Different involvement for aldolase isoenzymes in kidney glucose metabolism: aldolase B but not aldolase A colocalizes and forms a complex with FBPase

ARTICLE *in* JOURNAL OF CELLULAR PHYSIOLOGY · APRIL 2005

Impact Factor: 3.84 · DOI: 10.1002/jcp.20183 · Source: PubMed

JOURNAL OF CELLULAR PHYSIOLOGY 202:743–753 (2005)

Different Involvement for Aldolase Isoenzymes in Kidney Glucose Metabolism: Aldolase B but not Aldolase A Colocalizes and Forms a Complex With FBPase

ALEJANDRO J. YAÑEZ, HEIDE C. LUDWIG, ROMINA BERTINAT, CARLOS SPICHIGER, RODRIGO GATICA, GUSTAVO BERLIEN, OSCAR LEON, MONICA BRITO, ILONA I. CONCHA, AND JUAN C. SLEBE*

Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Casilla, Valdivia, Chile

The expression of aldolase A and B isoenzyme transcripts was confirmed by RT-PCR in rat kidney and their cell distribution was compared with characteristic enzymes of the gluconeogenic and glycolytic metabolic pathway: fructose-1,6-bisphosphatase (FBPase), phosphoenol pyruvate carboxykinase (PEPCK), and pyruvate kinase (PK). We detected aldolase A isoenzyme in the thin limb and collecting ducts of the medulla and in the distal tubules and glomerula of the cortex. The same pattern of distribution was found for PK, but not for aldolase B, PEPCK, and FBPase. In addition, co-localization studies confirmed that aldolase B, FBPase, and PEPCK are expressed in the same proximal cells. This segregated cell distribution of aldolase A and B with key glycolytic and gluconeogenic enzymes, respectively, suggests that these aldolase isoenzymes participate in different metabolic pathways. In order to test if FBPase interacts with aldolase B, FBPase was immobilized on agarose and subjected to binding experiments. The results show that only aldolase B is specifically bound to FBPase and that this interaction was specifically disrupted by 60 μM Fru-1,6-P_2. These data indicate the presence of a modulated enzyme–enzyme interaction between FBPase and isoenzyme B. They affirm that in kidney, aldolase B specifically participates, along the gluconeogenic pathway and aldolase A in glycolysis. J. Cell. Physiol. 202: 743–753, 2005. © 2004 Wiley-Liss, Inc.

Although there is no doubt about the major role of the liver in maintaining plasma glucose levels (Nordlie et al., 1999), recent studies indicate that the kidney makes an important contribution to endogenous glucose production in humans (Ekberg et al., 1999; Cersosimo et al., 2000; Gerich et al., 2001). The renal contribution is greater than one third of the whole body glucose production and is an important component of homeostasis of plasma glucose levels (Stumvoll et al., 1997; Ekberg et al., 1999; Conjard et al., 2002). Moreover, it has also been demonstrated that this glucose synthesis can be stimulated by hypoglycemia, adrenaline, and diabetes mellitus (Cersosimo et al., 2000, 2001; Conjard et al., 2002). These results demonstrate the physiological importance of renal gluconeogenesis and have stimulated studies on the regulatory mechanisms involved.

One of the key regulatory enzymes of the gluconeogenic and glyconeogenic pathways is fructose-1,6-bisphosphatase (FBPase; EC 3.1.3.11), which catalyzes the irreversible conversion of fructose-1,6-bisphosphate (Fru-1,6-P_2) to fructose 6-phosphate and inorganic phosphate (Tejwani, 1983; Pilkis and Granner, 1992). On the other hand, fructose-1,6-bisphosphate aldolase (aldolase; EC 4.1.2.13) catalyzes the reversible conversion of Fru-1,6-P_2 to glyceraldehyde 3-phosphate and dihydroxy-acetone phosphate and participates both in gluconeogenesis and glycolysis (Penhoet and Rutter, 1975). In mammalian tissues three FBPase isoenzymes have been found; one in the liver, another in muscle, and the third in brain (Majumder and Eisenberg, 1977; Tejwani, 1983; Cloix et al., 1997). In addition, three aldolase isoenzymes have been identified: aldolase A, the classical muscle enzyme; aldolase B, expressed predominantly in liver; and aldolase C, expressed predominantly in brain (Lebherz and Rutter, 1969; Penhoet et al., 1969).

The heterogeneous distribution of the metabolic enzymes within a single cellular compartment has been proposed as an important regulatory mechanism (Srere and Ovadi, 1990; Ovadi and Srere, 1996). For instance, the binding of glycolytic and gluconeogenic enzymes to cytoskeletal proteins has been shown by several techniques and the modulation of these interactions through ligands indicates that they could play a role in regulating enzyme activities and cellular functions (Durrieu et al., 1987; Shearwin et al., 1990; Knull and Walsh, 1992; Lehotzky et al., 1994). In many metabolic pathways specific interactions between sequential enzymes occur as static or dynamic complexes, that allow substrate channelling (Ovadi and Srere, 1996, 2000). This process has several catalytic advantages, including

Contract grant sponsor: FONDECYT; Contract grant number: 1010720; Contract grant sponsor: Dirección de Investigación, Universidad Austral de Chile; Contract grant number: DID-UACH 200178.

Oscar Leon's present address is Virology Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile.

*Correspondence to: Juan C. Slebe, Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile. E-mail: jslebe@uach.cl

Received 20 March 2004; Accepted 3 June 2004

DOI: 10.1002/jcp.20183

microcompartmentation of metabolites (Srere and Ovadi, 1990; Ovadi, 1991; Ovadi and Srere, 1996; Spivey and Ovadi, 1999).

The co-purification of plant cytosolic FBPase and aldolase has indicated that these two metabolically sequential enzymes might specifically associate in vivo (Moorhead et al., 1994). Kinetic studies show that muscle aldolase isoenzyme decreases the sensitivity of muscle FBPase towards AMP inhibition but does not affect the allosteric properties of the liver FBPase, supporting the idea of a specific interaction between the muscle isoenzymes (Rakus and Dzugaj, 2000; Rakus et al., 2003a,b). With gel penetration experiments, MacGregor et al. (1980) have detected an interaction between rabbit liver aldolase and FBPase. However, no direct evidence for an interaction between the liver isoenzymes has been obtained.

The compartmentalization of metabolic pathways in organelles contributes to the regulation of metabolism by separating synthetic and degradative processes. Alternatively, on the basis of microdissection studies (Schmid et al., 1977) intercellular compartmentalization has been proposed for the degradation and the synthesis of glucose in kidney. It has been postulated that intracellular compartmentalization might be important to prevent futile cycling, since glycolysis and gluconeogenesis both occur in the cytosol. Similarly, it has been suggested that liver hepatocytes located in the periportal zone perform gluconeogenesis, while glycolysis is restricted to hepatocytes in the perivenous zone (Katz et al., 1977; Jungermann and Kietzmann, 1996; Saez et al., 1996). Accordingly, our results on the immunohistochemical localization of FBPase in liver and in kidney tissues support the concept of metabolic zonation in both organs (Saez et al., 1996; Yañez et al., 2003a,b).

The sequences of the mammalian aldolases are highly conserved, but aldolase B is slightly more divergent with ~70% sequence identity to both aldolases A and C (Rottmann et al., 1984). On the other hand, aldolase A performs the Fru-1,6-P_2 cleavage more efficiently than aldolase B, as demonstrated by a 20 to 30-fold higher k_{cat} (Eagles and Iqbal, 1973). Aldolase B has a 10-fold lower Km for glyceraldehyde 3-phosphate and dihydroxyacetone phosphate than aldolase A (Penhoet et al., 1969). On the basis of the kinetic properties of these isoenzymes, it has been suggested (Penhoet et al., 1969; Eagles and Iqbal, 1973) that aldolase B has evolved to have a role in gluconeogenesis while aldolase A is more effective participating in glycolysis (Penhoet et al., 1969; Penhoet and Rutter, 1971; Eagles and Iqbal, 1973). This notion is strengthened by the fact that vertebrate skeletal muscle expresses almost exclusively aldolase A (Lebherz and Rutter, 1969). However, the involvement of a particular aldolase isoenzyme in a gluconeogenic or glycolytic complex remains to be established.

In order to associate the aldolase isoenzymes A and B to specific metabolic pathways in the kidney, we have extended our previous study on the distribution of FBPase and aldolase B (Saez et al., 1996; Saez and Slebe, 2000; Yañez et al., 2003a,b). The present study analyzes the localization of aldolases A and B within kidney tubular cells, and compares it with the localization of the gluconeogenic enzymes FBPase and PEPCK and the glycolytic enzyme, PK in order to answer the old question: Why isoenzymes? We demonstrate the interaction of FBPase with aldolase B by affinity chromatography. Our results support the notion that opposite glucose pathways are segregated in kidney, and indicate that the aldolase isoenzymes A and B play different functions in glucose metabolism.

