OptiPrep™ Application Sheet S41

Resolution of smooth endoplasmic reticulum (SER), SER domains, study of SER communication with other organelles and lipid droplets

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- **To access other Application Sheets** referred to in the text: return to the **2020SMemapp** file and select the appropriate S-number.

1. Background

It is widely recognized that the smooth endoplasmic reticulum contains specialized domains that are structurally and functionally associated with other membrane compartments. **Application Sheet S22** describes the fractionation of endoplasmic reticulum (ER), Golgi, plasma membrane and endosomes in continuous gradients of iodixanol, using relatively low *g*-forces for at least 12 h. The method is widely used; it highlights a paper by Woods et al [1], which describes this strategy as applied to 3T3 cells. Calreticulin shows a distinctive biphasic distribution in a 10-40% (w/v) iodixanol gradient but only the denser fraction also contains paxillin, which identifies this ER subfraction as perinuclear [1]. This functional and structural specialization of the ER is now widely recognized as more and more functional specializations have been discovered. Lynes and Simmen [2], amongst others, have reviewed some of these domain-specific functions; for example the peripheral ER that is closely associated with the plasma membrane, the mitochondria-associated membranes (MAM) and domains of the smooth ER that are associated with peroxisome biogenesis and the formation of lipid droplets.

2. MAM analysis

Lewin et al [3] prepared a standard rat liver homogenate (see **Application Sheet S05**) and loaded a post-nuclear supernatant (PNS) on to 20-40% iodixanol gradient. No other details about the gradient were given. The principal aim of their studies was to determine the localization of acyl-CoA synthetase 4. The gradient fractions were analyzed for wide range of markers including: acyl-CoA synthetase 1 (ER located); mitochondria were identified by acyl-CoA synthetase 5, glutamate dehydrogenase and glycerol-3-phosphate acyltransferase. MAM was identified by phosphatidylethanolamine methyltransferase. The gradient was able to resolve peroxisomes, mitochondria, MAM and endoplasmic reticulum. An approximate indication of the distribution of the major membrane compartments that were analyzed is given in Figure 1.

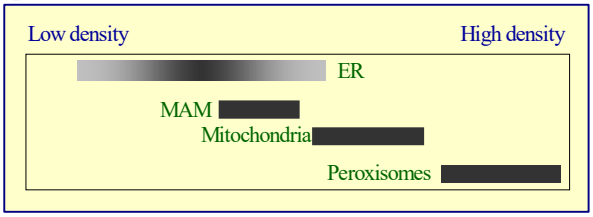

Figure 1. Approx. distribution of rat liver membranes in 20-40% iodixanol gradient – data adapted from ref 3.

In studies by Myhill et al [4], HeLa cells were homogenized in a routine HEPES- buffered 0.25 M sucrose solution containing 1 mM EDTA, using a ball-bearing homogenizer. A PNS was loaded on to a continuous 5-25% (w/v) iodixanol gradient (produced by diffusion from a 5%-interval step gradient) at approx. 120,000 *g* for 3 h. An approximate indication of the distribution of the major membrane compartments that were analyzed is given in Figure 2. A very noticeable difference between the two patterns is the relative banding positions of MAM to the ER and mitochondria. In the case of rat liver the MAM was well resolved from the mitochondria but overlapped the ER, while in the case of HeLa cells the MAM was well resolved from the ER but co-banded with the mitochondria. It is not clear if this is a distinction between the two sources or the difference in the density range of the iodixanol gradient, or both. The 20-40% (w/v)

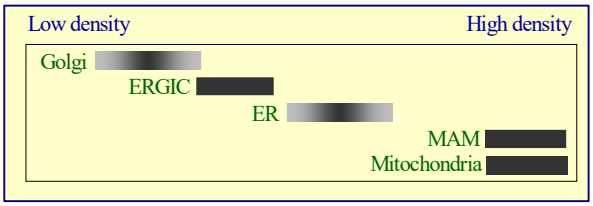

Figure 2. Approx. distribution of Hela cell membranes in 5-25% iodixanol gradient – data adapted from ref 4.

