Biochimica et Biophysica Acta, 690 (1982) 74–80
Elsevier Biomedical Press

BBA 71315

CHANGES IN MEMBRANE SURFACE PROPERTIES OF HEPATIC PEROXISOMES OF RATS UNDER SEVERAL CONDITIONS AS DETERMINED BY PARTITION IN AQUEOUS POLYMER TWO-PHASE SYSTEMS

SHUICHI HORIE [a], HIDEMI ISHII [b], HIROTAKE ORII [c] and TETSUYA SUGA [a]

[a] *Department of Clinical Biochemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03,* [b] *Department of Clinical Biochemistry, Faculty of Pharmaceutical Science, Teikyo University, Sagamiko-cho, Tsukui-gun, Kanagawa 199-01 and* [c] *Department of Radiology and Nuclear Medicine, Tokyo Metropolitan Institute of Medical Sciences, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113 (Japan)*

(Received January 5th, 1982)

Key words: Peroxisome; Surface property; Aqueous polymer; Cross-partition; Clofibrate; Diabetes; (Rat liver)

Changes in membrane surface properties of hepatic peroxisomes of rats under several conditions were observed by aqueous polymer two-phase systems, which contained 6% (w/w) dextran T 500, 6% (w/w) polyethyleneglycol 4000, 250 mmol sucrose/kg and various concentrations of sodium phosphate buffer. The partition of peroxisomes into the upper phase depended to a large extent on their membrane surface charge. The cross-points of peroxisomes shifted from 5.55 to 5.25 and 5.2 after the administration of clofibrate and aspirin for 2 weeks, respectively, although that of alloxan-diabetic rat peroxisomes was not altered. The hydrophobic properties of peroxisomes, examined by means of a partition containing polyethyleneglycol monostearate, were altered by diabetes and starvation, but no change occurred in rats treated with clofibrate or aspirin. In the liver of rats fed a high-fat diet, the partition of peroxisomes was the same as that of the control. These findings indicate that hypolipidemic drugs such as clofibrate and aspirin induce the proliferation of peroxisomes and lead to the alteration of the surface charge of peroxisomal membranes. Diabetes or fasting lead to an alteration mainly of the hydrophobic properties. Both changes are probably due to alteration of content and/or composition of the proteins and the phospholipids in peroxisomal membrane under the conditions used.

Introduction

Peroxisomes are bounded by a single limiting membrane and are characterized by their contents of H_2O_2-producing oxidases and H_2O_2-decomposing catalase [1]. Lazarow and De Duve [2] found a new fatty acyl-CoA oxidizing system in rat liver peroxisomes which increased markedly on treatment with clofibrate. Other hypolipidemic agents exert similar effects and so these compounds have been utilized in studies of the physiological role of peroxisomes [3–5]. In addition, morphological investigations have showed that these compounds lead to an alteration in the fine structure of the peroxisomal membrane and the accumulation of fibrillar materials in the peroxisomal matrix [6–9]. The hepatic peroxisomes of rats fed a high-fat diet proliferated, and this was associated with a marked increase in the activity of the β-oxidation system and carnitine acetyltransferase [10,11]. Moreover, under conditions of starvation or diabetes, peroxisomal β-oxidation activity rose very quickly [12,13]. The physical and chemical alterations in peroxisomal membranes under these conditions are not yet known.

In the present study, aqueous polymer two-

phase systems were used to investigate the surface properties of hepatic peroxisomes of rats under various conditions. The system is based on the phenomenon that a mixture of two different polymers will eventually separate into two layers and that particles in the mixture are distributed into the top, intermediate and bottom phases, depending on their surface properties [14]. This method is useful for evaluating such properties of a particle as the surface charge and hydrophobicity rather than size or density [15]. The properties of the membranes of intracellular organelles can be examined by these two-phase systems [16]. Since the polymers protect the various types of subcellular organelles [17], the method is suitable for the study of the surface condition of peroxisomes which have membranes that are susceptible to mechanical treatments [18]. Furthermore, it is known that the cross-points of substances measured by the cross-partition method in these two-phase systems have values close to their isoelectric points [19–21].

In the present study, we investigated the variations in the membrane surface properties of peroxisomes in livers of rats treated with clofibrate, aspirin, high-fat diet, starvation and alloxan, by means of dextran-polyethyleneglycol phase systems.