MATERIALS AND METHODS

Antibody production

Rabbit polyclonal antisera raised against purified pig muscle aldolase A and pig kidney aldolase B (Alarcón et al., 1971) and FBPase (Reyes et al., 1987) were prepared as described previously (Saez and Slebe, 2000; Yañez et al., 2003b). These antisera have a high specificity for aldolase A, aldolase B and for the hepatic FBPase, respectively (Saez et al., 1996; Saez and Slebe, 2000; Yañez et al., 2003a,b). Chicken egg yolk antibodies against FBPase and aldolase B were prepared by injecting two laying leghorn hens with the antigen (FBPase or aldolase B) subcutaneously, using the same protocol conditions as for rabbits (Saez et al., 1996). Simple water dilution was employed for the isolation of polyclonal IgY from egg yolk granules. The IgY isolation was performed by a modification of the method described by Akita and Nakai (1993). Sheep anti-rat PEPCK purified antibodies were generously gifted by Dr. Daryl Granner (Vanderbilt University, Nashville, TN). This antiserum has a high specificity for the cytosolic isoform of PEPCK and does not crossreact with the mitochondrial form (Beale et al., 1981; Zimmer and Magnuson, 1990). Chicken anti-rat monocarboxylate transporter 1 (MCT1) was purchased from Chemicon International, Temecula, CA while goat anti-rabbit PK was purchased from Rockland, Gilbertsville, PA.

Westem blot

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the Bio-Rad minigel apparatus instructions. 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. The proteins were electrophoretically transferred from polyacrylamide gels to nitrocellulose membranes and probed with the anti-aldolase serum. The antibody–antigen interactions were detected using peroxidase coupled to goat anti-rabbit IgG.

RT-PCR

Total RNA from rat kidney was isolated using the reagent TRIzol according to the manufacturer's instructions. One microgram of each sample was reverse-transcribed at 42°C for 60 min, followed by enzyme inactivation at 70°C for 15 min. The resulting cDNA samples were amplified by the PCR method, using the following primers: aldolase A sense, 5′-ATGCCCCACCCATACCCAGCA-3′ and antisense, 5′-TTAGTAGGCATGGTTAGAGATGAA-3′, from the published nucleotide sequence of *Rattus norvegicus* muscle aldolase cDNA and giving a PCR product of ~1,100 bp (gi:6978486); aldolase B sense, 5′-ATGGCTCACCGATTTCCAGCC-3′ and antisense, 5′-CTAGTAGGTGTAGGAGGCTGTG-3′, from the published nucleotide sequence of *Rattus norvegicus* liver aldolase cDNA and giving a PCR product of ~1,100 bp (gi:27476058); aldolase C sense, 5′-ATGCCCCACTCATACCCAGC-3′ and antisense, 5′-TCAGTAGGCATGGTTGGCCAC-3 from the published nucleotide sequence of *Rattus norvegicus* brain aldolase cDNA and giving a PCR product of ~1,100 bp (gi:6978488). As an internal control, 430 bp of the constitutively expressed housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (sense 5′-CCCCTTCCGCTGATGCCCC-3′ and antisense 5′-TGGGCCCTCGGCCGCCTG-3′), was also synthesized and used to normalize the amount of mRNA in each RT-PCR reaction. All primers were synthesized by Invitrogen (Gaithersburg, MD). Amplifications were carried out for 30 cycles by denaturing at 95°C for 30 sec, annealing at 54°C for 1 min, and extending at 72°C for 4 min, with a final extension at 72°C for 10 min. The PCR products

were analyzed by electrophoresis on 1.5% agarose gels containing ethidium bromide.

Tissue immunocytochemistry

Kidney and heart tissues obtained from adult Wistar rats (body weight 300–350 g) were fixed in Bouin's fluid, 4% (v/v) (Yañez et al., 2003a) for 24 h at room temperature. Fixed samples were dehydrated in ethanol and embedded in Paraplast Plus (Monoject Scientific, St. Louis, MO).

Immunostaining was performed according to the peroxidase/anti-peroxidase (PAP) method with the modifications described in the "LSAB kit" by DAKO Corporation (Carpenteria, CA). Briefly, dewaxed tissue sections were treated with absolute methanol and with 3% (v/v) H_2O_2 for 5 min, rehydrated, and washed with 0.05 M Tris-HCI, 0.15 M NaCl, pH 7.8. Incubation with the first antiserum was performed for 2 h at room temperature with rabbit polyclonal anti-FBPase (1:1,000), chicken polyclonal anti-aldolase B (1:500), rabbit polyclonal anti-PK (1:2,000), or rabbit polyclonal anti-aldolase A (1:1,000). In the second step, secondary antibodies were applied for 1 h followed by incubation for 30 min with streptavidin-peroxidase conjugate. The incubations were followed by three washings with PBS. Finally, the peroxidase reaction was developed using 3,3′-diaminobenzidine (DAB) and examined by light microscopy. Controls for the immunostaining procedure were prepared by substituting the first antibody with non-immune rabbit serum at the same dilutions.

Co-localization of aldolase B and FBPase was studied by double-label immunofluorescence. Deparaffinized sections of kidney were incubated for 2 h with the mixture of the first antibodies (rabbit anti-FBPase diluted 1/1,000 and chicken anti-aldolase B diluted 1/500). After washing, the sections were incubated for 1 h with a mixture of Alexa 594 conjugated anti-rabbit IgG (diluted 1/200) and Alexa 488 conjugated anti-chicken IgY (diluted 1/200) from Molecular Probes (Eugene, OR). Controls were performed by substituting the primary or secondary antibodies with buffer. Cross-reactivity of secondary antibodies was tested replacing the target primary antibody with normal serum or buffer. Stained sections were examined with a Zeiss (Thomwood, NY) laser scanning confocal microscope.

Purification of enzymes and enzymatic assays

FBPase was purified from pig kidney as described by Reyes et al. (1987) while aldolases A and B were purified from pig muscle and kidney as described by Alarcón et al. (1971). The purity of the enzymes was checked using 10% SDS–PAGE according to Laemmli (1970).

FBPase activity was determined spectrophotometrically at 30°C by following the rate of NADH formation at 340 nm in the presence of an excess of both glucose-6-phosphate dehydrogenase and phosphoglucose isomerase (Colombo et al., 1972). The reaction system (0.5 ml) contained 50 mM Tris-HCl buffer, pH 7.5, 0.1 mM EDTA, 5 mM $MgSO_4$, 50 μM Fru-1,6-P_2, 0.3 mM NAD^+, and 1.2 U of each auxiliary enzyme. The reaction was initiated by the addition of FBPase. One unit of FBPase activity was defined as the amount of enzyme that catalyses the formation of 1 μmol of Fru-6-P per min under the conditions described (Cárcamo et al., 2000).

Aldolase activity was determined spectrophotometrically at 30°C by following the rate of consumption of NADH at 340 nm in the presence of an excess of both glyceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase (Alarcón et al., 1971; Saez and Slebe, 2000). The reaction system (0.5 ml) contained 35 mM Tris-HCl buffer, pH 7.5, 0.1 mM EDTA, 3.3 mM 2-mercaptoethanol, 0.6 mM Fru-1,6-P_2, 0.1 mM NADH, 8.7 U of triose phosphate isomerase, and 3 U of glyceraldehyde-3-phosphate dehydrogenase. The reaction was initiated by the addition of aldolase. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 μmol of NAD^+/min under the conditions described.

The concentrations of FBPase and aldolase were determined by absorbance at 280 nm using an $\varepsilon^{1\ mg/ml}$ value of 0.755 for FBPase (Marcus and Hubert, 1968) and an $\varepsilon^{1\ mg/ml}$ value of 0.89 for aldolase (Penhoet and Rutter, 1971).

The partially purified aldolase B used for the affinity chromatography was obtained by following the first steps (acid and ammonium sulphate precipitations) of the purification procedure (Alarcón et al., 1971). The precipitate obtained between 52 and 65% ammonium sulphate saturation was redissolved in 20 mM Tris-HCl buffer, pH 8.0, containing 0.1 mM EDTA, and 0.5 mM 2-mercaptoethanol and dialyzed against the same buffer. The dialyzed solution was applied to the FBPase-Sepharose column.

