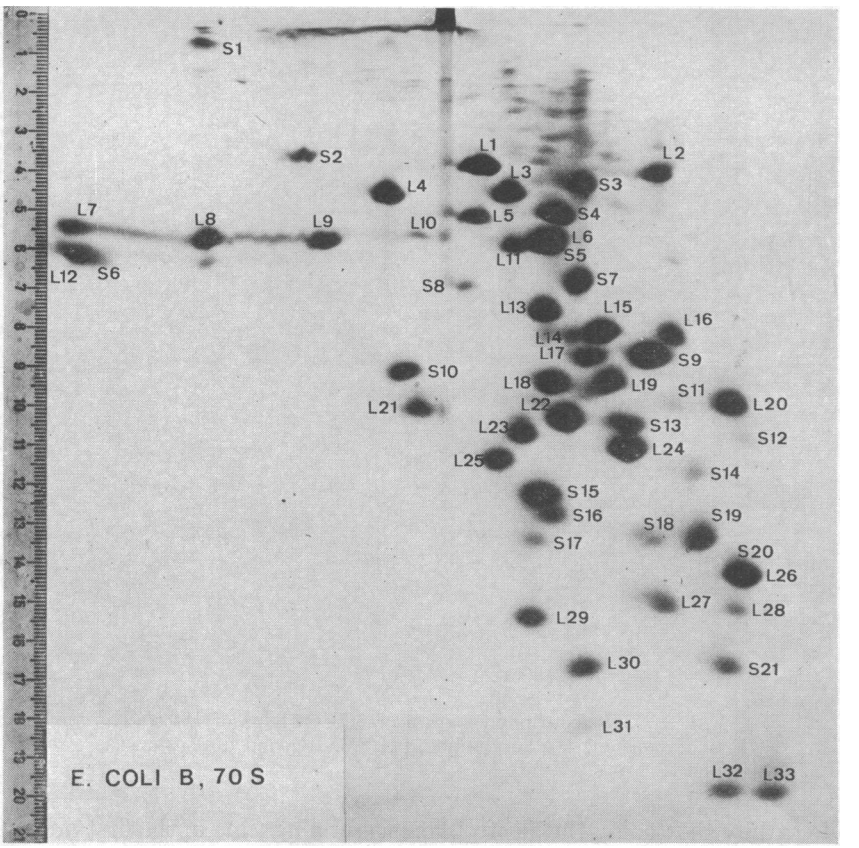

FIG. 4. 2D-electrophoretogram of 70S proteins of *E. coli* B. First dimension, 4% acrylamide, pH 8.6; second dimension as in Fig. 1.

70S ribosomes: 2D electrophoresis of the 70S proteins gives virtually the same results as superposition of the electropherograms of the 30S and 50S subunits. However, at high concentrations, certain spots of the 30S and 50S ribosomal proteins overlap one another (Fig. 4, spots S6, L12; S5, L6, L11; and S20, L26). In some cases this overlapping may be avoided, and the individual spots clearly marked off, by using smaller protein concentrations. Nevertheless, two of the proteins (S20, L26) always move in the same position.

It is clear from an examination of the electrophoretograms that the ribosomal protein spots stain with different intensities. Some stain deeply (black) while some stain faintly (grey). This is true for proteins of both the 30S and the 50S subunit. With a few exceptions, noted above, the difference in the degree of staining of the spots is reproducible from one run to another. These results generally agree with those of other investigators[4,5,10] and indicate that, in the case of some of the proteins, there is less than one copy per ribosome.

In the uppermost region of the electropherograms of untreated (i.e., not oxidized or reduced) proteins (Figs. 4, 5, and 7 *left*) one sees several shadowy spots. As these spots are in a region corresponding to proteins with molecular

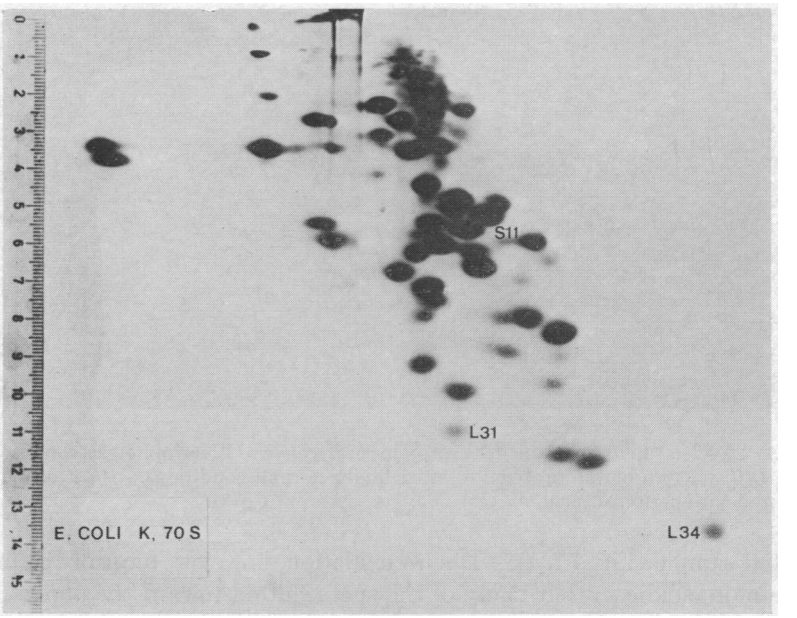

FIG. 5. 2D-electropherogram of 70S proteins of *E. coli* K. First dimension, 8% acrylamide, pH 8.6; running time, 25 hr. Second dimension as in Fig. 1.

