ASSOCIATION PRODUCTS OF NATIVE AND DERIVED RIBOSOMAL SUBUNITS OF *E. COLI* AND THEIR STABILITY DURING CENTRIFUGATION

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1. Introduction

During our studies of the ribosome cycle we have investigated the association behaviour of so-called native and derived ribosomal subunits of *E. coli* [1]. It was noted that the native sub-particles yield an association product sedimenting between 59 S and 63 S (designated 61 S ribosomes), whereas derived subunits associate to form 70 S ribosomes. The 50 S subunit appeared to determine the sedimentation rate of the association product.

Recently Infante et al. [2–4] showed that free sea urchin ribosomes dissociate during sucrose gradient centrifugation as soon as they reach a zone in which the hydrostatic pressure exceeds a certain value. The thus formed "pressure-derived" subunits appear as one peak before they are resolved and lag behind the expected position of undissociated ribosomes. When this phenomenon occurs, conclusions concerning the sedimentation coefficient of the particles involved are obviously erroneous.

In this paper we show that the product obtained by association of native *E. coli* ribosomal subunits also dissociates under the hydrostatic pressure caused by centrifugation and furthermore that the association products from native and derived subunits differ in pressure sensitivity rather than in true sedimentation rate. The stabilizing effect of $tRNA_{OH}$ is illustrated.

2. Materials and methods

The preparation of native and derived ribosomal subunits from *E. coli* Q13 has been described [1]. Labelled particles were obtained from bacteria grown in medium containing ^{3}H-uridine. 30 S subunits were free of 50 S subunits and the 50 S subunits contained less than 5% 30 S subunits and no 70 S ribosomes.

3. Results

Fig. 1. shows the results when reaction mixtures containing either labelled 30 S (fig. 1A) or labelled 50 S (fig. 1B) and an unlabelled partner are analyzed by centrifugation in a sucrose gradient. The 61 S association product is recognized in the absorbance profile as a distinct peak in fig. 1A, but the radioactivity is smeared out over the 40–70 S region. The specific radioactivity of the 61 S peak is far below the value expected for an association product (one third of that of the 30 S particles). Apparently this peak consists mainly of 50 S subunits with the 30 S particles lagging behind. A similar conclusion is reached when the radioactivity of the 61 S of fig. 1B is observed. The phenomenon is even more pronounced when a steeper gradient in the hydrostatic pressure is applied (fig. 3). In the experiment of fig. 1 this gradient is concave and runs from 0 atm at the meniscus (neglecting atmospheric pressure) to 674 atm at the bottom of the sucrose gradient (fig. 2, curve a). In the experiment of fig. 3 it runs from 0–1950 atm (fig. 2, curve b). Under the latter conditions the asso-