Affinity chromatography on FBPase-Sepharose

FBPase was coupled to Sepharose-4B using adipic acid dihydrazide and ethylene glycol bis(succinimidylsuccinate) (EGS) as spacer groups. The starting material was cyanogen bromide activated Sepharose-4B, hydrated and washed with 0.1 mM HCl. Eight milliliters of 50 mM adipic acid dihydrazide in 0.1 M $NaHCO_3$ were added to 3 ml of the Sepharose, the mixture was shaken at room temperature for 1 h and the reaction was allowed to proceed over night in the dark. The gel was washed successively with 1 M NaCl and distilled water. The EGS was then coupled to the gel, as described by León and Schulman (1987). Briefly, 1.5 ml of 0.2 M Hepes pH 7.8 and 4 ml of 18 mg/ml EGS dissolved in DMSO were added to the gel and the mixture was shaken for 10 min at room temperature. The gel was washed rapidly and successively with the following ice-cold solutions: 30% DMSO in 0.1 M sodium acetate pH 6.0; 0.1 M sodium acetate pH 6.0 and distilled water. Then, 2 ml of 0.2 M Hepes pH 7.8 and FBPase in 15 mM Hepes pH 7.5, 0.1 mM EDTA were added to 3 ml of the gel. The final concentration of FBPase was 1.2 mg/ml. The mixture was shaken gently at room temperature for 1 h. Finally the gel was washed first with 20 mM Tris-HCl pH 7.5, 0.1 mM EDTA, 1 M NaCl, and then with the same buffer without NaCl. The FBPase-Sepharose was stored at 4°C. For control experiments, Sepharose-4B coupled only to the spacer groups was prepared by the same method.

The amount of FBPase per ml of FBPase-Sepharose was determined by amino acid analysis. A 0.5 ml of settled FBPase-Sepharose was subjected to acid hydrolysis and amino acid analysis as previously described (Ludwig et al., 1999). For the standard, 0.5 ml of settled Sepharose-4B coupled to the spacer groups mixed with 125 μg of FBPase was subjected to the same procedure.

For the affinity chromatography a column of 0.7 × 8.0 cm with approximately 3 ml of FBPase-Sepharose was prepared and equilibrated with 20 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 0.5 mM 2-mercaptoethanol. The chromatography was performed at room temperature.

Soluble-particulate distribution of FBPase and aldolase

Renal tissue was obtained from Wistar rats. The kidney was homogenized in sucrose 0.25 M, Tris-HCl

EDTA 1 mM, $CaCl_2$ 3 mM PMSF 0.2 mM and the homogenate was centrifuged for 10 min at 480*g*. The supernatant designated low speed supernatant (LS) was centrifuged for 20 min at 25,300*g*, to produce the high speed supernatant (HS). Aliquots of the fractions LS and HS were pre-incubated with 0.1% (v/v) Triton X-100 at 20°C, for 20 min in sucrose buffer (above). Aldolase and FBPase activities of these fractions were then assayed.

RESULTS

Effect of Triton X-100 on FBPase and aldolase activities in kidney homogenates

We examined the ability of the detergent Triton X-100 to solubilize a particulate fraction of rat kidney FBPase and aldolase. Control experiments using pure aldolases and FBPase demonstrate that the action of Triton X-100 do not affect the enzymes activities (data not shown). As seen in Figure 1, preincubation of a low speed supernatant (LS) with 0.1% Triton X-100 at 20°C, for 20 min, resulted in a net increase in assayable FBPase activity of 70%, indicating an exposure of particulate FBPase. The detergent had no effect on the FBPase activity in the high speed supernatant (HS), suggesting that it does not contain particulate enzyme. By contrast, a decrease in total assayable aldolase activity of 50% was found after preincubation of the low speed supernatant (LS) with Triton X-100 (Fig. 1). As for FBPase, the detergent had no effect on the aldolase activity in the high speed supernatant (Fig. 1). Although the total assayable activity in the initial kidney homogenate had a variation of 10–25% within replicate experiments, Triton X-100 was found to enhance assayable FBPase activity approximately three-fold but to decrease aldolase activity approximately two-fold (data not shown). In control experiments, using Western blot analysis, we found that the protein level of FBPase and aldolases remains unchanged in LS, preincubated or not with Triton X-100 (data not shown), irrespective of the significant increase of the FBPase activity and decrease of aldolase activity. Similar results were obtained with HS. These data indicate that the dissociation of FBPase or aldolase from particulate elements within the kidney cells have important and opposite effects on the catalytic activity of these enzymes.

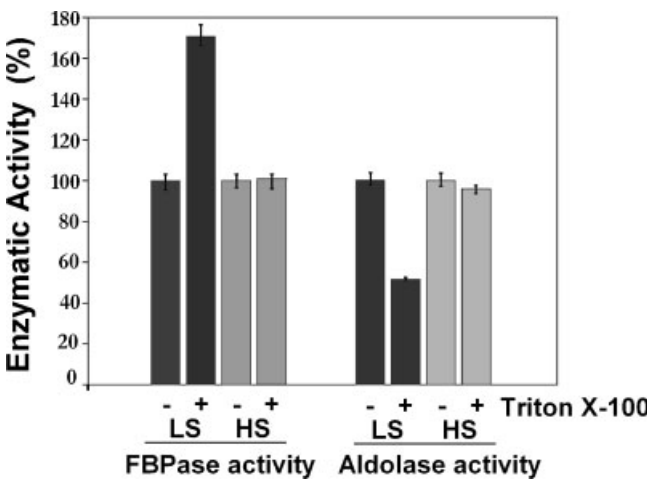

Fig. 1. Effect of Triton X-100 on FBPase and aldolase activities. Rat renal homogenate was centrifuged for 10 min at 480*g* and then the supernatant was centrifuged for 20 min at 25,300*g* obtaining a low speed (LS) and high speed supernatant (HS). Two aliquots of each supernatant were treated with or without 0.1% Triton X-100 at 20°C for 20 min, before assaying aldolase and FBPase activities. The initial FBPase and aldolase activities in LS were 12 and 3 U/g tissue, respectively.

Expression of aldolase A and aldolase B in kidney cells

Total RNA and cytosolic protein extract of whole rat kidney were used to study the aldolase isoenzymes gene expression in this organ. The RT-PCR shows the presence of mRNA of aldolases A and B in the tissue as a product of 1,100 bp (Fig. 2A). The level of mRNA of these aldolase isoenzymes was similar to the internal control of GAPDH (Fig. 2A). Aldolase C mRNA was not detected (Fig. 2A). Western blot analysis demonstrated the presence of similar immunoreactivity of aldolase A and aldolase B in the renal extract (Fig. 2B). These results together indicate that both aldolase isoenzymes are similarly expressed in renal tissue. Moreover, each antibody detected a single immunoreactive band, which migrated at the predicted subunit molecular weight (MW ~39,000). Aldolases A and B antibodies do not cross-react with purified aldolase B and A, respectively, demonstrating the high isoform specificity of the prepared antibodies.

Immunolocalization of gluconeogenic and glycolytic enzymes in rat kidney cells

The immunohistochemical analyses of FBPase and PEPCK (gluconeogenic enzymes), pyruvate kinase (glycolytic enzyme), and the aldolase isoenzymes A and B were performed in rat kidney sections. The distribution of aldolase B in rat kidney cortex was shown to be very similar to that of FBPase and PEPCK (Fig. 3A–I). Intense immunostaining of the enzymes was observed at the proximal straight and convoluted tubules located in the cortex, but not in cells located in the medulla of the kidney (Fig. 3A–I). A difference in the immunolocalization of these enzymes was the apical staining of FBPase and PEPCK in the proximal tubules compared with aldolase B. Also, the results show that these enzymes are in a similar abundance in the three segments (S_1, S_2, and S_3) of the proximal tubules. The very low immunoreaction detected in the distal tubules and medulla indicates a low or absent expression of aldolase B, FBPase, and PEPCK (Fig. 3A–I).

Interestingly, the localization of the isoenzyme A of aldolase was totally different, as it was mainly exp-

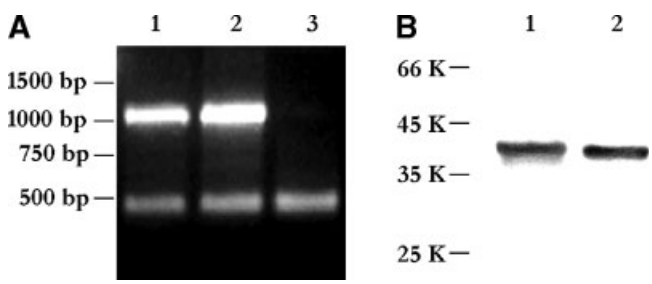

Fig. 2. Renal expression of aldolases A and B by RT-PCR and immuno-blot. **A**: Total RNA from kidney was isolated to obtain the cDNA. The RT-PCR was performed with primers specific for rat aldolase A, B and C, and GAPDH. The products obtained for aldolases A and B are shown in lanes 1 and 2, respectively. Lane 3 correspond to aldolase C. The expression level of these enzymes is compared to GAPDH product (480 bp). PCR controls were performed with the same primers and RNA. **B**: Total kidney protein extract (40 μg) was fractionated by SDS–PAGE followed by immunoblotting using rabbit anti-aldolase A antibody (1) and chicken anti-aldolase B antibody (2). The staining reaction was performed using peroxidase-labeled secondary antibody and 3,3-diaminobenzidine (DAB).

