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# Localization of surface peptide from ribosomal protein L7 on 80 S ribosome by biotinylation

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A surface topography of ribosomal peptides on ribosome particles was conducted by using *N'*,Hydroxysuccinimido-biotin (NHS-biotin) modification. All rat ribosomal proteins, except proteins L3 and L8, are biotinylated when the ribosome particle is the substrate. A surface peptide from protein L7 was determined from biotinylated ribosomes by high performance liquid chromatography and cyanogen bromide peptide mapping. It was found that only the tandem repeats of the $NH_2$-terminal segment of protein L7 are accessible to biotinylation. It is concluded that the $NH_2$-terminal-end of protein L7 should be exposed on the surface of ribosomal particles.

Biotinylation; Ribosomal protein; Ribosomal peptide; *N'*,Hydroxysuccinimido-biotin (NHS-biotin)

## 1. INTRODUCTION

The surface localization of ribosomal components on ribosomes has been deduced by immuno-electron microscopic studies [1], and mass distribution of neutron scattering studies [2]. Thus, the more detailed information at the molecular level on local arrangement of peptide fragments and/or amino acids or nucleotides becomes the second order of the prospect for the better understanding of the structure-function relationship of ribosomes. The epitope specificity of antibodies against *Escherichia coli* ribosomal proteins has been used for the purpose of probing the local arrangement of functional domains on the *E. coli* ribosomes [3,4,5]. Despite lacking the specific antibodies to eukaryotic ribosomal proteins, a chemical modification approach has been used to examine the surface localization of ribosomal proteins [6,7]. In the present study, we have extended the techniques and combined them with high performance liquid chromatography to examine the topographic distribution of a single ribosomal protein at the peptide level. Ribosomal protein L7 from rat ribosomes was purposely selected, and a chemically modified surface peptide was mapped on ribosomal particles through the analysis of the biotinylation.

## 2. MATERIALS AND METHODS

Ribosomes were prepared as described elesewhere [8]. The total extraction of ribosomal proteins from 80 S ribosomes was performed following the standard procedures [9].

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Biotinylation of ribosomes (rat liver) was performed in a solution containing 80 S ribosomes (90 $A_{260}$ units/ml); 50 mM KCl and 5 mM $MgCl_2$ (in some experiments various concentrations of $MgCl_2$ were used) in 0.1 M phosphate buffer, pH 7.0. After dialyzation at 4°C against 2 changes of this buffer, NHS-biotin (3.0 mg/100 $\mu$l *N',N*-dimethylformamide) was added to a concentration of 1 mg NHS-biotin/90 units of $OD_{260}$ ribosomes and allowed to react for one hour at 4°C. The mixture was centrifuged at low speed (10 000 rpm; 1 min); the supernatant containing ribosomes was subjected to protein extraction or pelleted down through a 1 M sucrose solution containing TKM buffer. Buffers containing primary amines should not be used for the biotinylation reaction of ribosomal particles [10].

The ribosomal proteins from biotinylated and non-biotinylated ribosomes were separated by high performance liquid chromatography on an octadecyl (C-18) reverse phase column (100 × 7.5 mm ID; Bio-Rad). The eluents were: buffer A, 0.1% aqueous TFA; Buffer B, 0.1% TFA in 60% acetonitrile; and a linear gradient of 0 – 60%. The eluate was monitored at 214 nm, 0.2 a.u.f.s., flow-rate 0.7 ml/min, temperature 30°C, and monitored at a wavelength of 214 nm.

Protein L7 was cleaved with cyanogen bromide [11] and the mixture of fragments of protein L7 was resolved by one-dimensional gel electrophoresis containing sodium dodecyl sulfate [12].

Samples of ribosomal proteins and biotinylated proteins were also resolved using both one-dimensional polyacrylamide gel (containing sodium dodecyl sulfate) [12] and micro-two-dimensional polyacrylamide gel electrophoresis [13]. Gels were transferred to nitrocellulose, blots were soaked with blocking solution containing 50 mM Tris-Hcl, pH 7.4, 150 mM NaCl (TBS) with 5% non-fat dried milk for 30 min, washed 3 times in TBS and further incubated with avidin-peroxidase complexes in a blocking solution. Chromogenic development was processed by using *P*-chloranapthol (0.5 mg/ml in 20% methanol) with 0.03% $H_2O_2$. The color was allowed to develop in subdued light.

