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Localization of the Fructose 1,6-Bisphosphatase at the Nuclear Periphery

Doris E. Sáez, Carlos D. Figueroa, Ilona I. Concha, and Juan C. Slebe

Instituto de Bioquímica (D.E.S., I.I.C., J.C.S.) and the Instituto de Histología y Patología (C.D.F.), Universidad Austral de Chile, Casilla 567, Valdivia, Chile

Abstract The localization of fructose 1,6-bisphosphatase (D-Fru-1,6-P_2-1-phosphohydrolase, EC 3.1.3.11) in rat kidney and liver was determined immunohistochemically using a polyclonal antibody raised against the enzyme purified from pig kidney. The immunohistochemical analysis revealed that the bisphosphatase was preferentially localized in hepatocytes of the periportal region of the liver and was absent from the perivenous region. Fructose-1,6-bisphosphatase was also preferentially localized in the cortex of the kidney proximal tubules and was absent in the glomeruli, loops of Henle, collecting and distal tubules, and in the renal medulla. As indicated by immunocytochemistry using light microscopy and confirmed with the use of reflection confocal microscopy, the enzyme was preferentially localized in a perinuclear position in the liver and the renal cells. Subcellular fractionation studies followed by enzyme activity assays revealed that a majority of the cellular fructose-1,6-bisphosphatase activity was associated to subcellular particulate structures. Overall, the data support the concept of metabolic zonation in liver as well as in kidney, and establish the concept that the Fructose-1,6-bisphosphatase is a particulate enzyme that can not be considered a soluble enzyme in the classical sense.

Key words: FBPase, gluconeogenesis, perinuclear association, metabolic zonation, immunolocalization, subcellular fractionation, confocal microscopy

Fructose-1,6-bisphosphatase (FBPase; D-Fru-1,6-P_2-1-phosphohydrolase, EC3.1.3.11), a key regulatory enzyme of the gluconeogenic pathway, catalyses the irreversible conversion of fructose-1,6-bisphosphate and H_2O to fructose-6-phosphate and inorganic phosphate [Benkovic and de Maine, 1982; Tejwani, 1983; Hers and Hue, 1983; van Schaftingen, 1987]. The FBPase enzyme isolated from different sources is a tetramer composed of identical subunits of molecular weight ranging from 36,000 to 41,000 and a divalent metal ion as Mg^{2+} or Mn^{2+} is required for its catalytic activity [Benkovic and de Maine, 1982; Tejwani, 1983; Rittenhouse et al., 1983]. Mammalian enzymes (liver and kidney) are over 85% homologous in amino acid sequence [Marcus et al., 1982; El-Maghrabi et al., 1988] and are regulated by two synergistic inhibitors: AMP and fructose 2,6-bisphosphate (Fru-2,6-P_2) [Tejwani, 1983; Hers and Hue, 1983; van Schaftingen, 1987]. These two small molecules are also involved in the regulation of glycolysis through their action as strong activators.

Regulation of glycolysis and gluconeogenesis is extremely important in organs and tissues, such as the liver and renal cortex, sharing both capabilities. It has been suggested that glucose in the liver periportal zone is mainly formed by gluconeogenesis and glycogenolysis while in the perivenous zone it is generated by glycolysis linked to liponeogenesis [Sasse et al., 1975]. Such a zonation would be analogous to the renal cortex heterogeneity, with gluconeogenesis being located in the proximal and glycolysis in the distal tubules [Schmidt et al., 1975].

Although the sequence of enzymatic reactions that constitute the gluconeogenic and the glycolytic pathways are well defined at the biochemical level, the dynamic, regulation, and spatial organization of these processes in living cells are not yet fully understood. The classical concept of the glycolytic pathway (or the gluconeogenic pathway) as a system of freely soluble components located in the cytoplasm of cells is controversial. Many studies indicate that glycolytic

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Address reprint requests to Juan Carlos Slebe, Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Campus Isla Teja, Casilla 567, Valdivia, Chile.

Part of this work has been presented in abstract form [Sáez et al., 1994].

enzymes do not exist in a soluble form only, they also may be (reversibly) associated with cellular structures [for reviews, see Masters et al., 1987; Srere, 1987]. Moreover, glycolytic enzymes are supposed to form clusters that allow channeling of substrates [Stephan et al., 1986; Neuzil et al., 1990; Tompa and Batke, 1990]. Similarly, it has been suggested that some gluconeogenic enzymes associate with the endoplasmic reticulum [Foemmel et al., 1975; Robinson and Karnovsky, 1983].