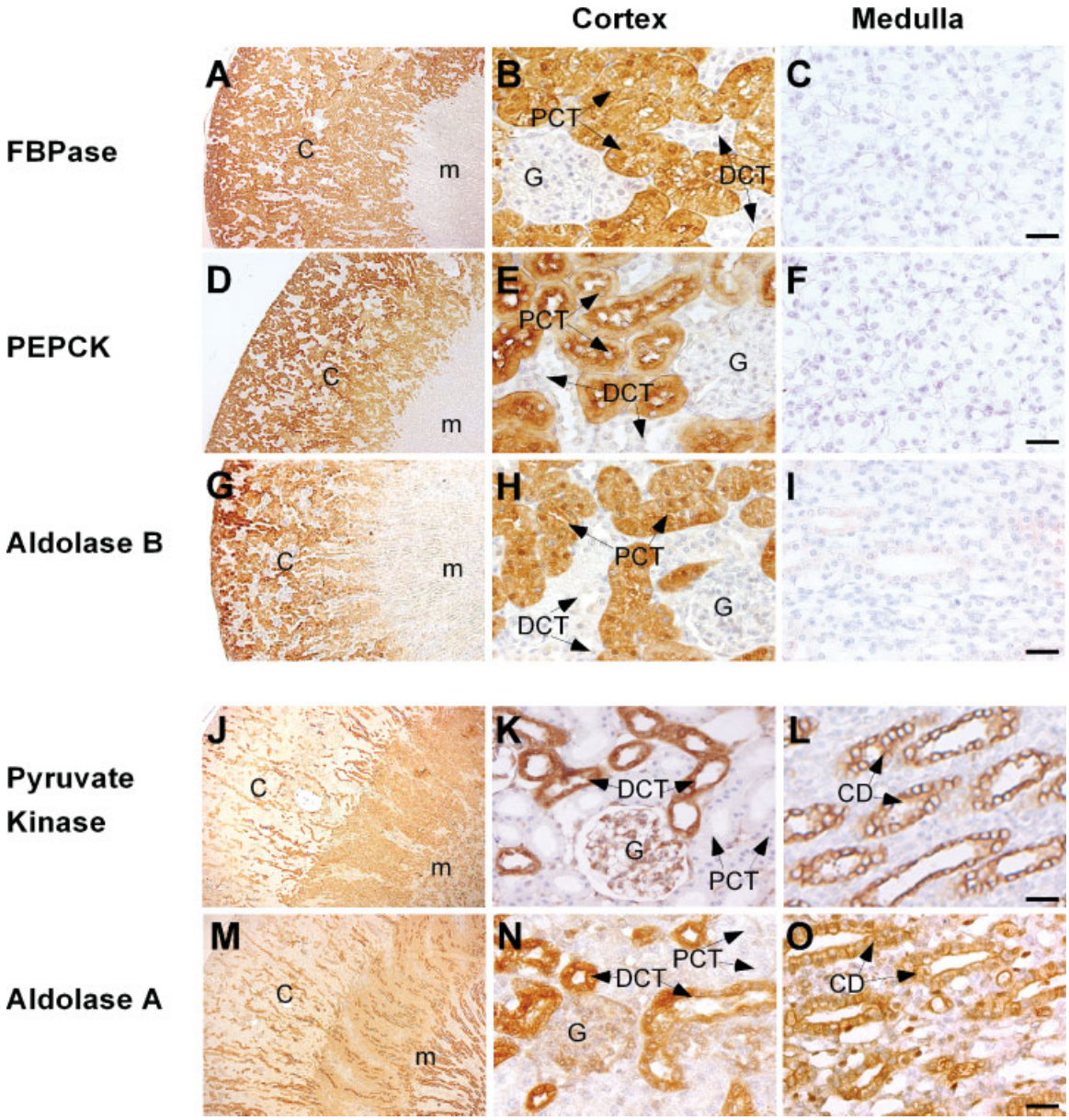

Fig. 3. Immunolocalization of FBPase, PEPCK, aldolase B, PK, and aldolase A in rat kidney cells. Renal tissue obtained from adult rat was fixed in Bouin's fluid, dehydrated in ethanol and embedded in paraffin. Immunostaining was performed according to the SAB method by DAKO. **A**–**C**: Anti-FBPase (1:1,000). **D**–**F**: Anti-PEPCK (1:10,000). **G**–**I**: Anti-aldolase B (1:500). **J**–**L**: Anti-PK (1:1,000). **M**–**O**: Anti-aldolase A (1:1,000). The peroxidase reaction was developed using DAB and examined by light microscopy. All the enzymes were detected in the kidney cortex and medulla at high magnification. C, cortex; M, medulla; PCT, proximal convoluted tubules; DCT, distal convoluted tubules; CD, collecting ducts; G, Glomerulus. Scale bars, 40 μm.

ressed in the medulla and additionally in the cortex (Fig. 3M–O). Particularly intense staining of aldolase A was observed at the glomerulus, distal tubules, loop of Henle and collecting tubules, showing a clear cellular separation in the expression of aldolase A and B isoenzymes in rat kidney (Fig. 3G–I, M–O). The distribution pattern of aldolase A in rat renal tissue was very similar to the localization pattern of PK (Fig. 3J–O), except that only aldolase A shows immunoreactivity in the loop of Henle (Fig. 3J,M). Immunostaining controls, in which the primary antibody was replaced by non-immune rabbit serum at the same dilution, gave no immune reaction (data not shown). In addition, we have observed that starvation, a condition that has been demonstrated to increase the renal gluconeogenesis and the uptake of lactate, glycerol and glutamine (Cersosimo et al., 2000; Conjard et al., 2002), did not alter the distribution pattern of these enzymes in kidney (data not shown).

In order to confirm the differential distribution of these enzymes in the kidney, we performed a series of co-localization experiments of aldolase A, aldolase B, FBPase, PEPCK and PK using fluorescence double-labeling confocal microscopy. Figure 4A clearly shows that PEPCK co-localizes with FBPase in the cytoplasm of the proximal tubule cells and also in a compartment close to the apical membrane of proximal tubule cells of the external cortex. Interestingly, in the same cells aldolase B also co-localizes with FBPase (Fig. 4B). A high degree of intracellular co-localization of these enzymes was found in the cortex in the three segments (S_1, S_2, and S_3) of the proximal tubules. Although both