## 3. RESULTS AND DISCUSSION

Over the years a substantial number of chemical modification reagents have been studied for their ability to interact with amino acid functional groups. These reagents are used to determine which amino acid side groups are so-called 'buried' and 'exposed' side chains.

The modification data, combined with primary sequence information, have yielded considerable general information for the construction of the tertiary structure of a molecule.

A number of workers have attempted to extend the use of chemical reagents to determine the general features of ribosome structure [6,7]. We have employed the approach and achieved success in defining the surface topography by exploiting the efficacy of the N′,hydroxysuccinimido-biotin (NHS-biotin) reagent. The treatment of ribosome particles with the NHS-biotin reagent modifies the lysine and free amino groups of ribosomal proteins where they are accessible. The reactivity and sensitivity of biotin detection are known to be within the 10 – 100 ng range [10]. The degree of accessibility to the biotin reagent reflects the total area exposed on the surface of the ribosome. Examining the distribution of biotinylated residues on ribosomal proteins would further reveal the topographic arrangement of ribosomes at the molecular residue level.

The biotinylated ribosomal proteins from treated ribosomes were easily detected after electrophoresis and electro-transfer to nitrocellulose from one-dimensional polyacrylamide gels containing sodium dodecyl sulfate. Almost every protein, except the large subunit protein L3, took the avidin-peroxidase-biotin chromogen staining (Fig. 1). The resolution power of one-dimensional gels for separating total ribosomal proteins is limited, and only a few proteins, L3, L4, L5 and L6 are distinguishable as single bands. Thus, the analysis of ribosomal proteins for biotinylated particles was further resolved by micro-two-dimensional polyacrylamide gel electrophoresis [13]. The Coomassie staining of the two-dimensional gel electrophoregram clearly shows the NHS-biotin effects which caused almost every protein spot to sail away from its original position (Fig. 2, panel B). The sailing 2D electrophoregram has made it difficult to verify the original identity of each spot. However, the sailing electrophoregram can be reviewed by carefully comparing with the standard electrophoregram. Two proteins, protein L3 and protein L8, were found not to change their positions at all, being unreactive to NHS-biotin reagent even though they have a high content of lysine residues [14]. The status of L3 agrees with the result of the one-dimensional gel while the new identity of protein L8 was concealed because of the resolution power of the two-dimensional gel. The results implied that proteins L3 and L8 might be buried inside of the particle. The identities of some of the biotinylated proteins were tentatively assigned to their parent proteins as indicated in Fig. 2D.

It has been demonstrated that ionic strength, particularly the concentration of magnesium ions, greatly affects the compactness of the ribosome structure [15,16]. Thus, the biotinylation of the ribosome was examined under various magnesium ionic concentrations

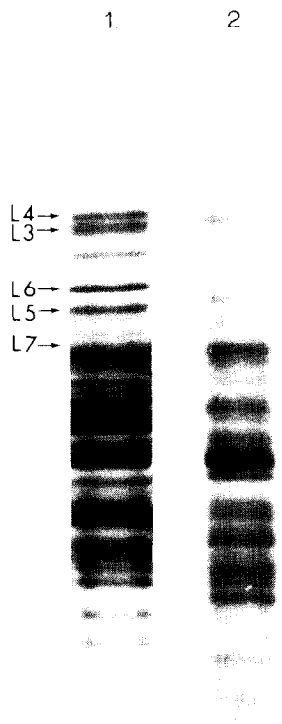



Fig. 1. One-dimensional polyacrylamide gel electrophoresis containing sodium dodecyl sulphite. Lane 1 was total ribosomal proteins from 80 S ribosomes stained with Coomassie blue R; lane 2 was an electro blot of total ribosomal proteins from biotinylated 80 S ribosomes, visualized by biotin-avidin chromogen staining. The analysis was of 10 μg of total ribosomal proteins for both lanes.

and no detectable differences were observed; protein L3 and L8 still remained unreactive. Whether there is comparable increase in the percentage of accessible sites for individual biotinylated ribosomal proteins still remains to be determined.

Application of high performance liquid chromatographic techniques to the separation of ribosomal proteins of different organisms has been reported [17]. Total proteins from 80 S ribosomes and biotinylated ribosomes were analyzed by high performance liquid chromatography. The separation is very efficient (Fig. 3). Whether biotinylated or non-biotinylated, in general there is no difference in the chromotography profile. Only a few peaks shifted (Fig. 3; as indicated by small arrows).