The fructose-1,6-bisphosphatase has been extensively studied in liver and kidney cells, and has been classically considered as a soluble enzyme, not bound to cellular extraction, but there are no data on the intracellular localization of the enzyme. It therefore appeared to us that a study of the possible association of FBPase with particulate structures might provide evidence bearing on the idea that major metabolic sequences are associated with cellular structures in vivo.

The aim of the present work is to localize FBPase by immunocytochemistry and analyze the distribution of the enzymatic activity by subcellular fractionation with the hope of gaining further insight into the physiological role of this enzyme.

METHODS

Antibody Production

A rabbit antiserum against purified kidney pig fructose-1,6-bisphosphatase [Reyes et al., 1987] was prepared. White rabbits received two subcutaneous injections at 1-week intervals of an emulsified mixture of purified fructose-1,6-bisphosphatase (2 mg) and Freund's complete adjuvant (first injection) or incomplete adjuvant (second injection). One week after the second injection, a booster injection of 4 mg of pure FBPase was given. Ten days after the booster injection the rabbits were bled, and the presence of antibodies was checked by Western blotting. Rabbit serum immunoglobulins were prepared by ammonium sulfate precipitation followed by DEAE-cellulose chromatography as described [Deutsch, 1967].

Western Blot

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) using a minigel apparatus. The final acrylamide monomer concentration in the slab gels was 10% (w/v) for the separating gel and 4% (w/v) for the stacking gel. Protein was transferred from polyacrylamide gels to nitrocellulose membranes electrophoretically using the procedure of Tsang et al. [1983]. The nitrocellulose bound protein was probed with the anti-FBPase serum, and the antibody-antigen interactions were detected using peroxidase coupled to goat anti-rabbit IgG.

Tissue Immunocytochemistry

Renal and hepatic tissue obtained from adult Holtzman rats (body weight 300 to 350 g) were fixed in Bouin's fluid, 4% (v/v) formal-saline or periodate-lysine-paraformaldehyde buffered at pH 7.4 [McLean and Nakane, 1974] for 24 h at room temperature. Fixed samples were dehydrated in ethanol and embedded in paraplast plus (Monoject Scientific, St. Louis, MO) or Hiscosec (Merck, Darmstadt, Germany).

Immunostaining was performed according to the peroxidase/anti-peroxidase (PAP) method [Sternberg et al., 1970] with the modifications described earlier [Figueroa et al., 1984, 1988]. Briefly, dewaxed tissue sections were treated with absolute methanol/10% (v/v) H_2O_2 for 15 min, rehydrated and washed with 0.05 M Tris, 0.15 M NaCl, pH 7.8, each. Anti-FBPase antibodies were used. Dilutions varied from 1/250 to 1/1,000 in the same buffer containing 1% (w/v) of immunoglobulin-free BSA. Incubation with the first antiserum was performed overnight at 22°C in a water bath that was used as a moist chamber. The second antibody (swine anti-rabbit IgG, Dako, Glostrup, Denmark) at a dilution of 1:80 and the PAP complex (Dako) at 1:100 were applied for 30 min each. The peroxidase activity was visualized by incubating the sections in 0.1% (w/v) of 3,3'-diaminobenzidine (Sigma, St. Louis, MO) and 0.03% (v/v) of H_2O_2 for 15 min in the dark.

Controls for the immunostaining procedure were prepared by omission of the first antibody and its replacement by non-immune rabbit serum at the same dilutions. Alternatively, the first antibody was preabsorbed overnight at 4°C with an excess of the same FBPase used for immunizations, and then centrifuged at 10,000*g* to remove the immune precipitates. In further controls, we used an antibody prepared against the C-terminal region of the low affinity facilitative glucose transporter GLUT2 (East Acres Biologicals, Southbridge, MA).

Stained sections were examined with a Zeiss (Thornwood, NY) laser scanning confocal microscope equipped with an argon ion laser (488 nm) illumination at the Centro de Equipo Mayor (CEM) of the Facultad de Ciencias, Universidad de Chile. The system was operated in the transmission mode for routine examination, and in the reflection contrast mode for scanning through the nuclei.

Subcellular Fractionation

Renal and hepatic tissue were obtained from Holtzmann rats. Fractionation by differential centrifugation into cytoplasmic extract (E), nuclear (N), heavy mitochondria (M), light mitochondria (L), microsomal (P), and supernatant (S) fractions was performed as described [de Duve et al., 1955]. Marker enzymes for peroxisomes (catalase), mitochondria (glutamate dehydrogenase), microsomal fractions (NADPH:cytochrome c reductase), lysosomes (acid phosphatase), and the soluble compartment (phosphoglucomutase), as well as protein, were measured as described [Bronfman et al., 1984].

