# THE RIBOSOMAL PROTEIN L8 IS A COMPLEX OF L7/L12 and L10

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## 1. Introduction

Most of the electrophoretic components from the 50S subunit of *E. coli* ribosomes that can be identified by two dimensional electrophoresis [1,2] have been purified and characterized in several laboratories (for a review see [3]). However, several of the putative proteins were not identified as unique species in this laboratory [4]. One in particular, L8, was recently found to behave as though it might be an aggregate containing L7/L12 and L10 [5].

We have now completed an analysis of the electrophoretic behaviour of L8, and show that it can be converted into the two well characterized components L7/L12 and L10. The reverse process, namely, reconstituting L8 from its constituent components has also been accomplished. The data persuade us that there is no unique protein species corresponding to L8 in the *E. coli* ribosome. The stability and specificity of the aggregate formed between L7/L12 and L10 suggest that these proteins are immediate neighbors bound to each other in the intact 50S subunit.

## 2. Experimental

Ribosomes and ribosomal subunits were prepared from *E. coli* MRE 600 as described previously [6]. Protein L6 was purified by nondenaturing methods [7]. A mixture of L7 and L12 was obtained by the procedure of Hamel et al. [8] while pure L7 and pure L12 were obtained by chromatography of this mixture of DEAE-cellulose as described by Möller et al. [9]. Finally, L10 was obtained essentially as described by Mora et al. [4].

The formation of protein aggregates was studied with mixtures containing different combinations of L6, L7, L10 and L12. These were dialyzed overnight against buffer 1 (20 mM sodium acetate, pH 4.5). Then they were dialyzed for 8 h against buffer 2 (20 mM Tris-HCl, 0.1 M NaCl, 5 mM $MgCl_2$, pH 7.6) before application to electrophoretic gels. All buffers contained 0.1 ml/liter β-mercaptoethanol, and the dialysis was carried out at 3–5°C.

Two-dimensional electrophoresis was carried out by the procedure of Kaltschmidt and Wittmann [1,2] as modified by Hardy [5]. Samples could be recovered from these gels by cutting out a plug containing the electrophoretic component of interest, and soaking the plug in an SDS solution over night. An alternative type of two-dimensional electrophoresis was also employed. This modified procedure consisted of putting the first dimension disc gel onto a normal 15% polyacrylamide-SDS slab gel and running the second dimension.

## 3. Results

A clear indication that 'L8' is a composite of L7/L12 and L10 can be obtained by comparing the electrophoretic pattern obtained with total 50S protein on standard two-dimensional gels and that obtained on gels containing SDS in the second dimension. L7 and L12 are resolved into two spots on the standard gels and 'L8' appears as two or more

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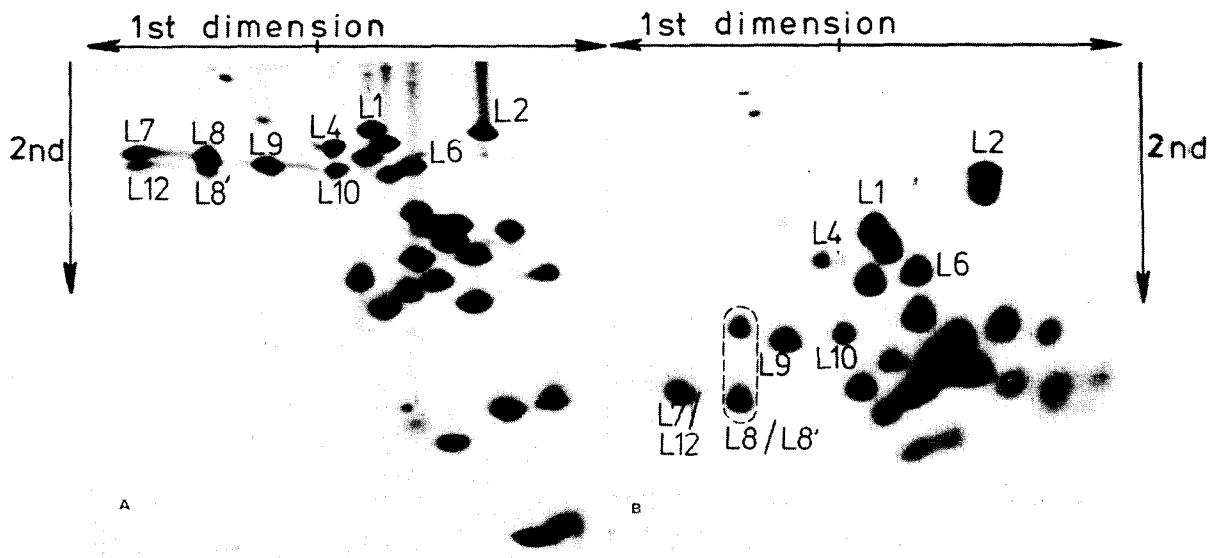