Materials and Methods

Animals and treatments. Male Wistar rats weighing about 180 g were used in all the studies. The rats were fed a diet containing 0.25% clofibrate or 1% aspirin ad libitum for 2 weeks, and high-fat fed rats received a 30% beef-oil diet for 1 week. Control rats were maintained on a standard diet. In the study of the fasted condition, food was withdrawn 3 days prior to the experiment. Diabetes mellitus was produced by subcutaneous injection of 150 mg/kg body weight of alloxan. Rats were killed 3 days after the injection. All animals had water ad libitum. The rats were decapitated and light mitochondrial fractions of the livers were prepared according to the method of De Duve et al. [22]; the liver homogenates were spun at 3300 $\times g$ for 10 min and the supernatants further spun at 12500 $\times g$ for 20 min. The resulting pellets were suspended in 0.25 M sucrose and designated the light mitochondrial fraction. The peroxisomal β-oxidation activity in the light mitochondrial fraction was 4.3-, 3.7-, 3.8-, 2.7- and 3.4-fold (means) the control level in the clofibrate, aspirin, high-fat diet, fasted and diabetic rats, respectively.

Partition experiments. The detailed procedures of aqueous polymer two-phase systems have been described previously [23]. Dextran T 500 ($M_r = 5 \cdot 10^5$, Pharmacia Fine Chemicals, Uppsala, Sweden) and polyethyleneglycol 4000 (PEG 4000, $M_r =$ 3000–3700, Nakarai Co., Tokyo) were used as shown in Figs. 1–4. To study hydrophobic interaction, Dextran T 500, PEG 4000 (M_r approx. 3000, Tokyo Kasei Co., Tokyo) and polyethyleneglycol monostearate (Tokyo Kasei Co.) were used. The fine compositions of the systems indicated in the figure legends were obtained by weighing out stock solutions. The systems were allowed to equilibrate in a cold-room (5°C). Ten grams of each system were put into test tubes, then 0.1-ml aliquots of the light mitochondrial fraction were added. This was equivalent to 3 mg of protein in each system, and the same concentration was used in all the experiments. They were inverted gently ten times to mix the contents, allowed to settle for 60 min in the cold-room, then 1.5 ml of the top phase (the polyethyleneglycol-rich phase) were carefully separated. The pH values of corresponding phases were measured at the same time. The activity of catalase, a peroxisomal marker enzyme, in the top phase was assayed and calculated as a percentage of total activity added [21]. In the presence of polymer, less than 6% of the catalase was solubilized from particles between pH 5.0 and 7.0 during these partition procedures, and the ratio was not changed by use of polyethyleneglycol ester (Fig. 5).

Enzyme assay. Catalase activity was determined spectrophotometrically by measuring the reduction of absorbance at 240 nm according to the method of Lück [24]. Under the experimental conditions of the phase systems, the catalase activity was not affected at all.

Results

Reasonable partition of intracellular particles was obtained when 6% (w/w) Dextran T 500-6% (w/w) PEG 4000 phases were used. The partition coefficient of the peroxisomes was expressed as the percentage recovery of peroxisomes in the top