Fructose-1,6-bisphosphatase activity was assayed spectrophotometrically at 30°C by following the rate of NADH formation [Colombo et al., 1972]. The assay system of 0.5 ml contained 50 mM Tris-HCl, pH 7.5, 50 μM fructose-1,6-bisphosphate, 5 mM $MgSO_4$, 0.1 mM EDTA, 0.3 mM NAD^+, 0.1–0.4 μg of fructose-1,6-bisphosphatase, 4 U/ml of phosphoglucose isomerase, and 0.8 U/ml of glucose-6-phosphate dehydrogenase, and Triton X-100 as indicated in Table I. The initial rate of NADH formation was measured at 340 nm on a Shimadzu UV-260 spectrophotometer. One unit of fructose-1,6-bisphosphatase activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of inorganic phosphate or fructose-6-phosphate per min under the conditions described.

RESULTS

Characterization of the Anti-FBPase Antibody

The specificity of the FBPase antibody was assessed by immunoblotting. The anti-FBPase antibody immunostained a single band in rat kidney extracts, with an apparent subunit molecular weight of approximately 36,500 (Fig. 1, lane 3). The immunoreactive band migrated identically to purified FBPase from pig kidney (Fig. 1, lane 4). Parallel Coomassie blue staining revealed the presence of greater than 40 different protein bands in the rat kidney sample (Fig. 1, lane 1), indicating the elevated specificity of the anti-FBPase antibody. Only one protein band was in the lane containing the purified FBPase from pig kidney (Fig. 1, lane 2), indicating the high degree of purity of the sample utilized to elicite the anti-FBPase antibody. Confirming the specificity of the anti-FBPase antibody, no reactivity was observed when the blots were incubated with a preabsorbed antibody or with preimmune serum (data not shown). Furthermore, the kinetic properties of the enzyme-antibody complex are similar to those showed by the native enzyme except that the allosteric inhibition by AMP is decreased (data not shown).

TABLE I. Subcellular Distribution of FBPase From Rat Liver and Kidney*

	Enzyme activity (U/g tissue)			
	Liver		Kidney	
Fraction	A	B	A	B
Initial homogenate (H)	2.4	8.4	6.4	17.8
Cytoplasmic extract (E)	3.9	7.8	11.5	18.2
Nuclear fraction (N)	0	0.1	0	0.3
Heavy mitochondria (M)	0	0.3	0	0.3
Light mitochondria (L)	0	0.4	0	1.0
Microsomal fraction (P)	0	0.2	0	0.2
High speed supernatant (S)	4.5	4.9	8.5	8.8

*A: Fructose-1,6-bisphosphatase activity in the fractions. B: The fractions were preincubated with 0.1% Triton X-100 at 20°C, for 20 min. The fructose-1,6-bisphosphatase activity was assayed in the presence of 0.004% Triton X-100.

Immunolocalization of FBPase in Rat Kidney and Liver Cells

Rat kidney sections were highly immunoreactive with the anti-FBPase antibody (Fig. 2A and B). Particularly intense staining was seen in the cortex, specifically in the proximal tubules. No immunoreactivity was observed, in glomeruli ascending and descending loops of Henle, collecting tubules, and distal tubules; staining was also absent in renal medulla (data not shown). In addition to the immunoreactive material associated to the cytoplasm and to the plasma membrane, FBPase immunoreactive cells showed a clear staining associated to the entire nucleus (Fig. 2A and B). Prominent nuclear staining was also seen in hepatocytes from periportal regions (Fig. 2D and E), although immunoreactive material was also present in the cytoplasm and associated to the cell membrane. No immunoreac-