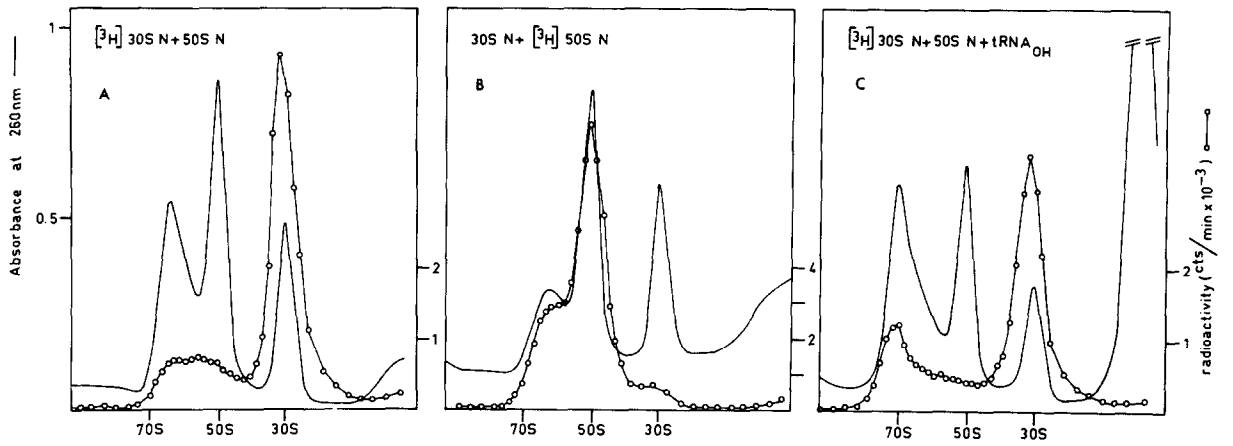

Fig. 1. Sedimentation analyses of combinations of labelled and unlabelled native ribosomal subunits after association. Reaction mixtures (total vol. 0.2 ml) of ribosomal subunits were incubated in 10 mM Mg acetate, 60 mM NH_4Cl, 6 mM β-mercaptoethanol and 10 mM Tris-HCl, pH 7.6 (standard buffer) at 37° for 30 min. A) contained 40 μg of ^{3}H-labelled 30 S and 80 μg of cold 50 S subunits; B) 40 μg of cold 30 S and 80 μg of ^{3}H-labelled 50 S subunits and C) as A but in addition 100 μg of uncharged tRNA. The mixtures were then centrifuged in 10–30% linear sucrose gradients for 15 hr at 22,000 rpm in a SW 27 rotor. Absorbance at 260 nm was monitored continuously in a Gilford spectrophotometer. Radioactivity of 5 drop fractions was counted in a liquid scintillation counter.

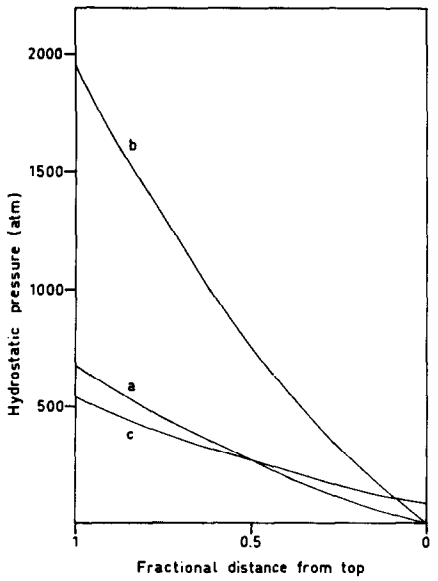

Fig. 2. Hydrostatic pressure gradients under different conditions of centrifugation. Curve a: Linear gradient of 19 ml (10–30% sucrose) spun at 22,000 rpm in a Beckman SW 27 rotor. Curve b: Linear gradient of 13 ml (10–30% sucrose) spun at 41,000 rpm in a Beckman SW 41 rotor. Curve c: Linear gradient of 570 ml (10–45% sucrose) with an overlay of 100 ml standard buffer, spun at 47,000 rpm in a B XIV zonal rotor of MSE.

ciation product no longer exists after sedimentation as the "61 S ribosomes" are virtually devoid of radioactivity (fig. 3A). This conclusion also follows from fig. 3B in which the specific radioactivity of the "61 S" shoulder corresponds fairly well with that of the 50 S peak. (The difference in absorbance profile between A and B of fig. 1 and 3 is due to slight variations in the associating abilities of subunit preparations.) The data are in line with the assumption that the ribosomes start to dissociate at a certain pressure. The steeper the pressure gradient, the sooner the ribosomes dissociate and the better the pressure-derived subunits can be resolved.

In order to verify whether hydrostatic pressure is the crucial factor determining dissociation of the association product during centrifugation, pressure gradients were applied as depicted in fig. 4, curves a, b and c. With the latter two gradients ribosomes are already exposed to significant hydrostatic pressures (275 and 475 atm, respectively) prior to sedimentation. If this initial pressure exceeds a certain value one may predict a sedimentation profile indistinguishable from that of a mixture of free 30 S and 50 S subunits. This prediction has been confirmed by the following experiments (figs. 5 and 6).