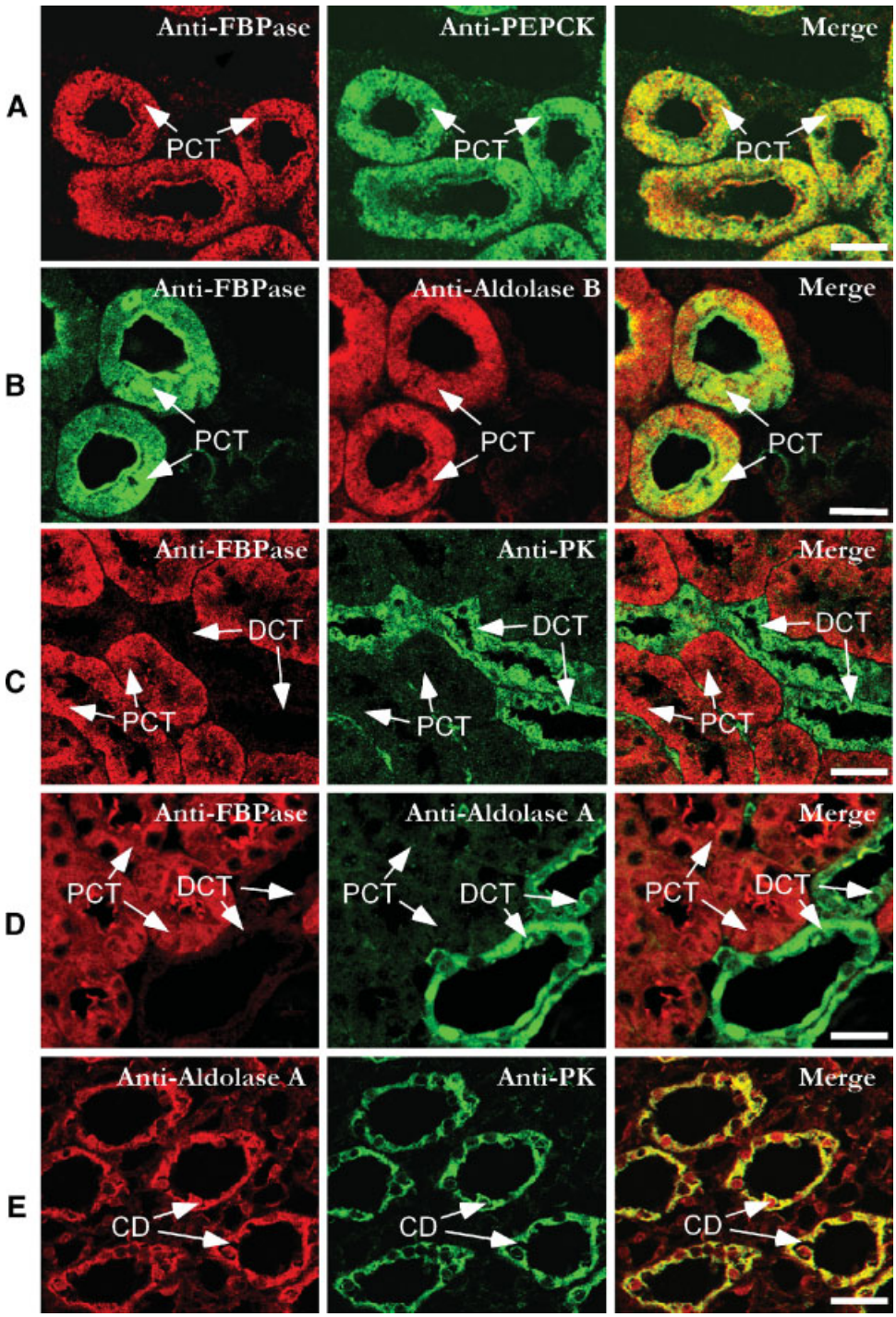

Fig. 4. Co-localization analysis in renal cortex by confocal microscopy. Rat kidney tissue was subjected to double-labeling immunofluorescence and confocal microscopy. Kidney cortex was incubated with: **A**: Rabbit polyclonal anti-FBPase (1:1,000; red channel) and sheep polyclonal anti-PEPCK (1:2,000; green channel). **B**: Chicken polyclonal anti-aldolase B (1:500; red channel) and rabbit polyclonal anti-FBPase (1:1,000; green channel). **C**: Rabbit polyclonal anti-FBPase (1:1,000; red channel) and goat polyclonal anti-PK (1:1,000; green channel). **D**: Chicken polyclonal anti-FBPase (1:1,000; red channel) and rabbit polyclonal anti-aldolase A (1:1,000; green channel). **E**: Kidney medulla was incubated with goat polyclonal anti-PK (1:1,000; green channel) and rabbit polyclonal anti-aldolase A (1:1,000; green channel). The merged images show both channels at the same time. PCT, proximal convoluted tubules; DCT, distal convoluted tubules; CD, collecting ducts; G, Glomerulus. Scale bars, 30 μm.

FBPase and aldolase B were observed in the cytoplasm, only FBPase, but not aldolase B, was detected at the apical membranes of the proximal tubules located at the external portion of the kidney cortex (Fig. 4B).

The glycolytic enzyme PK was detected in the glomerulus and distal tubules of the kidney cortex (Figs. 3K, 4C) and did not co-localize with FBPase (Fig. 4C), PEPCK or aldolase B (data not shown). Moreover, in the kidney cortex, aldolase A was mainly observed in the cytoplasm of the cells of the distal tubules and the collecting ducts. No staining was associated with the proximal tubules of the nephron, where FBPase, aldolase B, and PEPCK were localized (Figs. 3J–O, 4). As expected, aldolase A and PK co-

localized in the distal tubules of the nephron (data not shown). Although in the medulla, aldolase A was associated with the thin limb and collecting ducts (Fig. 3M,O), the isoenzyme shows a high degree of colocalization with PK only in the collecting ducts (Fig. 4E). Unexpectedly, aldolase A but not PK showed strong staining in the nucleus of some of these cells. Nuclear staining was also present in some cells of the distal tubules but was absent in cells of the proximal tubules, indicating that the reaction is specific. To unequivocally determine the presence of aldolase A inside of the nuclei, we performed a confocal analysis taking optical sections of 1 μm at 4 μm intervals through the kidney medulla (Fig. 5, upper part). The antibody staining was largely confined in the nuclei. To confirm the aldolase A capability to translocate into the nuclei, we characterized its localization in heart. Immunohistochemical detection in this organ, using DAB reaction, showed a strong staining inside the nuclei of cardiomyocytes (Fig. 5A, lower part). This subcellular localization was confirmed by immunofluorescence analysis (Fig. 5B, lower part). Specific staining was not detected in any of the negative controls without primary or secondary antibodies against either rabbit IgG or chicken IgY (data not shown).

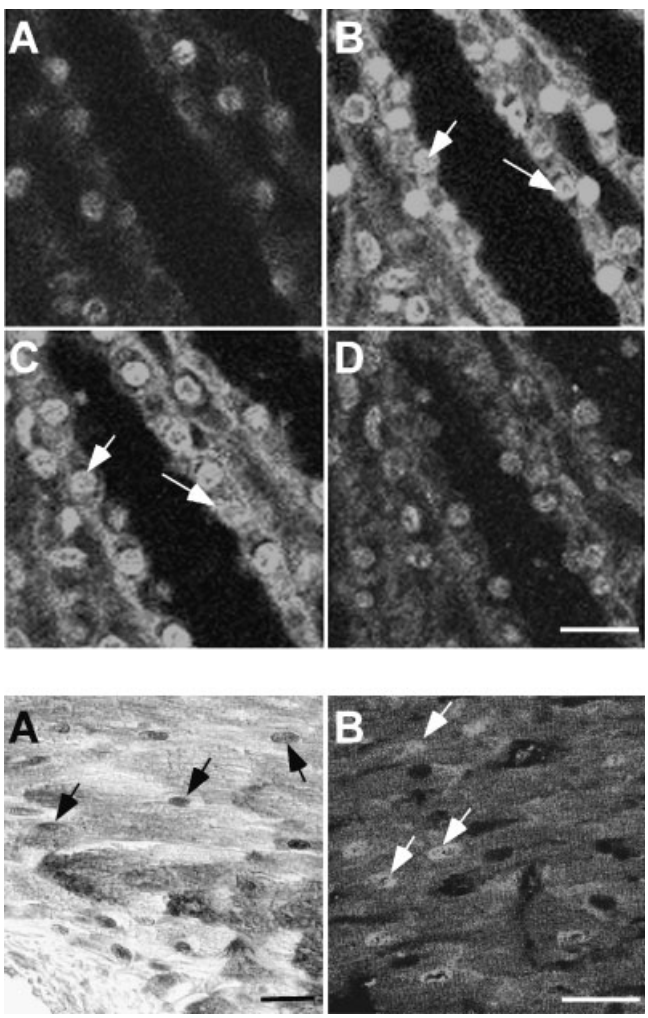

Fig. 5. Immunofluorescence analysis of aldolase A in rat kidney and heart cells. Upper part, **A**–**D**. Tissues obtained from adult rat were subjected to immunofluorescence and confocal analysis, using anti-aldolase A (1:500). Medullar kidney sections of 1 μm were obtained at 4 μm intervals. Lower part, **A**: Heart sections were incubated with anti-aldolase A and the immunostaining was performed using the peroxidase/DAB reaction. Lower part, **B**: Aldolase A was detected in rat heart cells using immunofluorescence labeling. Optical section of 1 μm was obtained showing the nuclear localization (arrows). Scale bars, 30 μm.

As described above, FBPase is in the cytoplasm and the apical cell membrane of proximal convoluted tubules of the external kidney cortex (Figs. 4A,B and 6A,B). However, in the internal proximal tubules, close to the medulla, FBPase appears also associated with the basolateral cell membrane compartment (Fig. 6C,D). Since we have previously established that the monocarboxylate transporter MCT1 is expressed specifically in segment S_1 of the proximal convoluted tubule in human kidney (Yañez et al., 2003b), in the present study we used MCT1 as a marker for the basolateral membranes of proximal tubules. Figure 6 shows a different expression of this transporter in the proximal tubules of the external cortex compared with those of the internal cortex. Moreover, both areas show an equivalent co-localization between FBPase and MCT1. In the S_1 segment of the external nephron, FBPase seems associated to the apical membrane region whereas in the S_1 segment of the internal nephron, FBPase is also observed in the basolateral membrane region.

Double staining of FBPase and DNA shows a nuclear localization of FBPase in the proximal tubule cells. However, only some of the cells exhibited co-localization of FBPase with propidium iodide. In agreement with previous data (Yañez et al., 2003a), we suggest that not all the proximal tubule cells are in the same metabolic state that allows the translocation of FBPase from the cytosol to the nucleus.

Affinity chromatography reveals a specific interaction between FBPase and aldolase B

The specific interaction between the sequential gluconeogenic enzymes, FBPase and aldolase, was investigated using FBPase immobilized on Sepharose. We chose to immobilize FBPase on CNBr-activated Sepharose 4B since this support has been widely used for the immobilization of other macromolecules. For the immobilization it was necessary to interpose a spacer arm between the matrix and the enzyme to prevent the loss of catalytic activity, observed when FBPase was coupled directly to CNBr-activated Sepharose (data not shown). The spacer arm chosen allowed the coupling of FBPase via amino groups. The immobilized enzyme (FBPase-Sepharose) exhibited 75% of the specific activity of the native enzyme and was stable for at least 1 week at 4°C. The amount of immobilized FBPase per unit volume of gel was estimated by amino acid analysis. Two separate experiments yielded identical results: 0.75 mg of enzyme per milliliter of settled gel.