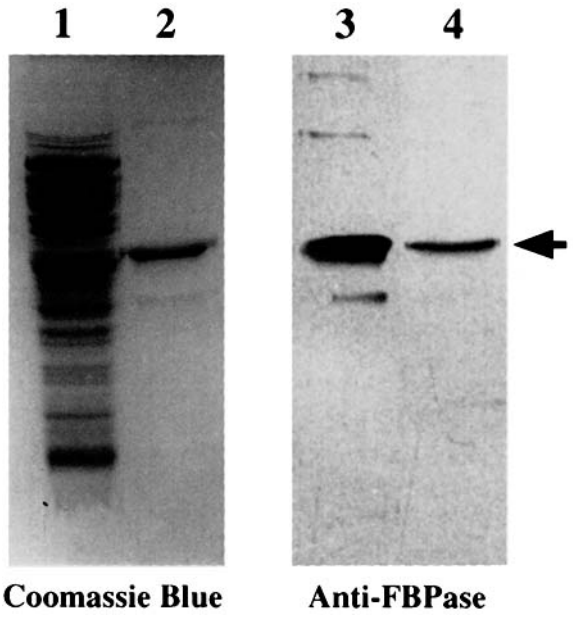

Fig. 1. Characterization of the anti-FBPase antibody. **Left:** Coomassie blue-stained acrylamide gel after SDS-PAGE. **Lane 1** contains 2.5 μg of pure FBPase. **Lane 2** contains 40 μg of a post-55°C step preparation of FBPase from rat liver obtained as described [Reyes et al., 1987]. **Right:** Western blot. Samples were fractionated by SDS-PAGE, transferred to nitrocellulose membranes, and reacted with the anti-FBPase antibody followed by a peroxidase-labeled secondary antibody as described in Methods. **Lane 3** contains 2.5 μg of pure FBPase. **Lane 4** contains the post 55°C sample of FBPase.

tive material was observed in the cells of the perivenous regions and in the endothelial cells and bile duct epithelial cells (data not shown). Confirming the specificity of the anti-FBPase antibody, no immunoreactivity was observed in samples probed with preimmune IgG (Fig. 2C,F).

The results of the immunolocalization experiments were consistent with the nuclear localization of FBPase in rat kidney and liver cells. As a control for the nuclear localization of FBPase, sections from rat liver were reacted with an antibody for the glucose transporter GLUT2, which is specifically expressed in liver [DeVos et al., 1995]. No nuclear staining was observed in these experiments with the immunoreactive material mainly associated with the plasma membrane (data not shown), the well known subcellular localization of GLUT2.

Perinuclear Localization of FBPase in Rat Kidney and Liver Cells

The immunostaining pattern with the anti-FBPase antibody seen with conventional light microscopy (Fig. 2) suggested the association of FBPase to the nuclei of rat liver and kidney cells. However, conventional microscopy lacks the power to differentiate between antigens that are located inside the nucleus from those having a perinuclear distribution. Further analysis using confocal microscopy clearly demonstrated a perinuclear localization of FBPase in both liver and kidney cells (Figs. 3 and 4). A composite figure, obtained by superimposing a confocal microscopy image (viewed in reflection contrast mode, green) on the conventional light microscopy image, showed the nuclear staining with the anti-FBPase antibody in liver cells of the periportal region (Fig. 3A). When the same field was viewed only in the reflection contrast mode (Fig. 3B) and a set of confocal images were taken at 0.25 μm intervals, it was clear that the antibody staining was confined to the nuclear periphery (Fig. 3C). A similar result indicating an association of FBPase to the nuclear periphery was obtained when kidney samples were analyzed by using the same technique (Fig. 4).

Subcellular Distribution of FBPase

The results of rat liver and kidney fractionation are shown in Table I. In two experiments, 1.0–1.2 g of tissue was fractionated by differential centrifugation as described in Methods, and the subcellular compartments were identified by their corresponding marker enzymes. In both tissues, the FBPase activity was clearly present in the initial homogenate, the cytoplasmic extract, and the high speed supernatant (Table I). No activity of FBPase was observed, however in the particulate subcellular fractions analysed that included the nuclear, light mitochondrial, heavy mitochondrial, and microsomal fractions. The amount of FBPase activity present in the total homogenate was similar to that present in the final high speed supernatant, indicating that no activity was apparently lost during the fractionation procedure. However, when the different fractions were treated with Triton X-100 prior to measuring FBPase activity, we observed a clear increase in the amount of FBPase activity present in the initial homogenate and in the cytoplasmic extract in cells from both liver and kidney. On the other hand, Triton X-100 had no effect on the amount of FBPase activity detected in the high speed supernatant. In liver cells, there was a 3.5- and a 2.0-fold increase in the amount of the FBPase activity in the initial homogenate and the cytoplasmic extract, respectively. The respective increases for the kidney