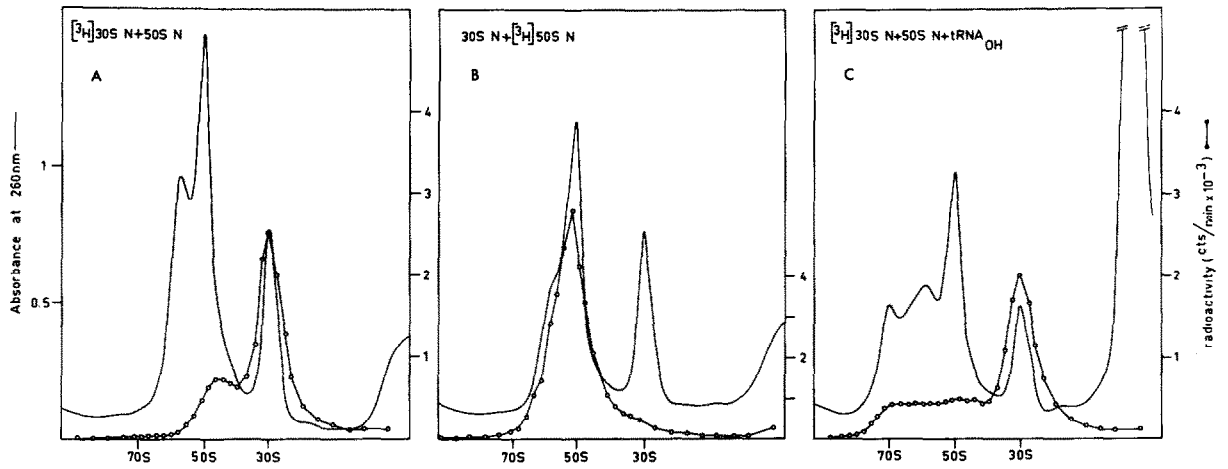

Fig. 3. Sedimentation analyses of combinations of labelled and unlabelled native ribosomal subunits after association. Reaction mixtures, A, B and C were identical to A, B and C in fig. 1. They were centrifuged for 4 hr at 41,000 rpm in a SW 41 rotor.

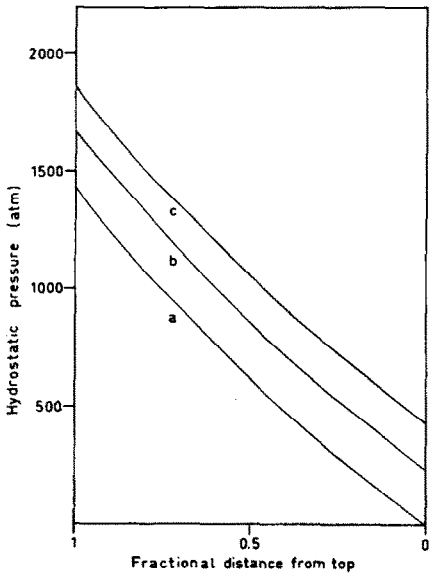

Fig. 4. Hydrostatic pressure gradients applied in fig. 5 and 6. For curves a, b and c compare legends of fig. 5A, B and C.

Reaction mixtures identical to those of fig. 1A were layered on top of sucrose gradients and subsequently covered with buffer overlays of varying heights. The pressure gradients in fig. 5A, B and C thus obtained in the SW 41 rotor after reaching maximal speed correspond to curves a, b and c of fig. 4. The results illustrated in fig. 5A are identical to those of fig. 3A. Fig. 5C shows independent sedimentation of subunits separated throughout the entire centrifugation. From fig. 5C it is concluded that 475 atm is sufficient and from fig. 5B that 275 atm is insufficient to cause complete dissociation of the association product. The same conclusion may be drawn from the experiment of fig. 6, in which the association product was first isolated and subsequently sedimented under the same conditions as in fig. 5A, B and C. The association product was isolated by fractionation of an association reaction mixture in a zonal rotor. The pressure gradient in this rotor (fig. 2C) is evidently not steep enough to complete the separation of the pressure-derived subunits. The particles in the 61 S region may therefore be compared with those of fig. 1 which contain an excess of 50 S subunits. The minor amount of free 30 S subunits present in this region during centrifugation reassociate with 50 S under atmospherical pressure. Consequently a purity test of the isolated association product performed by recentrifugation in the sucrose gradient (fig. 6A), reveals particles sedimenting at 61 S and at 50 S but not at 30 S.

That the association product is pressure sensitive, follows from the experiments of fig. 6B and C (pressure gradients shown in fig. 4b and c). Particles behaving like "61 S ribosomes" in fig. 6A sediment

