PERMEABILITY PROPERTIES OF RAT LIVER ENDOPLASMIC RETICULUM

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1. Introduction

The semipermeability of microsomal membranes has certain important functional implications, mainly since the accessibility of a number of substrates to the membrane-bound enzymes is shown to be a limiting factor [1–3]. Changes in membrane permeability by external or internal agents may be decisive for regulating the activity of various enzymic systems [4, 5]. Permeability properties also determine the size and density of the particle, which are the most important factors in the subfractionation of microsomes [6]. For the above reasons, the permeability of microsomal membranes was investigated in vitro, and experiments were performed to see whether the reaction products of one of the main microsomal enzymic systems, the hydroxylation complex, are actively transferred through the membrane during in vitro conditions.

2. Materials and methods

Starved rats weighing 180–200 g were used. Total microsomes from rat liver were prepared according to Ernster et al. [7] and were suspended in previously weighed tubes in an incubation medium containing the labeled solute and an appropriate amount of carrier in 0.25 M sucrose. After centrifugation for 2 hr at 105,000 *g*, the pellets were suspended in 3 ml of formic acid. The tubes were then reweighed, and the density of the suspension was accurately determined from a sample to obtain the exact total volume. 0.2 ml of the formic acid solution was added to 10 ml Bray solution [8] and the activity determined by liquid scintillation counting. The total water was estimated by measuring the wet-weight/dry-weight differences of pellets dried over H_2SO_4 at 80°. The amount of sucrose occluded in the pellet was determined from the sucrose accessible space of the pellet in each experiment, and the weight of the sucrose was then subtracted from the dry weight. The total water space of the pellet was likewise corrected by adding the volume occupied by the sucrose.

The extramicrosomal space, i.e. the water present in the space between the vesicles, was determined by using ^{14}C-labeled dextran (MW 80,000) in the presence of unlabeled carrier with a concentration of 15 mg/ml. Intramicrosomal water was obtained by subtracting the extramicrosomal water (dextran space) from the total water content of the pellet. The accessible microsomal space of other solutes was likewise obtained by subtracting the extramicrosomal water from the total space occupied by the solute in the pellet.

The radioactive substances were purchased from the Radiochemical Centre, Amersham. Protein was determined according to Lowry et al. [9]. Formaldehyde was estimated by the Nash reaction [10], and the hydroxylated product of naphthalene was separated by a polyethylene procedure [11].

3. Results and discussion

The water present in the microsomal pellet is distributed in the extramicrosomal and the intramicrosomal water space (table 1). There is 1.4 μl H_2O/ml dry weight in the intramicrosomal water compartment. The intramicrosomal space including sucrose-accessible space makes up 76% of the intra-

Table 1
Water compartments of liver microsomal pellet.

Water compartment	μl/mg dry weight	% of total	% of intramicrosomal water
Total microsomal water	3.30 ± 0.08	100	
Extramicrosomal water	1.86 ± 0.05	56	
Intramicrosomal water	1.44 ± 0.06	44	100
Sucrose space	1.10 ± 0.05		76
'Osmotic space' and hydration water	0.34 ± 0.03		24

The measurements were made as described in Materials and methods. The values represent the means of 39 experiments ± S.E.M.

microsomal water. The hydration water was estimated to be about 0.2 μl/mg dry weight by using the equilibrium density procedure in D_2O, described by Beaufay et al. [12]. Therefore, the amount of water which could be involved in the osmotic shifts in sucrose medium is very small, at least during ultracentrifugation.

Table 2
Apparent penetration of various substances through the microsomal membranes.

Substance	Intramicrosomal space (μl/mg dry weight)	% of total intramicrosomal water
Intramicrosomal water	1.44	100
Glycerol[a]	1.08	75
Glucose[b]	1.18	82
Sucrose[b]	1.09	76
Maltotriose[b]	0.95	66
Oubain[a]	1.19	83
Inulin[b]	0	0
Acetate[a]	−0.04	−3
Mevalonate[b]	0.09	6
Glucose-6-phosphate[b]	0–0.43	0–30

Every centrifuge tube contained in 11 ml microsomes from 2 g liver, carrier solutions in final concentration of 0.05 M, and the radioactive substance (5 μCi of ^3H and 0.5 μCi of ^{14}C labeled compounds). The results are the means of 6–11 experiments.
a ^3H label; b ^{14}C label.

The apparent permeability of microsomal membranes to various small molecules is shown in table 2. The non-charged substances tested (with molecular weights ranging from 80 to 580) penetrate the microsomal membranes more or less freely. The upper limit could not be determined exactly because of the lack of labeled uncharged water-soluble substances. This upper limit is lower than 5,000, however, as demonstrated by the non-permeable property of the inulin. On the other hand, charged substances, such as acetate, mevalonate, and glucose-6-phosphate (G6P) do not penetrate the membrane. The varying results obtained with G6P were caused by the enzymic hydrolysis of the sugar-phosphate during ultracentrifugation, following by the free penetration of the labeled glucose.