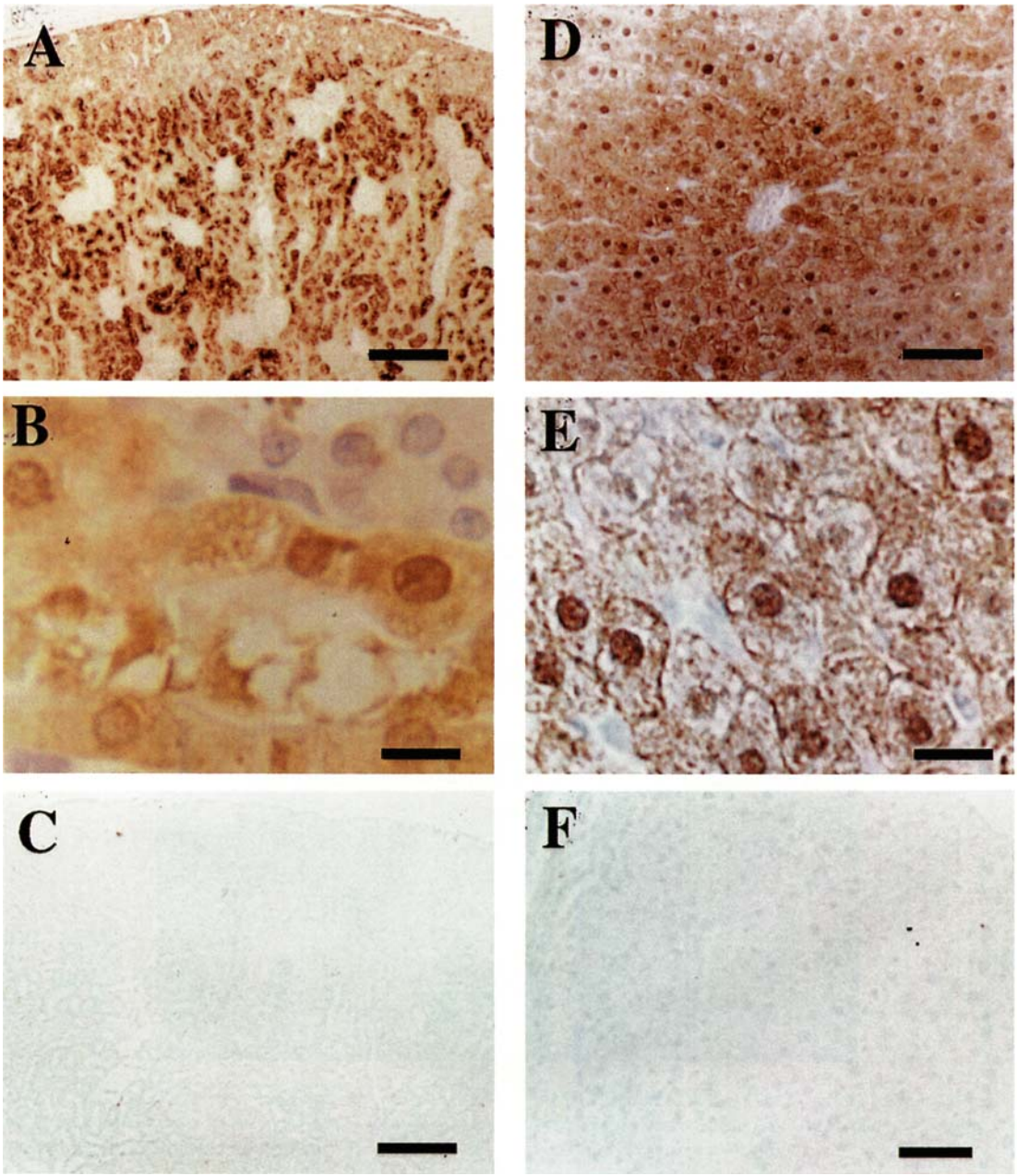

Fig. 2. Localization of FBPase in rat kidney and liver by conventional light microscopy. **A:** Nuclear staining of kidney proximal tubule cells reacted with the anti-FBPase antibody. Bar: 60 μm. **B:** Higher magnification of a kidney sample showing the nuclear staining with the anti-FBPase antibody. Bar: 10 μm. **C:** Control showing absence of immunoreactivity in kidney cells reacted with a preimmune IgG. Bar: 60 μm. **D:** Nuclear staining in rat hepatocytes surrounding the periportal region and reacted with the anti-FBPase antibody. Bar: 240 μm. **E:** Higher magnification of a liver sample showing the nuclear staining with the anti-FBPase antibody. Bar: 15 μm. **F:** Control showing absence of reactivity in rat liver cells reacted with a preimmune IgG. Bar: 240 μm.

cells were 2.0- and 1.6-fold. Only a very low level of FBPase activity was detected in the particulate fractions after treatment with Triton X-100. Control experiments indicated that the FBPase activity determination was not affected by the presence of Triton X-100, a result that is consistent with the lack of effect of Triton X-100 on the FBPase activity present in the high speed supernatant. Overall, the data are compatible with the unmasking, by Triton X-100, of a "latent" pool of FBPase activity associated to particulate elements present in the initial homog-