When purified aldolase B (0.6 mg) was applied to a chromatography column filled with 3 ml of FBPase-Sepharose gel in a 20 mM Tris-HCl buffer (pH 8.0) the enzyme was retained completely, as revealed by the absence of aldolase activity in the fractions collected (Fig. 7A). The bound aldolase was eluted with a linear gradient of Fru-1,6-P_2. As shown in Figure 7A, aldolase begins to be eluted from the affinity column at a Fru-1,6-P_2 concentration of 60 μM, indicating that the interaction between the enzymes is modulated by the substrate. Elution of total aldolase activity was also achieved with 30 mM NaCl (data not shown). In a control experiment, Sepharose 4B carrying only the spacer arm failed to retain aldolase (Fig. 7B). Moreover, no aldolase activity was eluted by 0.2 M NaCl. To test the specificity of this interaction we applied a sample of partially purified

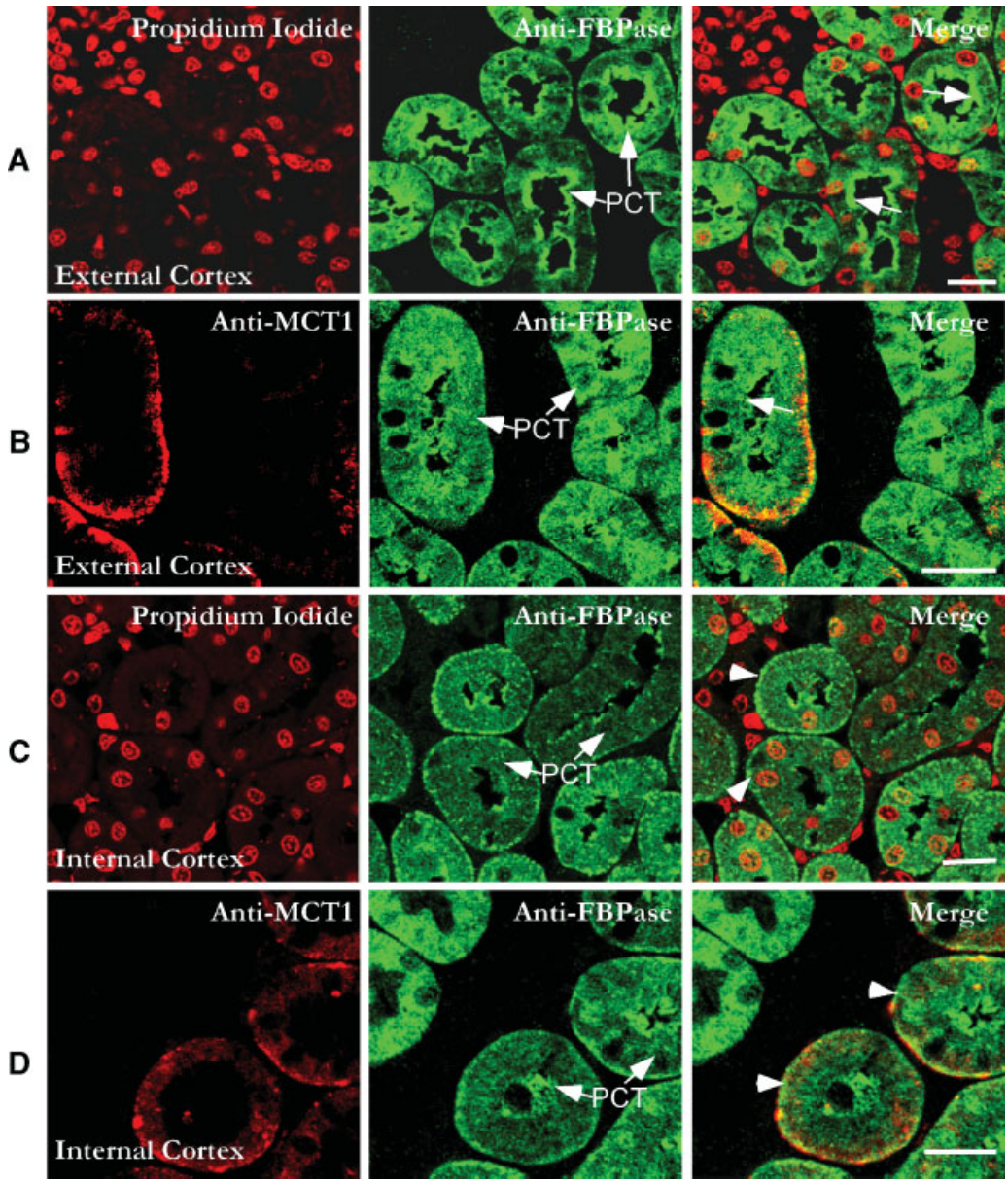

Fig. 6. Polarized localization of FBPase in renal cortex tubules. Rat kidney tissue was subjected to immunofluorescence labeling with antibodies against FBPase (green channel) and MCT1, and nuclear staining with propidium iodide (IP-red channel). **A**, **C**: External and internal cortex, respectively, was incubated with rabbit polyclonal anti-FBPase (1:1,000) and stained using goat polyclonal Alexa fluor-488 anti-rabbit (1:200), followed by incubation with propidium iodide. **B**, **D**: External and internal cortex, respectively, was incubated with chicken polyclonal anti-MCT1 (1:500; red channel) and rabbit polyclonal anti-FBPase (1:1,000; green channel). PCT, proximal convoluted tubules. The merged images show both channels at the same time showing apical (arrow) and basolateral (arrow head) on FBPase localization. Scale bars, 30 μm.

aldolase B, containing many different proteins including aldolase A, to the FBPase-Sepharose column. As expected, aldolase B was retained and eluted with 200 μM Fru 1,6-P_2 (Fig. 8). The protein eluted with substrate did not react with the aldolase A antibody (data not shown). The electrophoresis and immunoblot analysis revealed that only aldolase B was retained in the column, indicating a specific interaction between aldolase B and FBPase.

DISCUSSION

The results presented in this study provide direct evidence for a complex formation between the liver isoenzyme of FBPase and aldolase B. They lead us to propose that the aldolase isoenzymes A and B play different roles in glucose metabolism. Although glucose metabolic pathways are well defined at the biochemical level, the subcellular distribution, regulation and spatial organization of the enzymes in these pathways are poorly understood in kidney. In muscle, there is evidence that indicates that glycolytic enzymes are reversibly associated with the subcellular cytoskeletal network and that this interaction regulates their properties and the contraction process (Srere and Ovadi, 1990; Wojtas et al., 1997; Ovadi and Srere, 2000). Moreover, associations of some enzymes to subcellular structures are specifically modulated by salts and ligands, with the bound and free forms displaying different kinetic properties (Durrieu et al., 1987; Knull and Walsh, 1992; Lehotzky et al., 1994). The effects of

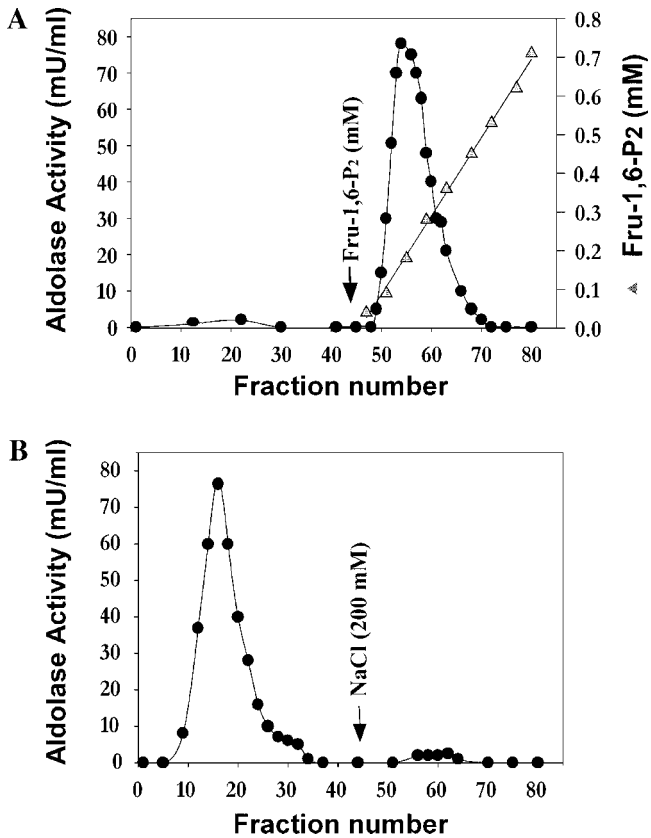

Fig. 7. Affinity chromatography of aldolase B on Sepharose-FBPase. **A**: FBPase (2 mg) was covalently bound to Sepharose-4B matrix and equilibrated with buffer E (20 mM Tris HCl pH 8.0, 0.1 mM EDTA, 0.5 mM 2-mercaptoethanol), to make the Sepharose-4B-FBPase matrix. Purified aldolase B was applied to the column and washed with buffer E. The column was eluted with a linear gradient between 0 and 0.8 mM FBP in buffer E. **B**: Control experiment with the chromatography of aldolase B in Sepharose-4B, shows no binding of aldolase B to the column.

