THE INFLUENCE OF CONCENTRATING METHODS ON ELECTRON MICROSCOPICAL IMAGING OF NEGATIVELY-STAINED 50 S RIBOSOMAL SUBUNITS OF *ESCHERICHIA COLI*

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1. Introduction

Electron microscopy of negatively-stained 50 S ribosomal subunits of *Escherichia coli* has revealed two principle projection forms, crown form and kidney form [1–4]. It is accepted, but not proved directly, that these forms represent projection forms of the same three dimensional particle structure, interchangeable by rotation of 90°. Crown forms were described as symmetrical [3,5] or as asymmetrical projections [4,6]. These forms are differing in the size and insertion of a rodlike appendage, which is clearly visible in asymmetrical but not in symmetrical projections. If artificial, which seems most likely, these differences could be introduced during isolation or by the preparation for electron microscopy. On basis of these crown forms different three dimensional models of the 50 S ribosomal subunit have been developed [4–6].

Depletion experiments on 50 S ribosomal subunits using various agents [6,7] revealed the rodlike appendage to be extremely sensitive to external influence; the structural products of the first step of depletion were in all cases similar to symmetrical crown projections.

This paper presents results showing that conditions without the depletion effect can cause conformational changes leading to symmetrical crown forms. Among methods in use for concentrating ribosomal suspensions, ethanol precipitation was found to influence imaging of 50 S ribosomal subunits.

2. Materials and methods

2.1. *Isolation of ribosomes and subunits*

50 S ribosomal subunits were obtained from 70 S tight couple ribosomes [8], isolated from *Escherichia coli* MRE 600, by dissociation in low magnesium concentration and subsequently purified from 30 S subunits by sucrose gradient centrifugation.

2.2. *Concentration methods*

70 S ribosomes or 50 S subunits were adjusted to 1 mg/ml and either pelleted by high speed centrifugation (200 000 × *g* for 2 h) or precipitated by polyethylenglycol (PEG) [9] and by ethanol [10], respectively, and subsequently pelleted by centrifugation (12 000 × *g* for 15 min). All pellets were resuspended to a ribosomal concentration of ~0.02 mg/ml.

2.3. *Electron microscopy*

50 S subunits were adsorbed onto carbon films (about 2 nm thick) by diffusion and negatively stained with 0.5% or 2% uranylacetate (not adjusted for pH). Microscopy was performed with a Philips 301 electron microscope at calibrated direct magnifications of 69 000 × or 91 000 ×.

2.4. *Image processing*

Selected single images of identical projection were arranged to square-arrays of at least 9, 16 or 25 different images and processed by light optical diffraction and filtering applying the FAIRS method [11]. For

reconstruction of images the central maxima of diffractograms were strongly attenuated, resulting in enhanced contour lines of particle images. Diffraction work was performed with a LOD 1 light optical diffractometer (Spindler und Hoyer, Göttingen).

3. Results and discussion

Typical projection forms of isolated 50 S ribosomal subunits as obtained by electron microscopy after negative staining were selected. Different images of identical forms were arranged to arrays of images and processed by FAIRS. Figure 1 shows the result of this work. Characteristic projection forms are represented by the central images of the optical reconstructions. Two clearly asymmetrical crown forms differing in the projection of the rod-like appendage were discriminated (fig.1a,b). Similar forms were found earlier [4,6], and interpreted as front and top views of the same particle form 4. Figure 1c shows a symmetrical crown form which is similar to forms descibed by various authors [1–3,5]. Figure 1d shows the kidney projection. While crown forms are considered to be front or back views, kidney forms are side views of the same structure [1–6].

Forced by needs to find identical images suitable for FAIRS a further projection of the 50 S ribosomal subunit could be discriminated. It is '6'-shaped and characterized by one prominent, curved appendage inserted on the side of the particle body (fig.1e). Concluded from size, shape and staining, this appendage corresponds with the central protuberance of crown forms. Therefore, this projection form is most likely a side projection.

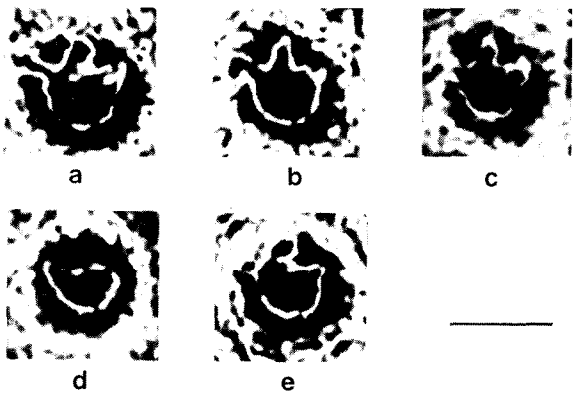

Fig.1. Characteristic projection forms of isolated 50 S ribosomal subunits. Representative central images of reconstructions after light optical diffraction and filtering of arrays of selected images (FAIRS). (a) and (b) different asymmetrical crown projections. (c) symmetrical crown projection. (d) kidney form. (e) 6-form. Bar represents 25 nm.

Screening fields of 50 S subunit preparations like this of fig.2a revealed highly different frequencies of described images. An evaluation of frequencies is listed in table 1. Asymmetrical crown forms were predominant, followed by symmetrical crown forms. Kidney forms and the '6'-shaped form were rare. A similar evaluation [3] is in contradiction to this finding, as these authors found a much higher amount of kidney forms.