Fig.1. Two dimensional gel electrophoresis of total protein from the 50S subunit. (A) The normal Kaltschmidt–Wittmann system. 4% polyacrylamide was used in the first dimension. (B) the first dimension was identical to (A) but followed by a second dimension in SDS and 15% polyacrylamide. The two spots corresponding to L8 are enclosed with a dashed line.

components here (fig.1a). However, in the presence of SDS, L7/L12 runs as a single component while the region which should contain 'L8' has two spots one at the vertical position of L7/L12 and one at the vertical position of L10 (fig.1b).

If two of the more intense 'L8' spots are recovered from the standard gel slab and electrophoresed a second time on an SDS slab gel, they are both resolved into two components: one corresponding to L10 and the other L7/L12 (fig.2). The other acidic proteins behave in a conservative manner when extracted and rerun on SDS gels (see fig.2). Such results indicate that the multiple spots corresponding to 'L8' are aggregates of L7 or L12 and L10.

This conclusion was verified by reconstituting 'L8' from mixtures of purified L7/L12 and L10 (fig.3). As can be seen in figure 4, 'L8' can be formed just by dialyzing L7/L12 with L10 in the cold. In contrast, mixtures of L7/L12 and L6 did not yield such composite aggregates (fig.5).

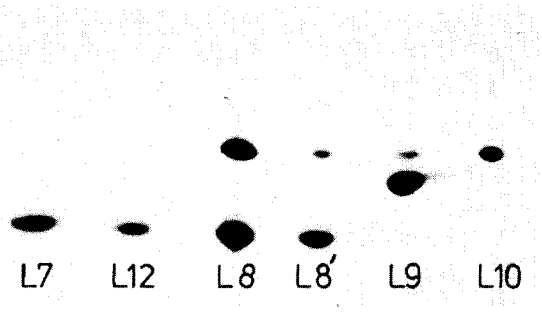


Fig.2. Single spots cut out from a gel identical to the one in fig.1a and run on a 15% polyacrylamide slab containing SDS. The proteins are from left to right L7, L12, L8, L8', L9 and L10.

## 4. Discussion

The data presented here identify 'L8' as an aggregate of L7/L12 and L10. The remarkable stability of this aggregate in buffers containing 6 M urea will account for the mistaken identification of 'L8' as a unique protein species [1,2]. However, we are at a loss to explain how this artifact escaped detection in the immunological screening described by Stöffler [10] or from two dimensional gels like the one in fig.1b [11].

We shall discuss the stoichiometry and physical properties of the L7/L12 aggregate with L10 in a