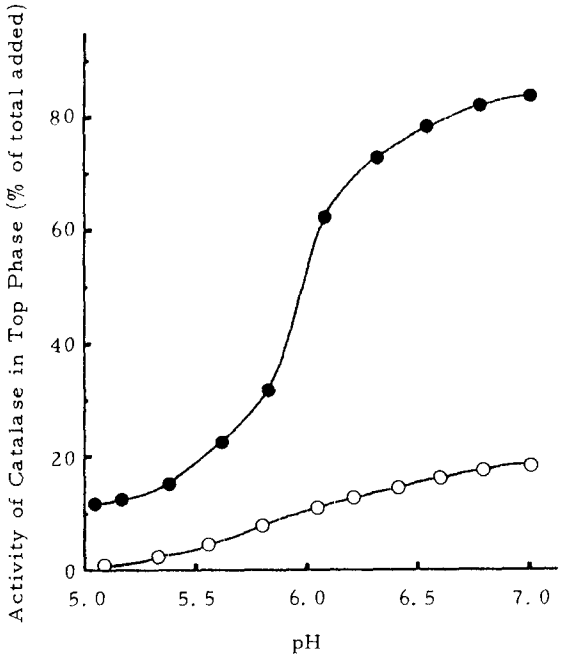

Fig. 1. Effect of pH on the partition of peroxisomes expressed as percentages of total catalase activity in the top phase. The light mitochondrial fraction was obtained as precipitate from rat liver homogenates by centrifuging at 3300× *g* for 10 min then at 12500× *g* for 20 min, suspended in 0.25 M sucrose and applied on the systems as sample. The systems contained 6% (w/w) dextran T 500, 6% (w/w) PEG 4000 and 250 mmol sucrose/kg + 10 mmol sodium phosphate buffer/kg (●) or 110 mmol sodium phosphate buffer/kg (○). pH values of corresponding phases were measured.

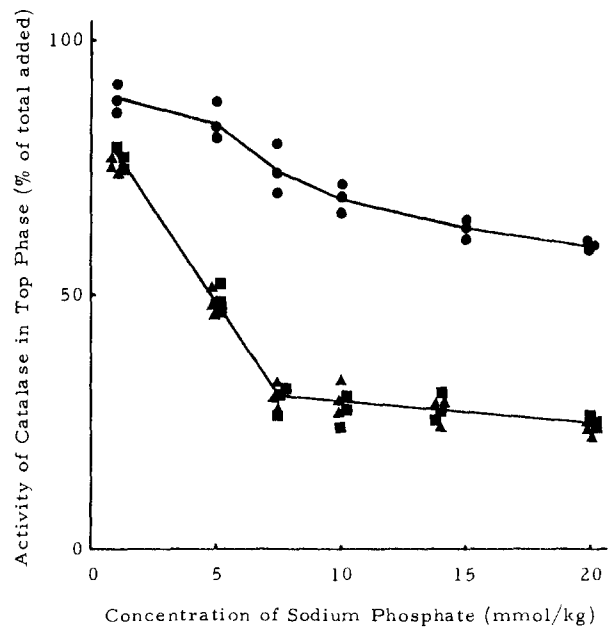

Fig. 2. Effect of the concentration of sodium phosphate buffer on the partition of peroxisomes expressed as percentages of total catalase activity in the top phase. Phase systems: 6% (w/w) dextran T 500, 6% (w/w) PEG 4000, 250 mmol sucrose/kg and 1, 5, 7.5, 10, 15 and 20 mmol sodium phosphate buffer/kg (pH 6.5). Rats were fed the diet containing either 0.25% clofibrate or 1% aspirin ad libitum for 2 weeks. ●——●, control rat; ■——■, clofibrate-treated rat; ▲——▲, aspirin-treated rat.

phase (% of total amount of peroxisomes added to the systems) calculated on the basis of catalase activity [21,23].

In general, an increase in pH causes an increase in the partition coefficients of red blood cells [25], and the same tendency was observed in the rat liver peroxisomes (Fig. 1). However, the increase in recovery from the upper phase with increasing pH was more marked in the partition systems with an isotonic sucrose solution than with an isotonic solution of sodium phosphate. Since it could therefore be presumed that the partition of peroxisomes was appreciably affected by the concentration of ions in the systems, we determined the partition coefficient at different concentrations of sodium phosphate under the same pH value of 6.5 (Fig. 2). The partition of peroxisomes in the top phase increased with decreasing concentration of sodium phosphate. Although an apparent distinction in the partition of peroxisomes was observed between control and clofibrate- or aspirin-treated rats at 10 and 20 mM/kg of sodium phosphate, the differences gradually decreased and became very small at a concentration of 1 mM/kg of sodium phosphate in the systems.