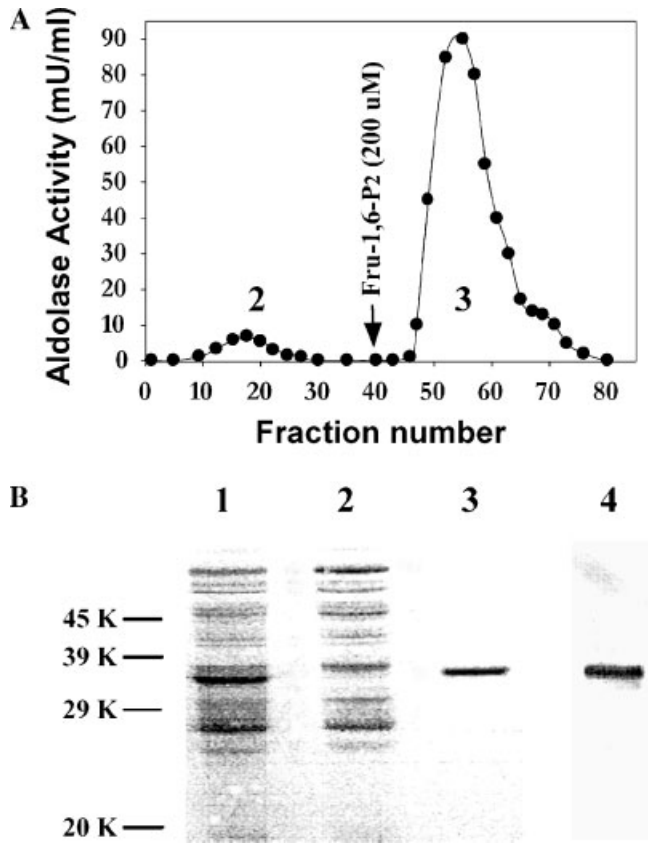

Fig. 8. Affinity chromatography reveal specific interaction between FBPase and aldolase B. **A**: The column containing the Sepharose-4B-FBPase matrix was equilibrated with buffer E. A partially purified extract from kidney (fraction A) was applied to the column and washed with the buffer E (fraction B). The retained aldolase was specifically eluted by addition of 200 μM Fru-1,6-P_2 (fraction C). The fractions collected from the column were assayed for aldolase activity. **B**: The fractions were analyzed by SDS–PAGE and stained with Coomassie blue. Lanes 1, 2, and 3 contain the fractions A, B and C, respectively. Lane 4 shows the Western blot of fraction C using rabbit anti-aldolase B antibodies. Arrows indicate protein standards.

Triton X-100 on FBPase and aldolase activity in the kidney homogenate (LS) support this notion. Interestingly, our observation is at odds with the traditional notion that gluconeogenic enzymes are located in the soluble portion of the cell. This heterogeneous distribution of aldolase and FBPase demonstrates that subcellular organization can represent a different mechanism to regulate the tissue metabolism.

Here, we convincingly demonstrate that aldolase B co-localizes with FBPase and PEPCK in the proximal tubule cells, whereas aldolase A and PK co-localize in the distal tubules and collecting ducts. Interestingly, aldolase A but not PK was clearly localized inside of the nuclei in kidney and muscle cells. Recently, we have demonstrated that FBPase is able to be translocated inside the nuclei of proximal tubule cells (Yañez et al., 2003a), results which are confirmed in the present study. As we have discussed (Yañez et al., 2003a), there are several possible functions for glycolytic and gluconeogenic enzymes in the nuclei. The most attractive idea is the putative role of these enzymes in DNA metabolism and glucose-induced gene regulation.

Differential metabolic functions for particular cells have been established in several organs (Bittar et al., 1996; Jungermann and Kietzmann, 1996; Saez et al., 1996; Saez and Slebe, 2000). Our results in kidney are in line with the concept of "liver metabolic zonation," supporting the view that physical separation of the glycolytic and gluconeogenic enzymes is required to avoid uneconomic substrate cycling in a single cell. Indeed, the synthesis of glucose by the proximal tubule segments of freshly microdissected nephrons and the expression of gluconeogenic enzymes FBPase, PEPCK and glucose-6-phosphatase in the proximal tubule (Guder and Schmidt, 1974; Schmidt et al., 1975; Hume et al., 1994; Saez et al., 1996; Conjard et al., 2001; Yañez et al., 2003b) sustain that this segment is a specialized compartment in which renal glucose production occurs. Moreover, the results obtained with PK are consistent with the distribution pattern of hexokinase and phosphofructokinase and indicate a specialized glycolytic function for the distal tubules (Schmidt et al., 1975; Vandewalle et al., 1981). That the nephron tubules have different functions correlates very well with the localization data of the aldolase isoenzymes and points to the participation of aldolase B in renal gluconeogenesis and glyconeogenesis and aldolase A in renal glycolysis. In this regard, a specific role has been reported for the glycerol-3-phosphate dehydrogenase (GPDH) isoenzymes in transgenic *Drosophila* (Luby-Phelps, 1994). This author has demonstrated that GPDH-1 and not GPDH-3 is critical for sequential binding and localization of aldolase and glyceraldehyde-3-phosphate dehydrogenase along the sarcomere. The presence of active

GPDH-3 in the cell is insufficient for muscle function because the formation of a proper isoenzyme complex is required to supply ATP to the myosin ATPase, for muscle contraction.

It has been established (Gerich et al., 2001) that renal glucose synthesis is particularly important in diabetes or in various conditions in which glucose homeostasis is disturbed. Treatment of type 2 diabetes has greatly improved due to the availability of new classes of oral antidiabetic drugs. Recently, glucose-6-phosphatase, FBPase, and PEPCK have become new targets for antidiabetic drugs (Kurukulasuriya et al., 2003), but bi-directional gluconeogenic enzymes have not been taken into account. Therefore, the specific participation of aldolase B in renal gluconeogenesis leads us to propose this isoenzyme as a new candidate for anti-hyperglycemic drugs.

Microdissection studies (Conjard et al., 2001) have established that the three segments of the proximal tubules (S_1, S_2, and S_3) can synthesize glucose at different rates from lactate. The S_2 and S_3 segments produce more glucose than the S_1 segment. The authors suggest that kidney nephrons have a heterogeneous expression of gluconeogenic enzymes at the proximal tubules. However, our data show that the expression levels of FBPase, aldolase B, and PEPCK, measured by immunohistochemistry, are similar along the proximal tubules. Thus, although we can conclude that the lower production rate from lactate, observed in the segment S_1 compared with the segment S_3, may not to be due to the influence of these enzymes, the involvement of other proteins cannot be ruled out. In humans, we have found that the expression level of MCT1 in the S_1 segment might be the reason for the difference in the glucose synthesis rate (Yañez et al., 2003b). In the present study, using MCT1 as a basolateral marker, we have found the same differential expression and localization of this transporter in rat kidney tubules. Interestingly, we also found that FBPase, but not aldolase B or PEPCK, exhibited different subcellular localizations along the rat proximal tubules. These results correlate with the effect of Triton X-100 and indicate that FBPase may play an important function in the metabolic heterogeneity observed in the proximal tubules.

This specific enzyme distribution pattern in the nephron, where FBPase colocalizes with aldolase B but not with aldolase A, led us to study the possible interaction between FBPase and aldolase B in kidney cells. Previously it has been demonstrated that sequential enzymes that operate within a metabolic pathway interact with each other to form highly organized complexes (Srere and Ovadi, 1990; Ovadi and Srere, 1996). However, there are methodological problems in demonstrating the existence of such complexes in vitro. The immobilization of proteins on agarose gels has been shown to be a successful method for the demonstration of the formation of complexes between weakly interacting enzymes (Ovadi and Srere, 2000). The results of the present study clearly demonstrate the existence of physical interactions between two sequential enzymes involved in renal gluconeogenesis. This interaction is disrupted by 60 μM Fru-1,6-P_2, probably due to the binding of the negatively charged substrate and/or to a conformational change elicited by the substrate. Such effects have been described for the interaction of several muscle enzymes with immobilized phosphofructokinase (Gerlach and Hofer, 1986). On the other hand, an increase of the ionic strength considerably reduces the interaction between FBPase and aldolase B, indicating that electrostatic forces are involved in the recognition process. A remarkable result was the finding that only aldolase B was retained on the FBPase-Sepharose column when a kidney extract fraction, containing many proteins, including aldolases A and B, was applied. These results provide compelling evidence for the existence of a specific protein–protein interaction between aldolase B and FBPase which is modulated by the substrate. It is clear that this specific association indicates a high degree of metabolic organization which can ensure the channeling of the substrate, preventing the loss of intermediates by diffusion, a mechanism which has been demonstrated in several systems (Orosz and Ovadi, 1987; Spivey and Ovadi, 1999; Ovadi and Srere, 2000). If the active sites of the enzymes are in close vicinity within the FBPase–aldolase B complex, Fru-1,6-P_2 could be transferred between these active sites without escaping into the bulk phase. These results affirm that in kidney aldolase A is uniquely involved in a glycolytic complex, while, aldolase B specifically participates along the gluconeogenic complex which is involved in the production of glucose.