The possibility must be taken into consideration that the products formed during an enzymatic process such as microsomal hydroxylation do not have the same localization as the molecules studied above in a passive transport process. In order to study the presence of an active transfer mechanism operating during

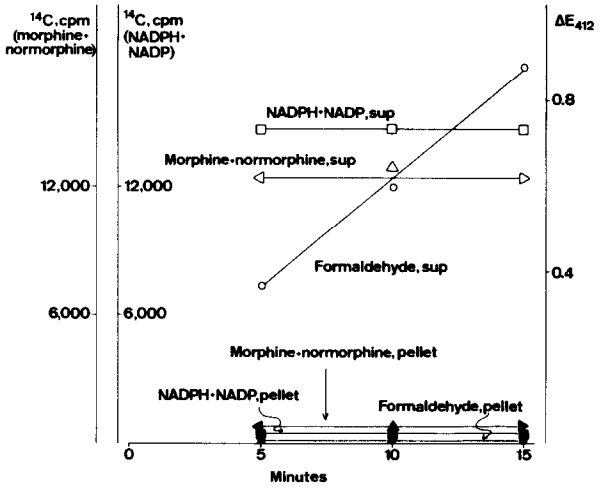

Fig. 1. Extramicrosomal appearance of products after morphine hydroxylation in vitro. In all experimental series, 2 tubes (A and B) were incubated for every time point. Incubation medium A in a final volume of 3 ml contained: tris-HCl buffer, pH 7.5, 0.1 M; $MgCl_2$, 5 mM; NADPH, 1 mM; morphine-HCl, 6 mM, and 0.1 ml ^{14}C-morphine-HCl (1 μCi/ml). Incubation medium B as medium A but 0.15 μCi ^{14}C-NADPH instead of labeled morphine. Incubation at 30° was stopped by cooling and the microsomes were sedimented by ultracentrifugation.

hydroxylation, microsomes were incubated in vitro in the presence of morphine and NADPH in conditions leading to demethylation and thereby to the appearance of normorphine and formaldehyde as well as NADP. For determining the distribution of the normorphine and NADP formed, labeled morphine and NADPH were added to the medium during incubation, and the total radioactivity was measured at various time-points both in the supernate and in the pellet. The total radioactivity gives only the sum of the substrate and the reaction product, but since close to 50% of the morphine is demethylated, an accumulation of normorphine and/or NADP in the lumen of the microsomes could easily be measured as an increased number of counts in the pellet and a decrease of counts in the supernate. As appears from fig. 1, no such change in the distribution of radioactivity occurred, nor was any decrease in the formaldehyde content of the pellet apparent.

On the other hand, when naphthalene hydroxylation was investigated, almost half of the product (1-naphthol) was recovered in the pellet (table 3, exp. 2a). This is in apparent conflict with the data of fig. 1 as well as with previous in vivo studies [13]. An explanation is given in table 3, exp. 1, which shows that both the hydrocarbon and its hydroxylated derivate are adsorbed by the microsomes. When 1-naphthol was produced enzymatically in vitro, the same amount of naphthol was found in the pellet as that recovered in the distribution experiment. Since naphthol formed in vivo is conjugated to the corresponding glucuronide [14], which is a much more hydrophilic compound, one would expect greater reliability from the in vivo experiments and more secondary adsorption of the product by the microsomal membranes in vitro. This interpretation is strongly supported in the experiment (table 3, exp. 2b) in which the incorporation of 10 mg uridine diphosphoglucuronic acid (UDPGA) significantly decreased the amount of naphthol in the pellet.

Table 3
Localization of ^{14}C-naphthalene and its hydroxylated product after incubation with liver microsomes.

	Percent of total counts	
	Supernate	Pellet
1) Distribution experiment		
a) microsomes + naphthalene	0.7	99.3
b) microsomes + naphthol	62	38
2) Detoxication in vitro		
a) without UDPGA	57	43
b) with UDPGA	83	17
3) Detoxication in vivo[a]	95	5

In the distribution experiments, microsomes from 1 g liver were suspended in 5 ml 0.25 M sucrose, together with 0.1 ml ^{14}C-naphthalene or ^{14}C-naphthol. The microsomes were separated from the medium by millipore filtration. In the experiments on in vitro detoxication, microsomes were incubated as described previously [11]. In exp. 2b, the incubation medium contained 10 mg UDPGA in a final volume of 4 ml. The incubation was interrupted by millipore filtration.
[a] Data taken from [13].

It would thus appear that the microsomal membranes are permeable to uncharged molecules of up to relatively high molecular weight but impermeable to negatively charged substances of as low a molecular weight as 90. Since sucrose freely penetrates microsomal membranes during ultracentrifugation procedure, no osmotic response and consequently no size changes in the microsomal vesicles should occur during gradient centrifugation. During in vitro hydroxylation reactions, the products and the glucuronidated form of the hydroxylated products do not show any appreciable accumulation in the intravesicular space, but they can be found in the extramicrosomal compartment.

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