The accessibility of an individual ribosomal protein to the biotin reagent was interesting. Protein L7 was purposely selected because (1) its primary structure has been determined [18]; (2) it has a special structural motif, 12 residues of tandem repeats at its $NH_2$-terminal end [18]; (3) its characteristics are displayed on a biotinylated 2D electrophoregram; and (4) it can be obtained easily as a single peak on the profile of high performance liquid chromotography (Fig. 3). Two biotinylated preparations of protein L7 were

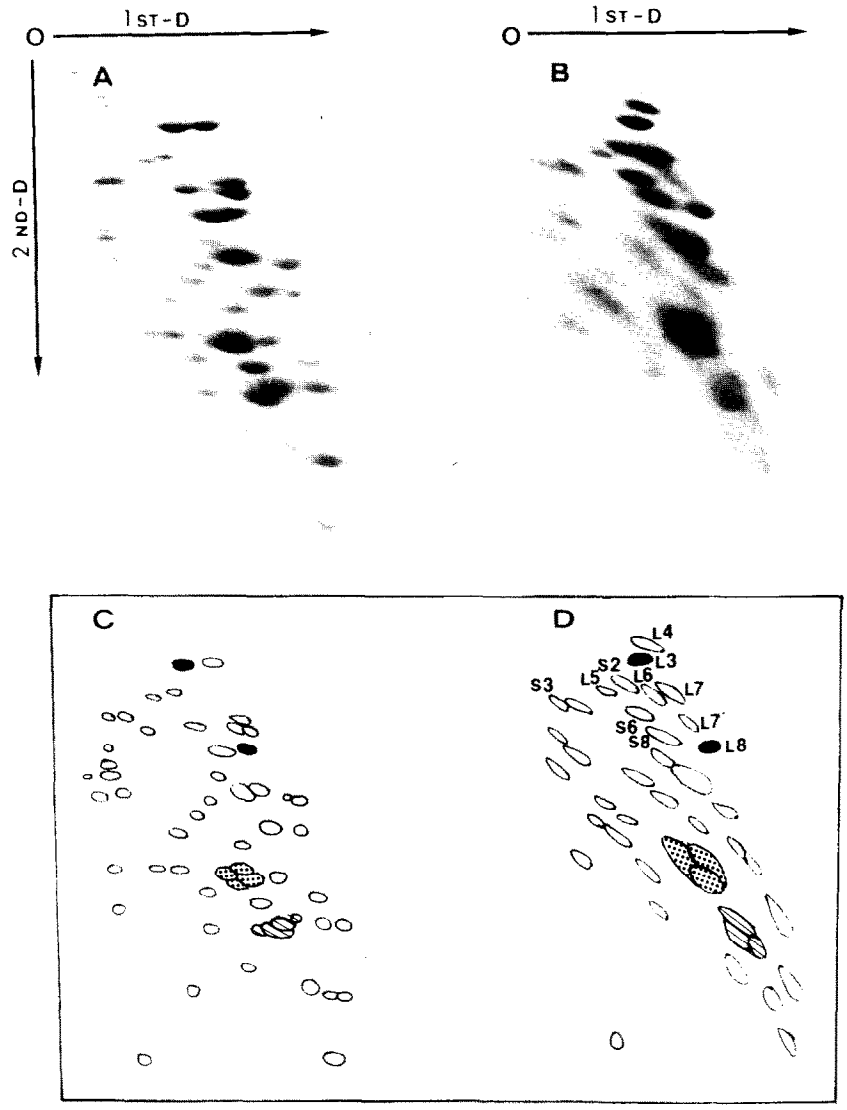
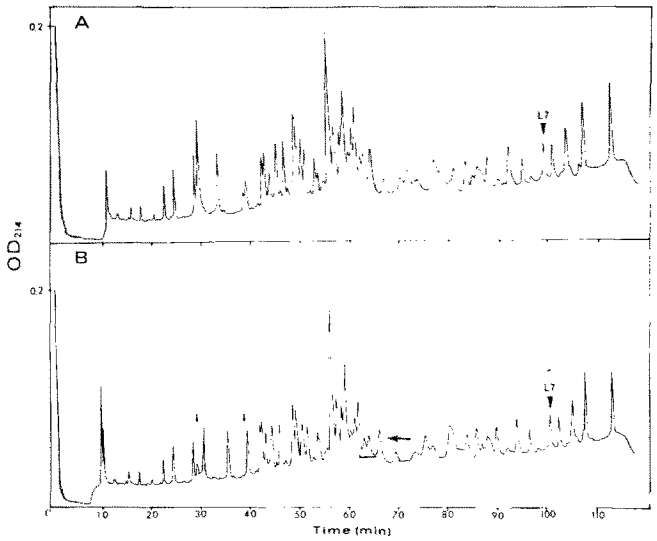