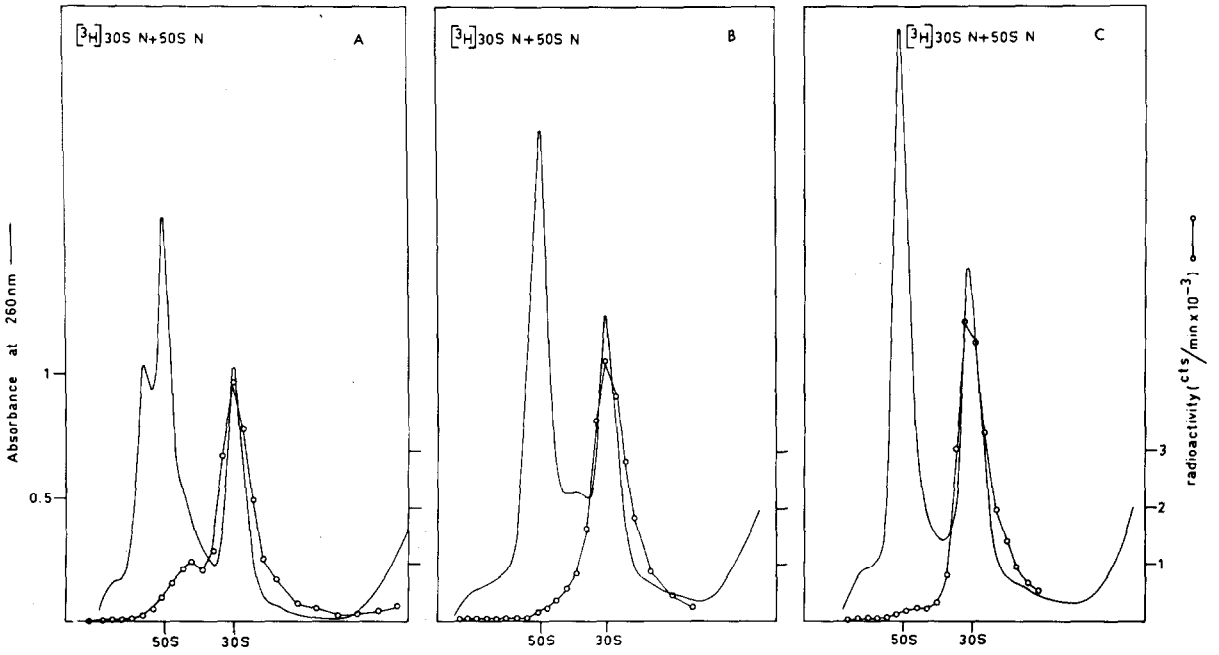

Fig. 5. Sedimentation analyses of the association product using different pressure gradients. Reaction mixtures identical to that of fig. 1A were layered on top of 8.5 ml sucrose gradients (10–30% sucrose) and covered with different amounts of buffer: A) no buffer, B) 2.25 ml and C) 4.5 ml buffer overlay. Centrifugation occurred for 2 hr and 40 min at 41,000 rpm in a SW 41 rotor.

in two separate peaks of 50 S and 30 S in fig. 6C. In fig. 6B a peak of the small subunits can be recognized and in the 50 S region two components are just visible.

In order to estimate the true sedimentation rate of the "61 S ribosomes" the sedimentation of these particles was compared with that of 70 S ribosomes under conditions when no pressure induced dissociation could be expected (0–75 atm pressure gradient). Both types of particles appeared to sediment at almost the same rate indicating that the sedimentation coefficient of 61 S is only apparent and that the real difference with 70 S ribosomes exists in pressure sensitivity.

Uncharged tRNA has a profound effect on the association of native subunits. In association experiments with ^{3}H-tRNA$_{OH}$ radioactive 70 S particles were formed as a major product. Incubation of isolated "61 S ribosomes" with ^{3}H-tRNA$_{OH}$ also resulted in the formation of radioactive 70 S ribosomes (unpublished experiments). In fig. 1C and 3C the association of labelled native 30 S with cold native 50 S and cold tRNA$_{OH}$ has been studied. Besides "61 S ribosomes" a distinct peak of 70 S ribosomes is recognizable in the absorbance profile. The experiments are best interpreted by the assumption that tRNA stabilizes the association product. The distribution of label, however, reveals that significant dissociation occurs particularly with the steep pressure gradient (fig. 3C).

As mentioned in the introduction derived ribosomal subunits associate to form 70 S particles (cf. [1]). When the association product of labelled 30 S derived and cold 50 S derived subunits was analyzed as in fig. 1 and 3 no dissociation of the 70 S product could be detected (data not illustrated). It may be concluded that this association product is much more pressure resistant than that formed from native subunits.