Fig. 3 shows the cross-points of peroxisomes in the clofibrate- and aspirin-treated rats determined by the cross-partition method. The cross-point, pH 5.55, of hepatic peroxisomes from the control rats coincided well with the value reported previously [23]. On the other hand, cross-points of peroxisomes from the clofibrate- and aspirin-treated rats were 5.25 and 5.2, respectively. It was found that the surface charge of peroxisomes of rats treated with the two hypolipidemic drugs changed in the more acidic condition. The cross-points of

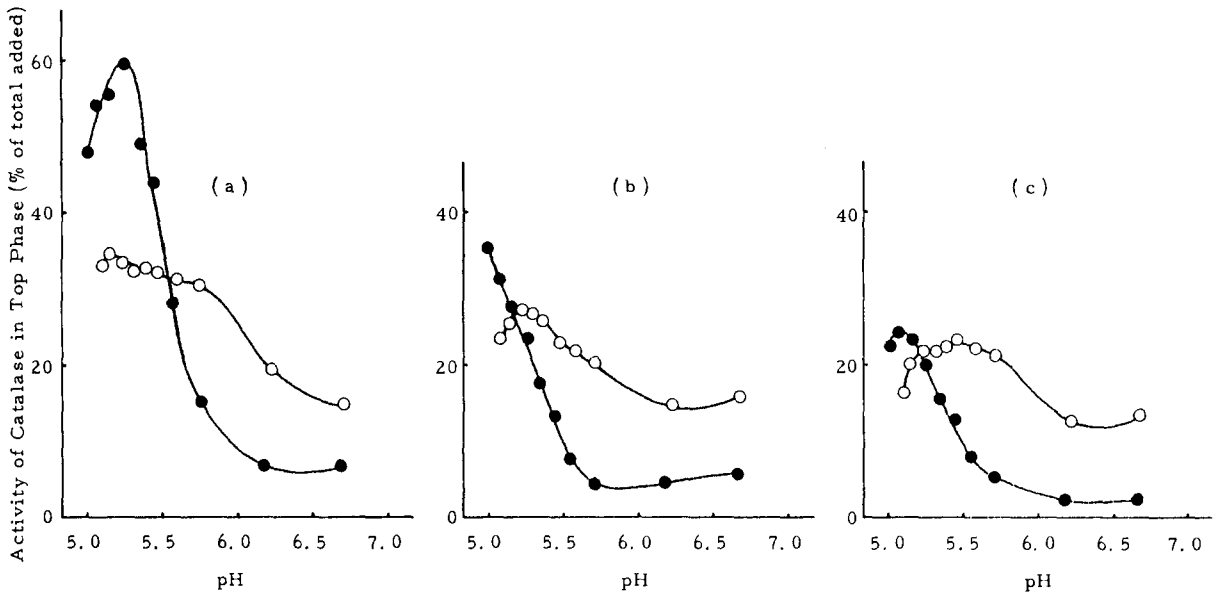

Fig. 3. Cross-points of peroxisomes from the liver of a rat treated with clofibrate and aspirin. The light mitochondrial fractions were applied to the systems, which contained 6% (w/w) dextran T 500, 6% (w/w) PEG 4000, 250 mmol sucrose/kg, 5 mmol sodium phosphate buffer/kg, and 100 mmol NaCl/kg (●) or 50 mmol Na_2SO_4/kg (○). Results are expressed as the percentage of total catalase activity in the top phase and each value is the mean of three experiments. pH values of corresponding phases were measured at the same time. Rats were fed a diet each containing 0.25% clofibrate or 1% aspirin ad libitum for 2 weeks. (a) Control rat. (b) Clofibrate-treated rat. (c) Aspirin-treated rat.

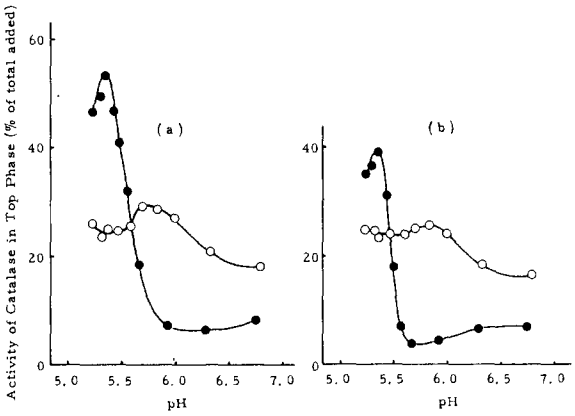

Fig. 4. Cross-points of peroxisomes from livers of rats fed a high-fat diet and with diabetes. The light mitochondrial fractions were applied to the systems, which contained 6% (w/w) dextran T 500, 6% (w/w) PEG 4000, 250 mmol sucrose/kg, 5 mmol sodium phosphate buffer/kg, and 100 mmol NaCl/kg (●) or 50 mmol Na_2SO_4/kg (○). Results are expressed as the percentage of total catalase activity in the top phase and each value is the mean of two experiments. pH values of corresponding phases were measured at the same time. High-fat diet fed rats received a diet containing 30% beef-oil for 1 week and the diabetes in rats was induced by the injection of alloxan (150 mg/kg, subcutaneously) 3 days before killing. (a) High-fat diet rat. (b) Diabetic rat.

