SUBCELLULAR DISTRIBUTION OF A RAT HEPATIC BINDING PROTEIN SPECIFIC FOR URIDINE DIPHOSPHOGLUCOSE

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SUMMARY

A binding protein, specific for UDP-glucose, is present in microsomal membranes of rat liver. Evidence is given for the localization of this protein in a Golgi-rich fraction. The whole membranous pathway for the biosynthesis of UDP-glucose is located in the same fraction and the relationship of the binding protein with the pathway is discussed, as well as its physiological significance.

INTRODUCTION

In previous reports, evidence has been presented that a UDP-glucose biosynthetic pathway is present in rat liver microsomal membranes (1), as well as a binding protein specific for UDP-glucose (2-3). Microsomal glucokinase, which is the first enzyme of this pathway, was located more precisely in a Golgi-rich fraction of rat liver (4-5), and, recently, the whole biosynthetic system for UDP-glucose was shown to occur also in the same fraction, prepared from smooth microsomes by means of a discontinuous sucrose gradient (6). In view of these results, it became meaningful to determine the submicrosomal location of the binding protein specific for UDP-glucose. The present paper documents the observation that this protein is present in the same Golgi-rich fraction as the biosynthetic pathway for UDP-glucose.

MATERIAL AND METHODS

Subcellular fractionation and enzyme assays.

A Golgi-rich fraction was prepared according to Morré (7), and checked for marker enzymes as previously described

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(5). The separation between rough and smooth reticulum, the further fractionation of the latter on a discontinuous sucrose density gradient and the characterization by marker enzymes were carried out under conditions recently reported (6).

Triton X-100 extracts.

All the membrane suspensions were diluted in order to give a protein content of 0.5 mg in a volume of 1 ml. Then, Triton X-100 was added in a final concentration of 0.1 % (v/v). After 30 min. at 4°C, the mixture was centrifuged 45 min. at 100 000 x g. The supernatant was the Triton X-100 extract.

Binding protein assay.

Conditions for the standard binding assay were the following : the incubation medium contained, in a final volume of 0.5 ml, 15 mM bicine buffer, pH 8.1, 1 mM $MnCl_2$, 0.15 mM UDP $|U^{14}C|$ glucose (0,375 µCi) and 190 µl of the Triton X-100 extract (about 0.1 mg of protein). After 0,10,20,30 min. at 22°C, aliquots (100 µl) of this medium were mixed with 2 ml of a suspension of Dextran-coated charcoal, prepared according to Bhalla *et al* (8). After centrifugation, the radioactivity of the clear supernatant was counted in a liquid-scintillation analyser (Tri-Carb Packard), using a scintillation fluid for aqueous samples.

In the experiments designed to evaluate the amount of non-specific bound radioactivity, EDTA, which inhibits the specific binding of UDP-glucose (2), was added to the incubation medium at a final concentration of 5 mM ; the inhibition was always about 90 %.

Protein assay.

Protein was determined by the method of Hartree (9).

RESULTS AND DISCUSSION

A Golgi-rich fraction prepared directly by the method of Morré exhibited a binding-protein activity of 8 nmoles/min/mg protein of the Triton X-100 extract. Unfortunately, the yield was very low, and it was therefore necessary to know whether the localization of the binding protein in the Golgi membranes was unique. The first step of this investigation was the separation of rough and smooth endoplasmic reticulum. Table I shows that the total binding activity was more than four times higher in smooth than in rough microsomes. The ratio between the corresponding specific activities was nearly 7. These results gave convincing evidence that the smooth endoplasmic reticulum was enriched in the UDP-glucose binding activity, as well as in the three enzymes involved in the UDP-glucose biosynthesis :

Table I : Distribution of binding activity in a typical preparation of rat liver rough and smooth endoplasmic reticulum

Fraction	Binding activity	
	Total (1)	Specific (2)
Smooth endoplasmic reticulum	4.3	3
Rough endoplasmic reticulum	1	0.44

(1) nmoles UDP-glucose/min/g liver

(2) nmoles UDP-glucose/min/mg protein of the Triton X-100 extract.

Table II : Distribution of binding activity after a typical fractionation of smooth endoplasmic reticulum on a discontinuous sucrose gradient

Fraction	Density	Binding activity	
		Total	Specific
1	1-1.05	0	0
2	1.08	0	0
3	1.11	3	5
4	1.13	0.7	2.6
5	1.14	0.5	2.6
6	1.16	0	0
7	1.17-1.18	0	0

The results are expressed as in Table I.

glucokinase, phosphoglucomutase and uridylyltransferase (6).

In order to localize this binding activity more precisely we used a sucrose density gradient which has been previously characterized (6). Binding activity was found only in three fractions of this gradient, densities of which are 1.11, 1.13 and 1.14, with a total recovery of 98 % (table II). 70 % were recovered in fraction 3 (d = 1.11), with the highest specific activity (5nmoles/min/mg protein of the Triton X-100 extract compared to 3 in the smooth endoplasmic reticulum). The distribution of marker enzymes for Golgi apparatus, endoplasmic reticulum and plasma membranes clearly demonstrated that fraction 3 was essentially composed of Golgi membranes (6). In the same fraction 60 % of the three enzymic activities which make up the pathway for UDP-glucose biosynthesis was found, along with the highest specific activities. This fraction produced, from ATP and glucose, an amount of UDP-glucose equivalent to the one obtained with a crude microsomal preparation (6).

All these data suggest that, in rat liver, the UDP-glucose binding protein is present in the same Golgi-rich fraction as the UDP-glucose biosynthetic pathway. The link between the two is UDP-glucose, and this similar localization supports the hypothesis that the binding protein is involved in the biosynthetic pathway for UDP-glucose, but it remains to be seen in what way. It could be a simple carrier for the transport of UDP-glucose to allow the access to the membranous glucosyltransferases (10). Alternatively, the binding protein could be implicated in the regulation of UDP-glucose biosynthesis, the binding of the metabolite inducing a change in the membrane conformation, as in the mechanism proposed for insulin action (11). Further investigations are in process to establish the role of the UDP-glucose binding protein in the Golgi membranes of rat liver.

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