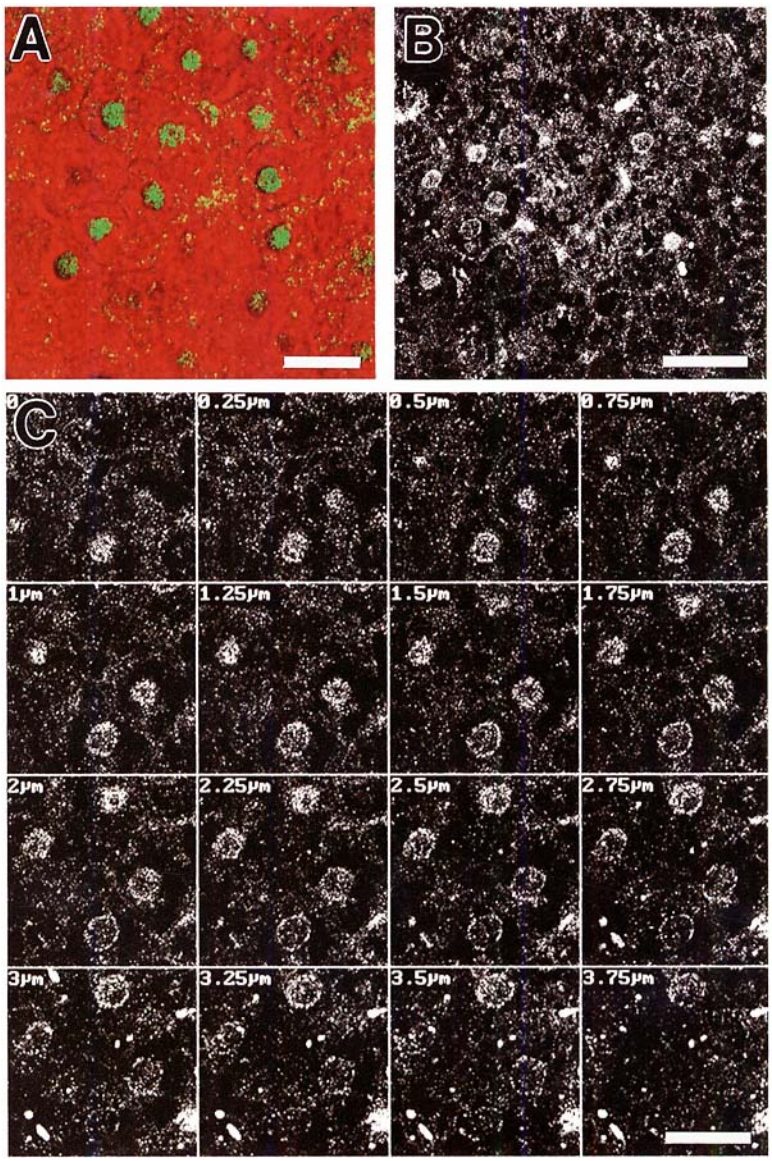

Fig. 3. Localization of FBPase at the nuclear periphery in liver cells by confocal microscopy. **A:** Composite figure obtained by superimposing a confocal microscopy image viewed in a reflection contrast mode (green) on the conventional light microscopy image (red), showing nuclear staining for FBPase in hepatocytes from the periportal regions. Bar: 10 μm. **B:** Same field as in A, viewed only in the reflection contrast mode. Four of these nuclei are examined by confocal microscopy in C. Bar: 10 μm. **C:** Series of confocal images, at 0.25 μm intervals, through the four nuclei from B. Bar: 25 μm.

enate that is also present in the cytoplasmic extract.

DISCUSSION

A central finding of the present study is that the gluconeogenic enzyme fructose 1,6-bisphosphatase has a predominant perinuclear localization in rat liver and kidney cells. This finding was substantiated by immunocytochemistry at light microscopy and was confirmed with the use of reflection confocal microscopy. The localization of the FBPase at the nuclear periphery contrasts with the traditional notion that this enzyme, as well as the majority of the glycolytic enzymes, are located in the soluble portion of the cell [Maretzki et al., 1989; Brooks and Storey, 1991]. They have been considered "soluble" enzymes since they are easily extracted in a soluble form from various cell types.

There is evidence, however, that certain enzymes of the glycolytic and gluconeogenic systems may be associated with specific subcellular