weights higher than 40,000, they might be caused by protein aggregates. To clarify this point, the proteins were either oxidized or reduced and alkylated. Most of the spots in the upper region disappeared after oxidation (Fig. 7 *right*); only a few spots around S1 remained. The disappearance of most of these spots suggests that they are indeed caused by aggregates of single protein components linked by S–S bonds. The number of spots visible after oxidation of the 70S proteins (Fig. 7 *right*) remains unchanged when compared with those of the

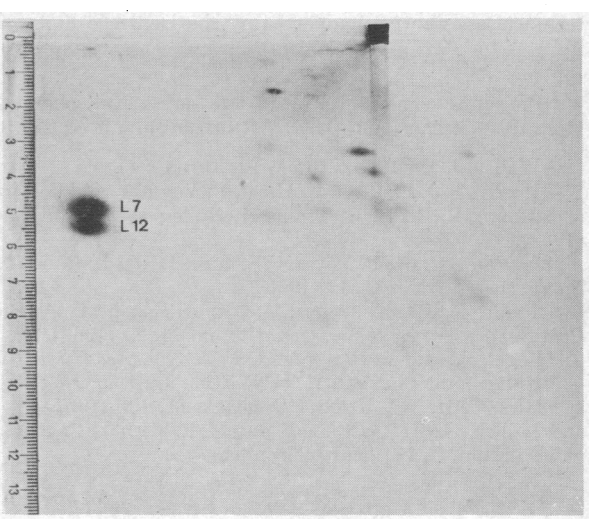

FIG. 6. 2D-electrophoretogram of protein fraction L7/-L12 on a background of added 70S proteins. First dimension, 8% acrylamide, pH 9.6. Second dimension as in Fig. 1.

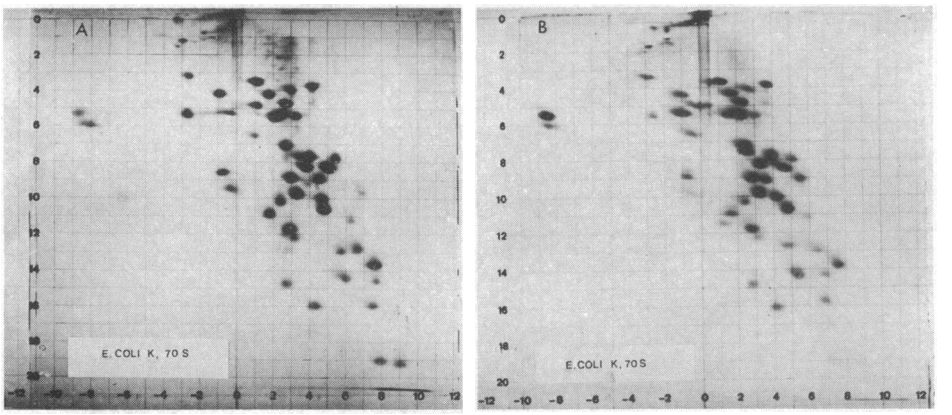

FIG. 7. 2D-electrophoretogram of 70S proteins of *E. coli* K. Electrophoresis conditions as in Fig. 1. *Left*, pattern of the proteins isolated under normal conditions. *Right*, same sample but oxidized with performic acid.[8]

untreated sample (Fig. 7 *left*). Due to oxidation, however, most of the proteins become more acidic so that many of the spots shifted toward the anode in both dimensions. The multiple spots in the vicinity of S1 may or may not be present depending on how the ribosomes and their proteins are prepared. These spots may be absent but spot S1 is always present. For this reason, spot S1 is thought to be a real 30S component, in agreement with a recent finding that this protein has a functional significance (C. G. Kurland, personal communication).

The results reported in this paper indicate that the 21 spots from the 30S and the 34 spots from the 50S subunits correspond to different ribosomal proteins. This conclusion, in accord with the results obtained in other laboratories by independent methods,[4,5] is supported by isolation and by chemical, physical, and immunological studies of the individual proteins.

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Abbreviation: 2D, two dimensional.

* Preceding communication of this series, Kaltschmidt, E., V. Rudloff, H. G. Janda, M. Cech, K. Nierhaus, and H. G. Wittmann, submitted to *Eur. J. Biochem.*

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