hepatic peroxisomes from the rats receiving the high-fat diet and from the diabetic rats were determined. As can be seen in Fig. 4, the values of 5.65 and 5.45 were found in the high-fat diet and diabetic rats, respectively. These changes in the cross-points were less than those in hypolipidemic drug-treated rats.

Fig. 5 shows the changes in the hydrophobic characteristics on the partition of hepatic peroxisomes in dextran-polyethyleneglycol phase systems containing polyethyleneglycol monostearate from the variously treated rats. The partitions were strongly dependent on the polyethyleneglycol monostearate content. The relative partition of peroxisomes in the top phase increased drastically in cases of fasted and diabetic rats, but partitioning in clofibrate-, aspirin- and high-fat diet-fed rats did not significantly change compared with the control levels. These results indicate that the hydrophobic properties changed in the membranes of hepatic peroxisomes from rats under the conditions of fasting and diabetes, although the membranes of hepatic peroxisomes from rats treated

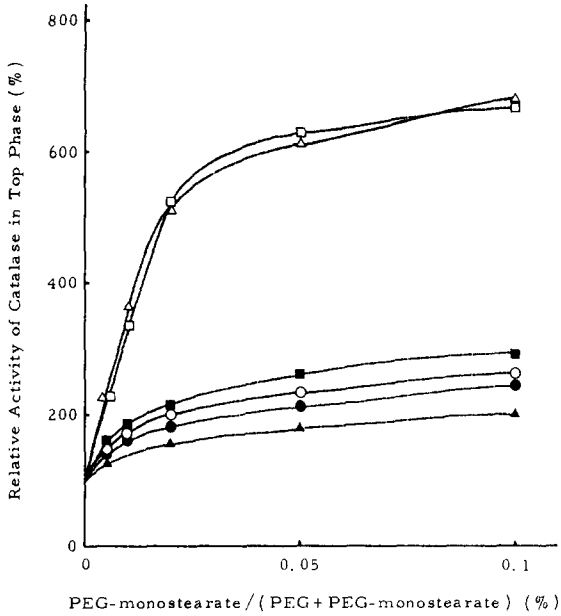

Fig. 5. Changes in the partition behaviors of peroxisomes as a function of the PEG-monostearate content. The system contained 6% (w/w) dextran T 500, 6% (w/w) PEG 4000 including various amounts of PEG-monostearate, 250 mmol sucrose/kg, 100 mmol Na_2SO_4/kg and 10 mmol sodium phosphate buffer/kg. Results are expressed as the percentages of total catalase activity in the top phase and each value in the figure is the mean of experiments as follows: control (5 experiments), clofibrate-treated (3), aspirin-treated (3), high-fat diet fed (2), diabetic (2) and fasted rats (2). Final pH values of 5.55 or 5.25 in the systems were prepared for the experiments of control (●), high-fat diet fed (○), diabetic (△) and fasted rats (□) or for the experiments of clofibrate-treated (■) and aspirin-treated rats (▲), respectively.

with peroxisome-proliferators were not influenced in their hydrophobicity.

Discussion

It is well known that liver peroxisomes of the rat proliferate on administration of clofibrate and aspirin [2–4], and electron microscopic observations have indicated that the hepatic peroxisomes of the rat and the mouse altered in size, shape and enzyme content after the administration of these compounds [3–5]. However, the changes in the properties of peroxisomal membranes under these conditions have not yet been established.