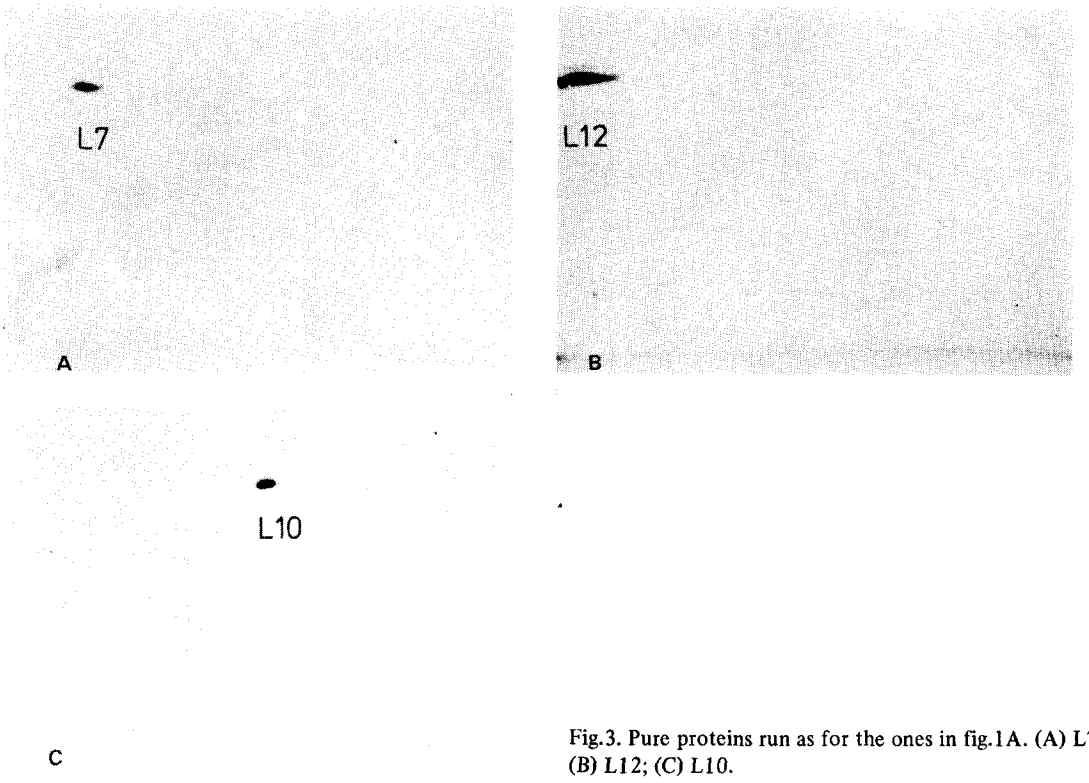


Fig.3. Pure proteins run as for the ones in fig.1A. (A) L7; (B) L12; (C) L10.

subsequent communication. Here, it is sufficient to point out that the complex does seem to be a specific one. The evidence for this suggestion is as follows: First, the very fact that a significant amount of 'L8' can be identified within the mxiture of more than thirty 50S proteins indicates that neither L7/L12 nor L10 are so 'sticky' that they will associate with any and all of the proteins in the mixture. Second, attempts to form complexes between L7 or L12 and other 50S proteins have so far failed.

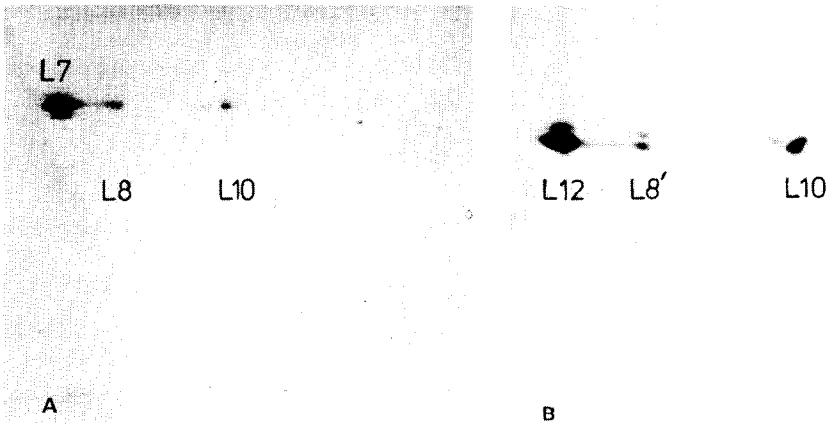


Fig.4. L7 (A) and L12 (B) mixed with L10, dialyzed as described and subsequently run like fig.1A. The L8 spot is formed.

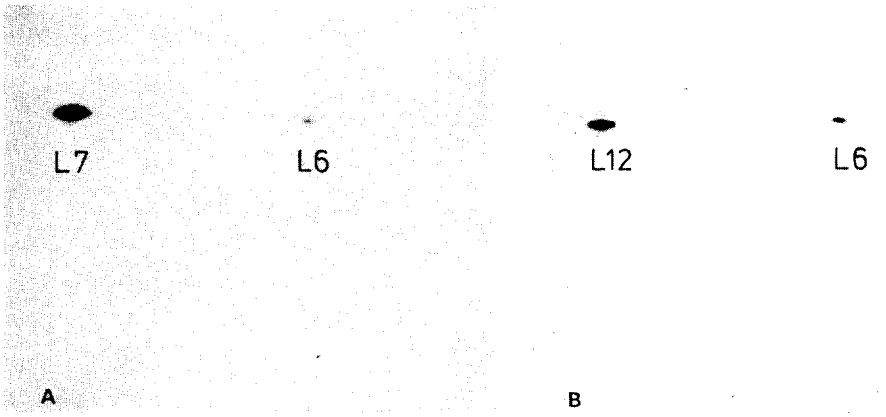


Fig.5. L7 (A) and L12 (B) mixed with L6, dialyzed and run at conditions identical to the ones in fig.1A. No new spots are generated.

This specificity is also reflected in the in vitro assembly of these proteins into the 50S subunit. Thus, it has been shown [12,13] that L7/L12 does not bind properly to a 50S core particle lacking L10 or L6. Similarly, Stöffler et al. [14] have shown that antibodies raised against L10 or L6 will block the reassembly of L7/L12 into the 50S subunit. Such observations suggest that these proteins interact directly or indirectly with each other in forming the finished subunit. Accordingly, it seems likely that the complex of L7/L12 and L10 represents a protein neighborhood of the functional 50S ribosomal subunit.

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