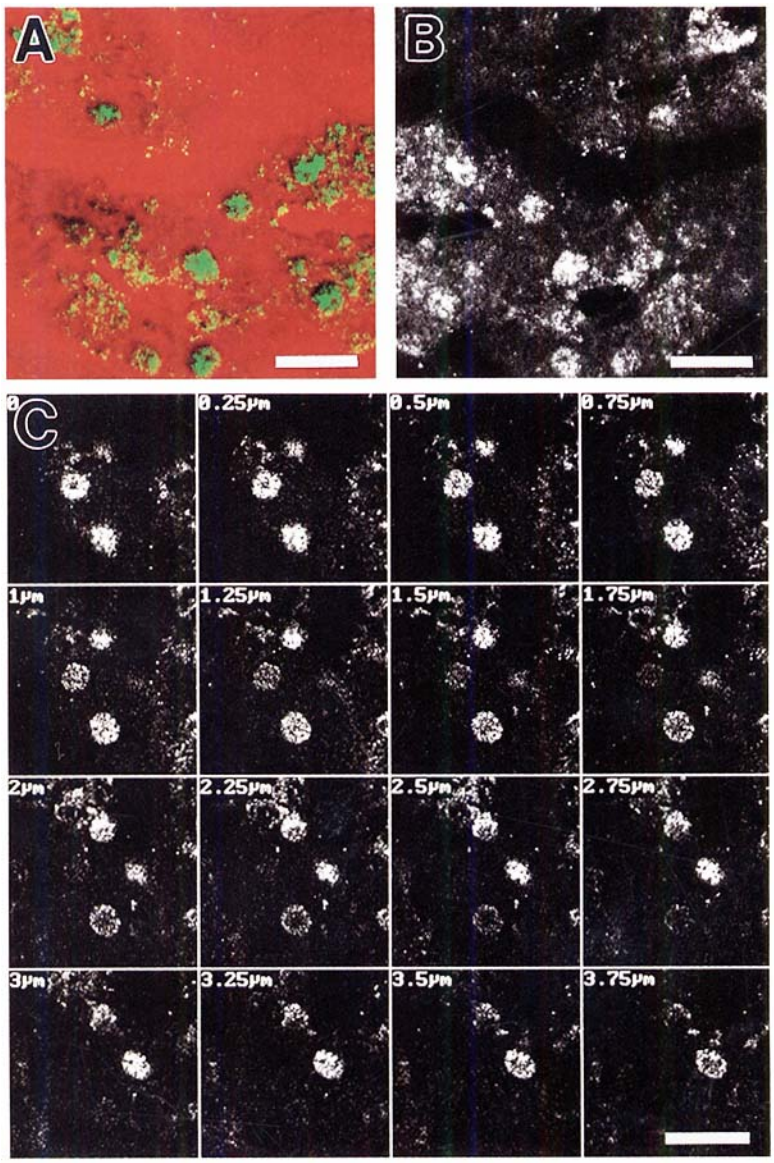

Fig. 4. Localization of FBPase at the nuclear periphery in kidney cells by confocal microscopy. **A:** Composite figure obtained by superimposing a confocal microscopy image viewed in a reflection contrast mode (green) on the conventional light microscopy image (red), showing nuclear staining for FBPase in proximal tubules cells. Bar: 10 μm. **B:** Same field as in A, viewed only in the reflection contrast mode. Four of these nuclei are examined by confocal microscopy in C. Bar: 10 μm. **C:** Series of confocal images, at 0.25 μm intervals, through the four nuclei from B. Bar: 25 μm.

particulate elements [Srere, 1987; Masters et al., 1987; Shearwin et al., 1990] and that the complexes of sequential metabolic enzymes are often bound to structural elements of the cell. Glucose-6-phosphatase is a membrane-bound protein housed within the endoplasmic reticulum [Hers and de Duve, 1950; Arion et al., 1980; Robinson and Karnovsky, 1983] and current evidence indicates that hydrolysis of glucose-6-phosphate by the glucose-6-phosphatase system in intact hepatic endoplasmic reticulum membrane preparations involves four integral components of the membrane [Arion and Canfield, 1993]. Similarly, the glycolytic type I, II, and III isozymes of hexokinase have been recently shown to be associated with specific cellular organelles [Preller and Wilson, 1992, and references therein].

There is also convincing arguments for the existence of specific enzyme-enzyme interactions among globular cytosolic enzymes [Srivastava and Bernhard, 1986; Srere, 1987]. Data are

available indicating the association, apparently physiologically relevant, between purified fructose-1,6-bisphosphate and fructose 1,6-bisphosphate aldolase from rabbit liver [MacGregor et al., 1980] and pig kidney [Sáez and Slebe, 1994] (unpublished results). Consistent with the existence of a highly specific association between the two enzymes is the report by Moorhead et al. [1994], indicating the copurification of cytosolic FBPase and aldolase from endosperm of germinating castor oil seeds. Formation of a complex between these metabolically sequential enzymes could facilitate chanelling of fructose 1,6-bisphosphate and/or alter the kinetic properties of the enzymes and thereby contribute to the gluconeogenic flux. The idea of extensive organization of metabolic pathways and metabolic channelling has been amply discussed [Ureta, 1978; Ureta and Radojkovic, 1987; Srivastava and Bernhard, 1986; Srere, 1987; Ovádi, 1988, 1991].

