Fractionation of rat liver proteins involved in translocation of newly synthesized proteins at the endoplasmic reticulum membrane

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The initial stages of protein export from eukaryotic cells involve targeting of a transient signal sequence to the endoplasmic reticulum (ER) membrane followed by translocation into the lumen of the ER. Whilst it is agreed that the nascent polypeptide threads co-translationally into the ER on exit from the ribosome, it has remained a matter of contention whether or not the rough ER membrane (RER) is functionally different from the smooth ER membrane (SER) in protein translocation.

Kreibich et al. (1978a,b) have shown that RER but not SER contains the ‘ribophorin’ I and II glycoproteins which are proposed to be the ribosomal binding sites on the membrane. Hortsch & Meyer (1985) have shown by immunoblotting with a monoclonal antibody raised against the SRP receptor (or ‘docking protein’) from dog pancreas that this protein has a distribution of 95:5 in RER and SER respectively. By contrast, Bielinska et al. (1979) reported that rat liver SER were more active than rat liver RER in translocation as were bovine adrenal cortex SER, an organ containing 95% SER, whilst pure golgi membranes (Rogers et al., 1982) were inactive.

We have prepared microsomal membranes from rat liver by two methods. In each case, two rats (male Wistar strain, 200-250 g) were starved for 24 h before decapitation. Livers were removed quickly, washed in chilled homogenization buffer (0.25M-sucrose, 3mM-imidazole pH 7.4, 1mM-phenylmethylsulphonylfluoride and 100 units of Trypsin/ml) blotted and minced before homogenization in a Potter-Elvehjem homogenizer unit (1:1, w/v) followed by centrifugation for 10 min at 11 000 g. at 4°C to remove unbroken cells, nuclei and mitochondria. In method I, the postmitochondrial supernatant (PMS) was layered on to a preformed eight-step continuous sucrose gradient made by successive layering of 3.5 ml of 2.5M-sucrose, 1.98M-, 1.75M-, 1.58M-, 1.38M-, 1.18M-, 0.99M- and 0.8M-sucrose in 3mM-imidazole, pH 7.4. Overnight centrifugation (for 16 h at 11 000 g. at 4°C) yielded 12 fractions (2.5 ml each) of density from 1.04 to 1.24.

Method II was the method of Kreibich et al. (1978) and involved adjusting the PMS to 1.35M-sucrose, laying over 2M-sucrose and 1.55M-sucrose before overlaying with 1M-sucrose in 3mM-imidazole and centrifuging overnight (for 16 h at 120 000 g. at 4°C). Bands of RER and SER could then be removed from the 1.5/2.0M- and 1.35/1.0M-sucrose interfaces respectively.

The distribution of a 46 000-M, labelled integral membrane protein of the ER whose counterpart in dog pancreas (45kDa) has been reported previously to bind signal peptide in a specific and saturatable manner (i.e. can be competed out by an excess of unlabelled peptide) Robinson et al., 1985) was determined by photoaffinity labelling of membrane fractions (750 g. of protein) using a radiolabelled photoactivatable 19-residue consensus signal peptide under u.v. light in the presence and absence of excess, unlabelled consensus peptide (1 M) (Robinson et al., 1985). It was observed in the isopycnic fractions that a 46 000-M, protein adduct, labelling of which was blocked by 1 M unlabelled peptide, was abundant in the low density (SER) fractions (Fig. 1a, tracks 5-8). In the high-density fractions (RER), specific labelling was obscured by a non-specifically labelled 46 000-M, protein contaminant (Fig. 1a, tracks 1-4). This contaminant, however, remained in the pelleted material when the specific 46 000 M, protein was solubilized in octyl glucoside at concentrations above its critical micellar concentration (75 mm) followed by centrifugation for 15 min at 386 000 g. at 4°C. The detergent extracts of rat liver RER and SER membrane fractions (prepared by method II) are shown in Fig. 1(a) (tracks 15-18). Proteins covalently labelled in dog microsomal membranes are shown for comparison in Fig. 1(a) (tracks 19 and 20). Production of only one labelled adduct of 46 000 M, is prevented by unlabelled signal peptide.

Immunoblotting with an anti-docking protein monoclonal antibody (a gift from Dr. D. Meyer, EMBL, Heidelberg, F.R.G.) of rat liver microsomal fractions obtained from the continuous gradient (Fig. 1b, tracks 1-7) and step-gradient (Fig. 1b, tracks 8 and 9) are compared with an immunoblot of dog pancreatic microsomes (Fig. 1b, track 10). Dog pancreatic microsomes show two bands, one at 73 000 M, (docking protein) and a breakdown product at 27 000 M. Although additional proteins blot in rat liver fractions, the 73 000 M, and 27 000 M, bands are seen to be most concentrated in high-density (Fig. 1b, track 1) and low-density (Fig. 1b, tracks 6 and 7), and in both fractions from the step gradient (Fig. 1b, tracks 8 and 9). Thus, the distribution of immunoreactive protein is similar to that of the labelled 46 000-M, protein-signal peptide adduct, although this distribution is different from that reported by Hortsch & Meyer (1985), who localized docking protein predominantly in RER.

Whilst it is not known why docking protein and the 46 000-M, protein should peak at either end of the gradient, it is significant that their distribution is identical in rat liver microsomes, and may imply that the two proteins are related in some physical manner in the membranes. It is conceivable that the protein that specifically links to the signal peptide may be involved in the actual movement of nascent proteins through the membrane after the initial targeting event involving SRP and docking protein.

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Fig. 1. Photoaffinity labelling and immunoblot analysis of rat liver microsomal membranes

Rat liver microsomal membranes, separated by method I (a, tracks 1–14) or method II (a, tracks 15–18), or dog pancreatic rough microsomes (a, tracks 19 and 20), were photoreacted with radioactive azidophenacyl signal peptide in the presence (tracks 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20) or absence (tracks 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19) of unlabelled peptide, and subjected to SDS/polyacrylamide-gel electrophoresis on 7–16% linear gradients. Rat liver microsomal membranes prepared by method II were extracted into octyl glucoside (75 mM) after irradiation before analysis, as described in the text. Immunoblots of rat liver subfractions prepared by method I (b, tracks 1–7) or method II (b, tracks 8 and 9), and dog pancreatic rough microsomes (b, track 10) were prepared from similar SDS/polyacrylamide-gel electrophoresis gels with anti-docking protein monoclonal antibody, using ¹²⁵I-labelled goat anti-mouse F(ab')₂ (1 x 10⁶ c.p.m. per nitrocellulose sheet). Autoradiographs are shown.