4. Discussion

The distinct behaviour of the association products

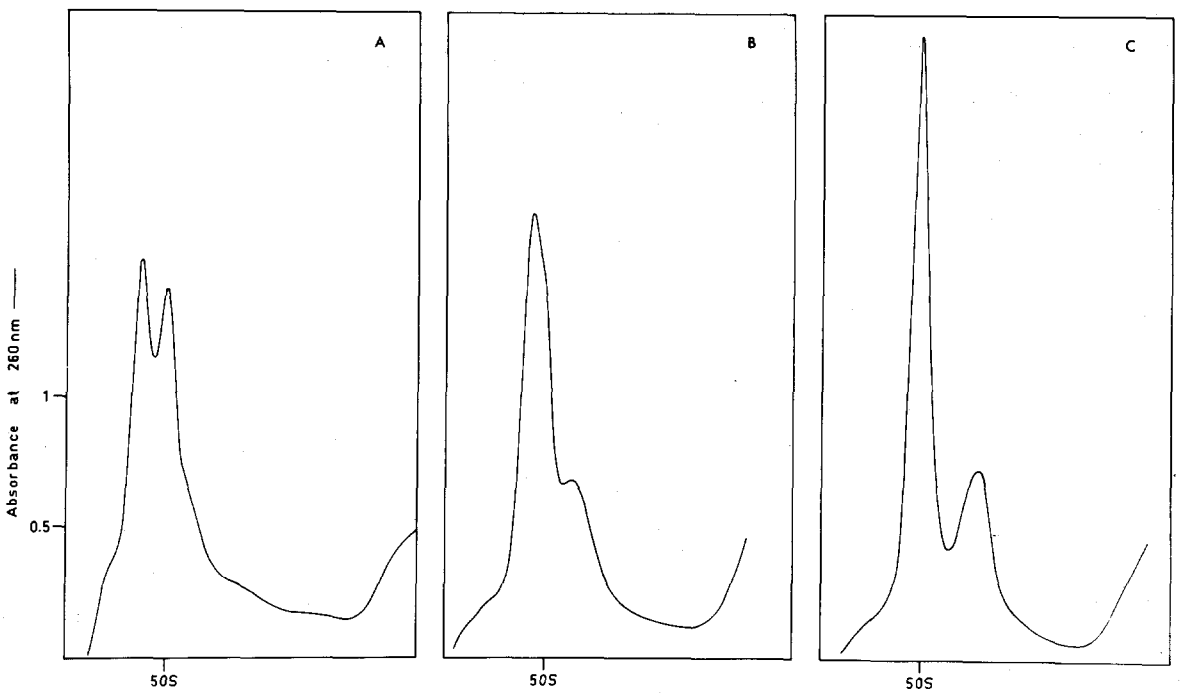

Fig. 6. Sedimentation analyses of the isolated association product of native subunits. The association product was isolated as described in the text and 80 μg was submitted to sedimentation analysis as in fig. 5A, B and C.

obtained from native and derived *E. coli* ribosomal subunits is an intriguing phenomenon. Although the true sedimentation coefficients of the products may not differ very much, the difference in pressure sensitivity is striking. The nature of the 50 S and not that of the 30 S subunits, determines the behaviour of the association products [1] and it remains for future research to clarify why derived 50 S particles yield a more pressure-stable product than native 50 S subunits. It has already been shown [1] that $tRNA_{OH}$ or aminoacyl-tRNA are not responsible for the difference in stability. Schreier and Noll [5] have described a number of pre- and posttranslocational steps which were characterized by vice versa conversions of 60 S to 70 S *E. coli* ribosomes. It is of obvious interest to learn to what extent pressure-induced dissociation has played a role in their analyses.

The data of this paper demonstrate that pressure-induced dissociation, described for the first time for sea urchin ribosomes by Infante et al. [2–4] can also occur with *E. coli* ribosomes. As pointed out by these authors, criteria other than sedimentation are demanded to establish conformational changes in monoribosomes. Evidence obtained in our laboratory suggests that differences in conformation of *E. coli* ribosomes can be recorded by polyacrylamide gel electrophoresis of intact ribosomes [6].

Recent sedimentation studies of *E. coli* ribosomes at low speed in the analytical ultracentrifuge by Spirin [7] revealed concentration effects on the sedimentation rate. These results were interpreted in terms of a dynamic equilibrium between ribosomal couples and subunits. A more extensive discussion of such a putative equilibrium is given by Infante [4].

References

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