iodixanol gradient used by Lewin et al [3] covers the range 1.127-1.223 g/ml while that used by Myhill et al [4] was 1.054-1.151 g/ml. The latter gradient has also been used to study (a) the localization of the ER oxidoreductin (Ero1α) to MAM in HEK cells, which was found the be dependent on the oxidizing conditions in the ER [5] and (b) that in HeLa cells Rab32 regulates the properties of MAM, notably Ca^{2+} and the enrichment of calnexin [6]. Discontinuous gradients of 10-30% (w/v) iodixanol, similar to those used by Myhill et al [4] and Gilady et al [5] also resolved Golgi, ER and MAM [7] and revealed that palmitoylation of the trans-membrane thioredoxin family protein (TMX) and calnexin influence their enrichment in MAM. Moreover the palmitoylation of calnexin influenced its functional properties [8].

- Reviews of some of the methodology for the study of MAM is provided in refs 9 and 10.
- Iodixanol gradients have also been used for the clear resolution of SER, principally from lysosomes, but also other organelles such as peroxisomes and mitochondria, subsequent to an initial sucrose gradient fractionation. The methodology, developed by Radhakrishnan et al for CHO-K1 cells [11,12], has been extended to HEK cells [13-15], Niemann-Pick type C cells [16], HepG2 cells [17], HeLa cells [18] and mouse liver [19]
- For more recent publications on MAM see refs 20 and 21.

3. ER-Golgi transport (COPII containing vesicles)

Iodixanol gradients are able to isolate the donor membrane vesicles that bud from the endoplasmic reticulum: Gorur et al [22] removed the intact ER membranes by sedimentation at 7000 *g* and subsequently concentrated the vesicles by flotation from the supernatant (adjusted to 22% iodixanol) layer through an upper 18% iodixanol layer (250,000 *g* for 90 min). The total gradient volume was <0.25 ml. On a larger scale Ding et al [23] layered 3 ml of a post-nuclear supernatant over a 9 ml 5-30% iodixanol gradient (200,000 *g* for 4 h) separating the dense calnexin-containing ER from the much lighter COPII vesicles. Recent publications on these ER-Golgi interactions can be found in refs 24 and 25.

4. Lipid droplets

Lipid droplets have been observed to be associated with the ER for over thirty years but it is only relatively recently that they have been shown to be involved in viral infection and a number of lipid-associated diseases. Presently, rather few papers have been published in which an OptiPrep™-based method has been used in their isolation. Because of the growing interest in these particles however a short summary of the methodology is included here. The most complete information comes from a paper by Heid et al [26]. Human hepatocellular carcinoma cells were homogenized by nitrogen cavitation and the PNS was adjusted to 30% (w/v) iodixanol. It was overlaid with layers of 20% and 10% iodixanol and centrifuged either at 190,000 *g* for 3 h or 220,000*g* for 2 h. The lipid droplets were concentrated close to the top of the gradient. The same three layer flotation gradient was adopted by Suzuki et al [27] for HeLa cells and by Akil et al [28] for Huh7 cells; the centrifugation conditions were however rather different: 166,000 *g* for 5 h and 200,000 *g* for 16 h respectively. A similar flotation approach was used by Buers et al [29] for studies on macrophages, the gradient however spanned a higher density range, it comprised 40%, 30% and 20% (w/v) iodixanol, but a much reduced *g*-force of 10,000 *g* for only 1h. In all cases the lipid droplets were recovered from the top of the gradient. Flotation through 10%, 20%, 30% (w/v) iodixanol discontinuous gradients has also been reported in refs 30-33.

More recently Jayson et al [34], Hashani et al [35] and Schott et al [36], using iodixanol gradients, have investigated the lipid droplets from human mammary carcinoma cells, skeletal muscle tissue and mouse hepatocytes respectively.

5. References

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