Cerebral microsomes. IV. On the attachment of ribosomes to the microsomal membranes of rat cerebral cortex

The isolation and characterization of cerebral ribosomes and the delineation of their functional capacity have been the object of several recent studies (for review, see Datta[5]). Similarly, the aggregation of ribosomal monomers into polysomal clusters[4,8,16], the size distribution of the latter[2], the sensitivity of purified preparations of ribosomes and polysomes toward ions and the *in vitro* capacity of either population to incorporate radioactive precursors have been examined[2,4,8,12,15]. Intracellularly, nervous tissue ribosomes and polysomes have been shown to exist both free[6,14] and in association with the membranes of the endoplasmic reticulum (rough microsomal membranes)[1,7,8,10,11,14] but, although an extensive study of the ribosome–membrane interaction in hepatic tissue has appeared[9], the mode of attachment of cerebral ribosomes to microsomal membranes has hitherto not been investigated.

The present communication describes the effect of ethylenediamine tetraacetic acid (EDTA) on membrane-bound ribosomes isolated from rat cerebral cortex. It is shown that, unlike hepatic membrane-bound ribosomes, the cerebral membrane-bound ribosomes are not released by the chelating agent in two stages, with the small subunit detaching first. Rather, a concerted and virtually synchronous detachment of both subunits seems to occur. Once detached, the two subunits exhibit differential sensitivity to EDTA, the smaller subunit being more labile.

The experiments were carried out with microsomes prepared as previously described[1,10], except that the sucrose-free isolation medium was supplemented with 20 m*M* Tris (pH 7.2), 4 m*M* Mg^{2+} and 25 m*M* K^+ (solution C) or 20 m*M* Tris (pH 7.2) and 10 m*M* Mg^{2+} (solution E)[11]. As shown elsewhere[11], the microsomal pellet isolated in solution C consists of free ribo- and polysomes as well as of membrane-bound ribosomes and smooth membranes, whereas the pellet isolated in solution E lacks free RNA-containing particles and contains only membrane-bound RNA as well as smooth membranes. The latter pellet thus compares to the twice gradient-sedimented hepatic microsomal pellet used by Sabatini *et al.*[9]. Since microsomes isolated, respectively, in solution E and suspended in 20 m*M* Tris, 2 m*M* Mg^{2+} and 15 m*M* Na^+ and those isolated in solution C and subsequently 'purified' of free ribosomes by sedimentation through a 5–20% gradient of sucrose in 20 m*M* Tris and 2 m*M* Mg^{2+} (63,500 × *g*, 2 h), gave almost identical dissociation patterns, only the results obtained with the former are presented.

For control purposes, the EDTA-elicited dissociation of free cerebral ribosomes[7,16] was also examined, as this served the purpose of calibrating the sedimentation values of the experimental subunit peaks. Ribosomes were prepared from microsomal pellets isolated in solution C according to Zomzely *et al.*[15], except that the concentration of deoxycholate was raised to 0.37%. The ribosomal pellet was suspended in 1 m*M* Tris (pH 7), containing 1 m*M* Mg^{2+}. EDTA (Na_2-form, pH 7.0, 0.4 *M*) was added to microsomal or ribosomal suspensions derived from 1 g of original cortex. The samples were kept on ice for 10 min, layered on gradients and centrifuged for 8 h and 45 min at 63,500 × *g* (Spinco SW-25.1 rotor). The concentrations of

EDTA (see Fig. 1) should be corrected for the presence of 2 mM Mg^{2+} in the microsomal suspension medium.

Gradients were pumped through a hole in the bottom of the centrifuge tube and were continuously monitored at 254 mμ (LKB Uvicord, model 8300 A provided with a rectangular flow-type cell, 4 mm path length, attached to an LKB recorder, model 6520 A). The effluent was drop-collected in an LKB UltroRac fraction collector.