The immunohistochemical data indicating the perinuclear localization of FBPase in rat liver and kidney cells appear to be at odds with the homogenization data indicating the presence of FBPase activity in the high speed supernatant in these cells and the almost total absence of enzymatic activity in the corresponding nuclear fraction. These two observations can be reconciled if we considerer the possibility that FBPase is associated to the perinuclear structures through weak interactions that are easily broken during the disruption of the ordered intracellular structure and environment that results from the homogenization process [McConkey, 1982]. Alternatively, it is also possible that the primary effect of the homogenization process is the disruption of the delicate anatomic integrity of the subcellular components associated to the nuclear periphery to which the FBPase is associated, without necessarily destroying the interaction between the FBPase and these subcellular structures. Support for this hypothesis is provided by the results of the fractionation studies that showed that the activity of FBPase present in the initial homogenate and in the cytoplasmic extract fraction were greatly increased by incubating these fractions in the presence of Triton X-100 prior to performing the enzymatic assay, while this treatment had no effect on the activity present in the high speed supernatant. The data can be interpreted as indicating that Triton X-100 unmasked a pool of FBPase still bound to subcellular particulate structures and that at least two thirds of the FBPase present in the liver and kidney cells is probably bound to subcellular structures by interactions that are preserved during homogenization but are broken in the presence of Triton X-100.

Overall, our data indicate that the presence of a given enzymatic activity in a high speed supernatant is not a definitive proof that such enzyme corresponds to a "soluble enzyme," and that the combined use of enzyme activity measurements and immunolocalization assays will provide a better understanding of the subcellular distribution of the enzyme in situ. These considerations are of great relevance considering that type III isozyme of hexokinase has been localized at the nuclear periphery [Preller and Wilson, 1992], although no enzyme activity was detectable in the particulate cellular fraction after subcellular fractionation. It is expected that reflection confocal microscopy will have an expanded role in the subcellular localization of other enzymes of the carbohydrate metabolism.

The immunohistochemical data showing that the FBPase is preferentially localized in the hepatocytes from periportal regions in the liver and in the kidney proximal tubules are consistent with the concept of metabolic zonation in both organs. The model of "metabolic zonation" proposes that gluconeogenesis and glycolysis are spacially separated in periportal and pericentral (perivenous) hepatocytes [Jungermann and Sasse, 1978; Jungermann and Katz, 1989], and also in the proximal and distal kidney tubules. The model is based on the observation that periportal and perivenous liver cells, and proximal and distal kidney tubules, present different patterns of enzyme activity and implies that the preferential location of metabolic pathways in separate distinct zones of the liver and kidney is of major importance for organ function.

Enzyme activities corresponding to the gluconeogenic enzymes fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase have been predominantly localized in the periportal region of the liver lobule [Katz et al., 1977a,b; Jungermann and Katz, 1989] and in the proximal tubules in the kidney [Schmid et al., 1977; Lawrence et al., 1986]. In the case of FBPase, evidence obtained from tissue microdissection indicates that its concentration is approximately 2–3 times higher in periportal than in perivenous liver tissue, whereas the glycolytic glucokinase (hexokinase IV or D) varies in the opposite direction [Katz et al., 1977b; Kress and Katz, 1993]. On the other

hand, Wals et al. [1988] found that the concentration of fructose-2,6-bisphosphate was nearly equal in the two zones, a result that was interpreted as indicating that there is no differences in the glycolytic capacity between the periportal and the perivenous regions of the liver. However, because fructose-2,6-bisphosphate activates phosphofructokinase and inhibits fructose-1,6-bisphosphatase, it is expected that it would be present in both the perivenous and periportal zone. Under normal conditions, the concentration of Fru-2,6-P_2 fluctuates in a wide range and, therefore, the modulatory effect of this sugar bisphosphate is believed to be physiologically relevant for the control of the two reciprocal processes, glycolysis and gluconeogenesis [Xue et al., 1994]. The result of similar studies using the microdissection and oil-well techniques indicated that the activity of FBPase was high in the proximal convoluted tubule of the kidney, whereas the highest activity of phosphofructokinase was found in the thick ascending limb of Henle's loop [Schmidt et al., 1975; Schmid et al., 1977]. The data indicated a clear separation of the glucose synthesizing and degrading pathways within the nephron, which is maintained in conditions that stimulate gluconeogenesis. In the present study, we provided complementary immunohistochemical data indicating that the enzyme fructose 1,6-bisphosphatase is localized in situ in the periportal hepatocyte of the liver and in the proximal tubules of the kidney.

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