Rat liver peroxisomes possess a cyanide-insensitive fatty acyl-CoA oxidizing system which differs from the mitochondrial system. Peroxisomal β-oxidation activity increased either with or without accompanied proliferation of peroxisomes under various conditions [2,10,13,26–29]. Therefore, a significant information about the hepatic peroxisomes will be obtained when the alterations of the membranes under these conditions are examined. In order to clarify the alteration in membrane surface properties of hepatic peroxisomes under conditions which induce an increase in the activity of peroxisomal β-oxidation (such as treatment with clofibrate, aspirin, and high-fat diet, in addition to fasting and diabetes) we have adopted aqueous polymer two-phase systems with the measurement of the activity of catalase which is a marker enzyme of peroxisomes.

The partition coefficient of a substance in an aqueous polymer two-phase system is affected both by the surface charge and hydrophobicity of the substance [15]. The partition coefficients of the hepatic peroxisomes were, therefore, measured at various ionic concentrations. The coefficients of the peroxisomes from clofibrate- and aspirin-treated rats were found to be lower than that of the control at each concentration of sodium phosphate in the system, although it was constant above 7.5 mM sodium phosphate. Similar observations were also made using lithium phosphate (measured at pH 6.0) instead of sodium phosphate. In the presence of an ion the partition coefficient was mainly reflected by the surface charge of the particle. Without ions, the partition coefficient was mainly reflected by the hydrophobicity of the particle [30]. These facts, and the finding that marked differences in the coefficients of hepatic peroxisomes were found between the control and treated rats at higher concentrations of ion in the systems and that minor differences were found when the ion concentration was extrapolated to zero in the systems, indicate that the surface charge of hepatic peroxisomes from the drug-treated rats is very different from that of the control, and that the hydrophobicity of peroxisomes from the drug-treated rats may also be altered by these compounds, albeit slightly.

To confirm the changes in the surface charge of peroxisomes under the various treatments of the

rats, the cross-points of the hepatic peroxisomes were determined by the cross-partition method. It has been well known that the cross-point is close to the isoelectric point of proteins and rat liver mitochondria [19–21]. From the results of cross-partition it was found that clofibrate and aspirin affect the surface charge of the hepatic peroxisomes while high-fat diet or diabetes do not. It has been reported that the administration of clofibrate or aspirin to rats resulted in a rapid proliferation of peroxisomes in the liver [1,4], suggesting a possibility that new peroxisomes have different membrane characteristics. In the liver from rats fed the high-fat diet the surface charge of peroxisomes remained unaltered though the peroxisomes proliferated [10]. It appeared that peroxisomes in the liver of rat fed the high-fat diet had a composition similar to that of the control.

Another important factor which influences the partition in the aqueous polymer two-phase systems is the hydrophobicity of the particles [31]. Shanbhag and Axelsson [30] reported a new method which is useful for measuring hydrophobic interaction between aliphatic hydrocarbon chains and proteins in aqueous environment. In order to eliminate the charge factor, the system contains a relatively high concentration of Na_2SO_4 and partition is carried out at the isoelectric pH of the substance. These conditions create a phase system with a potential between phases and a net charge of the partitioned substance both close to zero. Furthermore, using the same composition of partition systems, but with only one of the system containing a polymer-bound uncharged ligand, the corresponding phases became more apolar. In this study, to measure the hydrophobic properties of peroxisomal membrane under various conditions of rats, phase systems containing polyethyleneglycol monostearate and high concentration of Na_2SO_4 at pH 5.55 or 5.25 were applied. Over 100 mM Na_2SO_4 in the systems allowed recovery of peroxisomes almost equal to the partition ratio among the various conditions of rats, when polyethyleneglycol monostearate is not added to the systems.

All of the hepatic peroxisomes of rats under the various conditions exhibited higher affinity for the upper (polyethyleneglycol-rich) phase when polyethyleneglycol monostearate was added. Peroxisomes from rats with starvation and diabetes especially showed much higher values. These changes in the hydrophobicity of the peroxisomal membrane may be based on the alteration of the surface compositions and not accompanied by a charge factor. The results from cross- and affinity-partition suggest that no direct correlation exists between the alteration of the surface charge and of the hydrophobic properties in the membrane biogenesis of liver peroxisomes.