The original isolates were treated by means used for concentrating ribosomes. No significant differences in frequencies and imaging of particles were found, when high-speed centrifugation or PEG precipitation were applied (table 1). However, ethanol precipitation produced different frequencies of particle projections (fig.2b, table 1). Asymmetrical crown projections became infrequent, while symmetrical crown forms, lacking the pronounced rodlike appendage, were the dominant type of image. There was also a considerable change in the amount of the side projections, which increased. This feature was independent of ethanol concentration (40% or 50%) and magnesium concentration (10 mM, 15 mM or 65 mM). Even when 50 S subunits were isolated out of ethanol precipitated 70 S ribosomes, coinciding results were obtained.

It might be possible, that ethanol treatment alters the adsorption properties of ribosomal particles without changing their structure. However, as it was shown, that the type of projection does not depend on adsorption at all [12], this explanation seems to be unlikely. There are no hints for protein depletion under the applied conditions. Therefore, conformational changes are likely. It was shown, that copies of ribosomal proteins L7/L12 are located in the rodlike appendage [6]. These proteins are elongated in situ [6], and in solution [13,14], contain high amount of α-helical structure [15,16], and revealed sequence homologies to myosin [17]. On basis of these qualities it might be speculated:

1. That these proteins enable or facilitate the rodlike

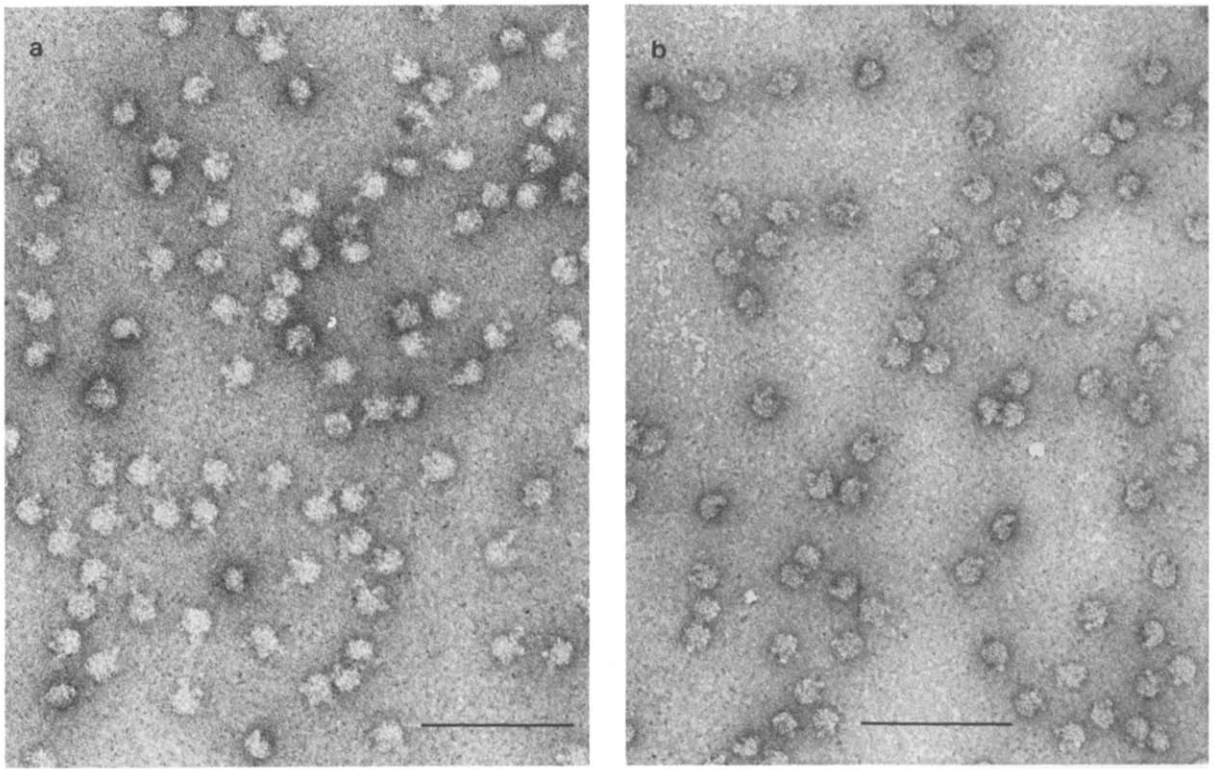

Fig.2. Fields of 50 S ribosomal subunits after negative staining. (a) Untreated population; (b) population, precipitated by ethanol (50%, Mg^{2+}-concentration in buffer was 15 mM) as described in section 2. Bars represent 100 nm.

Table 1
Procentual distribution of 50 S ribosomal subunit projection forms

Projection forms (fig.1)	Untreated	High speed centrif.	PEG precipn.	Ethanol precipn.
1a,b	60	55	55	10
1c	30	35	30	60
1d	5	5	10	20
1e	5	5	5	10
Evaluated particle images	714[a]	681[a]	638[a]	532[a]
Identified images	524[a]	527[a]	453[a]	418[a]

[a] Absolute values

appendage to flexibility, in contrast to the assumption of [4]; the two different asymmetrical crown forms (fig.1a,b) could in this way be explained as identical projections, which is supported by the unchanged imaging of the other appendages.

2. That these proteins enable a retraction of the appendage, resulting in symmetrical crown forms (fig.1c). This conformational change could account for loss in biological activity of ethanol-treated ribosomes [9].

Essential consequence for the structure of the 50 S ribosomal subunit is, that both crown projections may represent different conformations of a particle. Obviously, this change can be generated by external factors. It seems likely, that the more complex, asymmetrical crown forms, abundant in highly active preparations are close to the native particle structure.

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