Fig. 2. Two-dimensional polyacrylamide gel electrophoresis of 80 S ribosomal proteins. Electrophoresis was from left (cathol) to right in the first dimension, and from top to bottom in the second. The origin is marked 'O'.




carried out, one biotinylated in the free state, and the other obtained from biotinylated 80 S particles by high performance liquid chromatography. The patterns of cyanogen bromide cleavage maps from the 2 preparations were compared (Fig. 4). The cleavage maps of these 2 biotinylated L7 proteins have great differences; the former one has a total of 5 peptides extensively modified by the NHS-biotin reagent, while the latter shows only one peptide that is biotinylated. The biotinylated peptide belongs to the $NH_2$-terminal segment from $NH_2$-terminal residue to position at Met-76

Fig. 3. Separation of 80 S ribosomal proteins by high performance liquid chromotography. Panel (A) is the separation of total proteins from 80 S ribosomes; and Panel (B) shows the separation of total proteins from biotinylated 80 S under the same condition as (A).

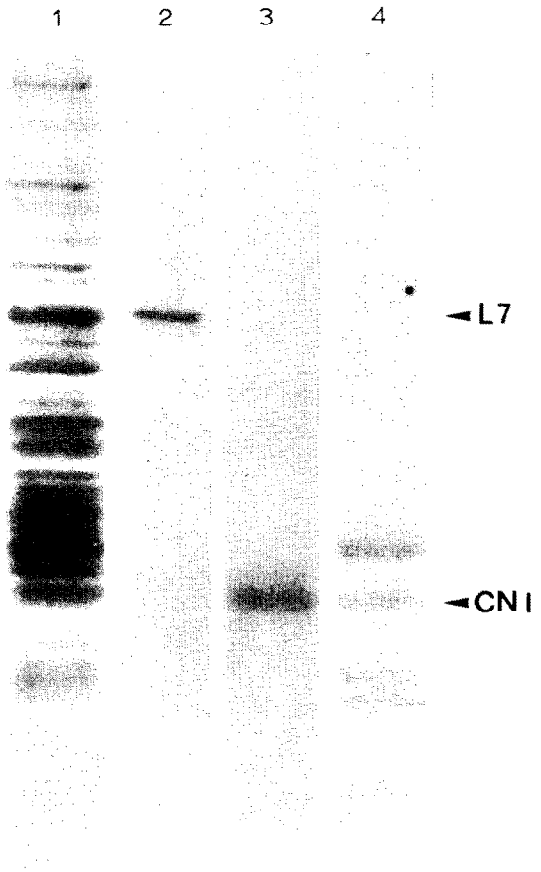



Fig. 4. Nitrocellulose blot of biotinylated ribosomal protein L7 and its peptides. The blot was detected using avidin–peroxidase complexes with *p*-chloronapthol chromogen system. Lane 1 shows the total proteins extracted from biotinylated 80 S ribosomes; lane 2 is biotinylated L7 isolated from biotinylated 80 S ribosomes by HPLC; lane 3 is the cyanogen bromide-cleaved product of that of biotinylated protein L7; and lane 4 is the cyanogen bromide-cleaved product of L7 which was biotinylated in the free state. Each band in lane 4 was properly identified from previous experiments (reference [18], and unpublished data). The CN-1 fragment is a 75 amino acid residue peptide from the $NH_2$-terminal of protein L7.

where 4 tandem repeat units reside [18]. The possible roles of these repeat units have been proposed [18]. They are considered to be (1) in binding of ribosomal RNA through its basic side chain interaction, and/or (2) in contacting of membrane through its predicted amphipathic structure. Our data indicate clearly that the structure is exposed to the surface of ribosomal particles as its basic residues are susceptible to modification. It is unlikely that the units serve to bind to the ribosomal RNA, thus, the possibility of interacting with endoplasmic reticulum membranes and serving as ribosome anchor site remains to be explored.

Our results are very encouraging. They offer the possibility of using biotin incorporation as an effective probe for surface topographic studies. We are now catalogueing the individual surface sites for each ribosomal protein through biotinylation.

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