In conclusion, from the present study of peroxisomes by aqueous polymer two-phase systems, three kinds of changes in properties of peroxisomal surface membrane were recognized, (i) the increase of polarity of the surface as in the cases of clofibrate and aspirin, where no significant change in the hydrophobicity appeared, (ii) changes in the hydrophobicity of the surface, as in the cases of starvation and diabetes, where no significant change in the polarity appeared, and (iii) no change in either the hydrophobicity or the polarity as in the case of high-fat diet. Eriksson and Albertsson [15] have shown that the partition of liposomes was much affected by the lipid composition and that the phospholipid played a dominant role in the partition of liposomes. Therefore, these changes in membrane surface properties of peroxisomes will reflect the alteration of membrane compositions such as phospholipids and proteins. This partition technique can thus be useful for the characterization of the surface properties of intracellular particles, such as charge and hydrophobicity, if the appropriate phase systems are selected.

References

1 De Duve, C. and Baudhuin, P. (1966) Physiol. Rev. 46, 323–357
2 Lazarow, P.B. and De Duve, C. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2043–2046
3 Lazarow, P.B. (1977) Science 197, 580–581
4 Ishii, H. and Suga, T. (1979) Biochem. Pharmacol. 28, 2829–2833
5 Watanabe, T. and Suga, T. (1979) J. Biochem. 87, 1595–1601
6 Hruban, Z., Swift, H. and Slesers, A. (1966) Lab. Invest. 15, 1884–1901
7 Svoboda, D.J. and Azarnoff, D.L. (1966) J. Cell Biol. 30, 442–450
8 Svoboda, D., Grady, H. and Azarnoff, D. (1967) J. Cell Biol. 35, 127–152

9 Hruban, Z., Gotoh, M., Slesers, A. and Chou, S.-F. (1974) Lab. Invest. 30, 64–75
10 Ishii, H., Fukumori, N., Horie, S. and Suga, T. (1980) Biochim. Biophys. Acta 617, 1–11
11 Neat, C.E., Thomassen, M.S. and Osumundsen, H. (1981) Biochem. J. 196, 149–159
12 Ishii, H., Horie, S. and Suga, T. (1980) J. Biochem. 87, 1855–1858
13 Horie, S., Ishii, H. and Suga, T. (1981) J. Biochem. 90, 1691–1696
14 Albertsson, P.-A. (1972) Partition of Cell Particles and Macromolecules, 2nd edn., Wiley-Interscience, New York
15 Eriksson, E. and Albertsson, P.-A. (1978) Biochim. Biophys. Acta 507, 425–432
16 Walter, H. (1975) Methods Cell Biol. 9, 25–50
17 Walter, H. (1969) Modern Separation Methods of Macromolecules and Particles (Gerritsen, T., ed.), Vol. II, pp. 121–145, Wiley-Interscience, New York
18 Popov, Ch.S. (1977) Dokl. Bolg. Akad. Nauk. 30, 755–757
19 Albertsson, P.-A., Sasakawa, S. and Walter, H. (1970) Nature 228, 1329–1330
20 Walter, H., Sasakawa, S. and Albertsson, P.-A. (1972) Biochemistry 11, 3880–3883
21 Ericson, I. (1974) Biochim. Biophys. Acta 356, 100–107
22 De Duve, C., Pressman, B.C., Gianette, R., Wattiaux, R. and Appelmans, F. (1955) Biochem. J. 60, 604–617
23 Horie, S., Ishii, H., Nakazawa, H., Suga, T. and Orii, H. (1979) Biochim. Biophys. Acta 585, 435–443
24 Lück, H. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H.-U., ed.), pp. 885–894, Academic Press, New York
25 Walter, H., Krob, E.J. and Garza, R. (1968) Biochim. Biophys. Acta 165, 507–514
26 Osumi, T. and Hashimoto, T. (1978) Biochem. Biophys. Res. Commun. 83, 479–485
27 Hryb, D.J. and Hogg, J.F. (1979) Biochem. Biophys. Res. Commun. 87, 1200–1206
28 Osumundsen, H., Neat, C.E. and Norum, K.R. (1979) FEBS Lett. 99, 292–296
29 Horie, S., Ishii, H. and Suga, T. (1981) Life Sci. 29, 1649–1656
30 Shanbhag, V.P. and Axelsson, C.-G. (1975) Eur. J. Biochem. 60, 17–22
31 Johansson, G. (1974) Mol. Cell. Biochem. 4, 169–180