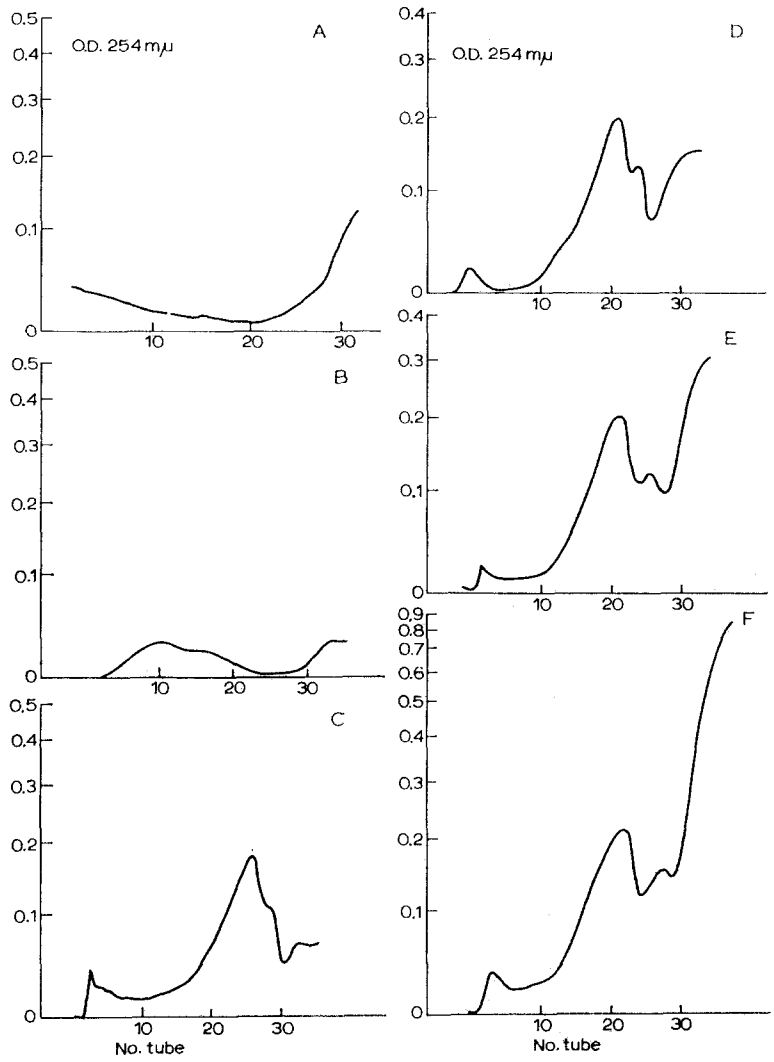

Fig. 1. Sedimentation profiles of microsomal preparations isolated from rat cerebral cortex in 20 mM Tris (pH 7.2) and 10 mM Mg^{2+} and centrifuged in a 5–20% sucrose gradient. A, Microsomes were suspended in 20 mM Tris (pH 7.2) and 10 mM Mg^{2+}. The sucrose gradient contained 2 mM Mg^{2+}. Centrifugation: 2 h at 63,500 × g (Spinco SW-25.1 rotor). B, Microsomes were suspended in 20 mM Tris (pH 7.2), 2 mM Mg^{2+} and 15 mM Na^{+} and were centrifuged for 8 h and 45 min at 63,500 × g (Spinco rotor SW-25.1) in an ion-free gradient. C–F, EDTA was added to the suspension medium as follows: C, 10 mM; D, 40 mM; E, 100 mM and F, 200 mM. Centrifugation was as in B.

Figs. 1A and B show the sedimentation profiles of microsomes sedimented, respectively, for 2 h in a gradient containing 2 m*M* Mg^{2+} and for 8 h and 45 min in an ion-free gradient. The absence of free ribosomes and ribosomal aggregates in the former preparation is clearly indicated (Fig. 1A); the pattern of Fig. 1B reveals small amounts of rather heavy material in the gradient, probably the result of spontaneous release of particulate RNA during the prolonged centrifugation in an ion-free medium.

The progressive dissociation of membrane-bound ribosomes by EDTA is shown in Fig. 1C–F. The peaks were compared to subunit standards obtained by dissociating free ribosomes in the presence of 20 m*M* EDTA (see above). This yielded a subunit pattern, closely resembling the hepatic one[9], *i.e.* an approximately 2 to 1 ratio of the large to the small subunit.

Upon addition of 10 m*M* EDTA to 1 g tissue equivalent of microsomes, a particulate material sedimenting as a large subunit together with material appearing as a shoulder in the descending, slow-sedimenting limb, was released. Increasing EDTA to 40 m*M* revealed a secondary peak in lieu of the shoulder, with sedimentation characteristics of the small subunit. Beginning at 40 m*M* EDTA considerable destruction of particulate RNA occurred, preferentially at the expense of the small subunit (Figs. 1E and F). At 400 m*M* EDTA (not shown) only the large subunit peak remained; it was destroyed by 600 m*M* EDTA.

The sedimentation profiles obtained suggest a mechanism of ribosomal release in which detachment of the ribosome and its dissociation into subunits are virtually synchronous events. Whether this peculiar property of cerebral ribosomes relates to the known *in vitro* differences between cerebral and hepatic protein synthetic systems[2,4,8,12] cannot be stated at this time. Another possibility should be entertained, namely that the apparent specificity of ribosome–membrane interactions, noted in other systems[13] as well, is coded for, and conferred by the membrane[3,9,10].

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