Our results contribute to explain how the kidney handles intermediates of competing metabolic pathways. We conclude that the characteristics of the two aldolase isoenzymes, A and B, and the relationships that they establish are tailored to suite specific metabolic roles of the tissues/cells in which they are expressed.

ACKNOWLEDGMENTS

We are thankful to Dr. Alejandro Claude from Universidad Austral de Chile and Dr. Valerie Smith from Gatty Marine Laboratory, University of St. Andrews, Scotland for their critical reading of the manuscript. We thank Dr. Daryl Granner from the Vanderbilt University, Nashville, TN for providing us the sheep anti-rat PEPCK serum and Dr. Felipe Barros from Centro de Estudios Científicos, Valdivia, for the access to the confocal microscope granted by Fundación Andes to CECS.

LITERATURE CITED

Akita EM, Nakai S. 1993. Production and purification of Fab' fragments from chicken egg yolk immunoglobulin Y (IgY). J Immunol Meth 162:155–164.

Alarcón O, Gonzalez F, Flores H, Marcus F. 1971. Isolation of crystalline pig kidney aldolase B. Biochim Biophys Acta 227:460–463.

Beale EG, Katzen CS, Granner DK. 1981. Regulation of rat liver phosphoenolpyruvate carboxykinase (GTP) messenger ribonucleic acid activity by N6, O2′-dibutyryladenosine 3′,5′-phosphate. Biochemistry 20:4878–4883.

Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ. 1996. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J Cereb Blood Flow Metab 16:1079–1089.

Cárcamo JG, Yañez AJ, Ludwig HC, Leon O, Pinto RO, Reyes AM, Slebe JC. 2000. The C1-C2 interface residue lysine 50 of pig kidney fructose-1, 6-bisphosphatase has a crucial role in the cooperative signal transmission of the AMP inhibition. Eur J Biochem 267:2242–2251.

Cersosimo E, Garlick P, Ferretti J. 2000. Renal substrate metabolism and gluconeogenesis during hypoglycemia in humans. Diabetes 49:1186–1193.

Cersosimo E, Garlick P, Ferretti J. 2001. Abnormal glucose handling by the kidney in response to hypoglycemia in type 1 diabetes. Diabetes 50:2087–2093.

Cloix JF, Beaulieu E, Hevor T. 1997. Various fructose-1,6-bisphosphatase mRNAs in mouse brain, liver, kidney, and heart. Neuroreport 8:617–622.

Colombo G, Hubert E, Marcus F. 1972. Selective alteration of the regulatory properties of fructose 1,6-diphosphatase by modification with pyridoxal 5′-phosphate. Biochemistry 11:1798–1803.

Conjard A, Martin M, Guitton J, Baverel G, Ferrier B. 2001. Gluconeogenesis from glutamine and lactate in the isolated human renal proximal tubule: Longitudinal heterogeneity and lack of response to adrenaline. Biochem J 360: 371–377.

Conjard A, Brun V, Martin M, Baverel G, Ferrier B. 2002. Effect of starvation on glutamine ammoniagenesis and gluconeogenesis in isolated mouse kidney tubules. Biochem J 368:301–308.

Durrieu C, Bernier-Valentin F, Rousset B. 1987. Microtubules bind glyceraldehyde 3-phosphate dehydrogenase and modulate its enzyme activity and quaternary structure. Arch Biochem Biophys 252:32–40.

Eagles PA, Iqbal M. 1973. A comparative study of aldolase from human muscle and liver. Biochem J 133:429–439.

Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J. 1999. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48:292–298.

Gerich JE, Meyer C, Woerle HJ, Stumvoll M. 2001. Renal gluconeogenesis: Its importance in human glucose homeostasis. Diabetes Care 24:382–391.
Gerlach G, Hofer HW. 1986. Interaction of immobilized phosphofructokinase with soluble muscle proteins. Biochim Biophys Acta 881:398–404.
Guder WG, Schmidt U. 1974. The localization of gluconeogenesis in rat nephron. Determination of phosphoenolpyruvate carboxykinase in microdissected tubules. Hoppe Seylers Z Physiol Chem 355:273–278.
Hume R, Bell JE, Hallas A, Burchell A. 1994. Immunohistochemical localisation of glucose-6-phosphatase in developing human kidney. Histochemistry 101: 413–417.
Jungermann K, Kietzmann T. 1996. Zonation of parenchymal and nonparenchymal metabolism in liver. Annu Rev Nutr 16:179–203.
Katz N, Teutsch HF, Jungermann K, Sasse D. 1977. Heterogeneous reciprocal localization of fructose-1,6-bisphosphatase and of glucokinase in microdissected periportal and perivenous rat liver tissue. FEBS Lett 83:272–276.
Knull HR, Walsh JL. 1992. Association of glycolytic enzymes with the cytoskeleton. Curr Top Cell Regul 33:15–30.
Kurukulasuriya R, Link JT, Madar DJ, Pei Z, Richards SJ, Rohde JJ, Souers AJ, Szczepankiewicz BG. 2003. Potential drug targets and progress towards pharmacologic inhibition of hepatic glucose production. Curr Med Chem 10: 123–153.
Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.
Lebherz HG, Rutter WJ. 1969. Distribution of fructose diphosphate aldolase variants in biological systems. Biochemistry 8:109–121.
Lehotzky A, Palfia Z, Kovacs J, Molnar A, Ovadi J. 1994. Ligand-modulated cross-bridging of microtubules by phosphofructokinase. Biochem Biophys Res Commun 204:585–591.
Leon O, Schulman LO. 1987. Covalent coupling of the variable loop of the elongator methionine tRNA to a specific lysine residue in *Escherichia coli* methionyl-tRNA synthetase. Biochemistry 26:1933–1940.
Luby-Phelps K. 1994. Physical properties of cytoplasm. Curr Opin Cell Biol 6: 3–9.
Ludwig HC, Herrera R, Reyes AM, Hubert E, Slebe JC. 1999. Suppression of kinetic AMP cooperativity of fructose-1,6-bisphosphatase by carbamoylation of lysine 50. J Protein Chem 18:533–545.
MacGregor JS, Singh VN, Davoust S, Melloni E, Pontremoli S, Horecker BL. 1980. Evidence for formation of a rabbit liver aldolase—Rabbit liver fructose-1,6-bisphosphatase complex. Proc Natl Acad Sci USA 77:3889–3892.
Majumder AL, Eisenberg F Jr. 1977. Unequivocal demonstration of fructose-1,6-bisphosphatase in mammalian brain. Proc Natl Acad Sci USA 74:3222–3225.
Marcus F, Hubert E. 1968. Functional consequences of modification of kidney fructose 1,6-diphosphatase by pyridoxal 5′-phosphate. J Biol Chem 243:4923–4926.
Moorhead GB, Hodgson RJ, Plaxton WC. 1994. Copurification of cytosolic fructose-1,6-bisphosphatase and cytosolic aldolase from endosperm of germinating castor oil seeds. Arch Biochem Biophys 312:326–335.
Nordlie RC, Foster JD, Lange AJ. 1999. Regulation of glucose production by the liver. Annu Rev Nutr 19:379–406.
Orosz F, Ovadi J. 1987. A simple approach to identify the mechanism of intermediate transfer: Enzyme system related to triose phosphate metabolism. Biochim Biophys Acta 915:53–59.
Ovadi J. 1991. Physiological significance of metabolic channelling. J Theor Biol 152:1–22.
Ovadi J, Srere PA. 1996. Metabolic consequences of enzyme interactions. Cell Biochem Funct 14:249–258.
Ovadi J, Srere PA. 2000. Macromolecular compartmentation and channeling. Int Rev Cytol 192:255–280.
Penhoet EE, Rutter WJ. 1971. Catalytic and immunochemical properties of homomeric and heteromeric combinations of aldolase subunits. J Biol Chem 246:318–323.
Penhoet EE, Rutter WJ. 1975. Detection and isolation of mammalian fructose-diphosphate aldolases. Meth Enzymol 42:240–249.
Penhoet EE, Kochman M, Rutter WJ. 1969. Iosl ation of fructose diphosphate aldolases A, B, and C. Biochemistry 8:4391–4395.
Pilkis SJ, Granner DK. 1992. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annu Rev Physiol 54:885–909.
Rakus D, Dzugaj A. 2000. Muscle aldolase decreases muscle FBPase sensitivity toward AMP inhibition. Biochem Biophys Res Commun 275:611–616.
Rakus D, Mamczur P, Gizak A, Dus D, Dzugaj A. 2003a. Colocalization of muscle FBPase and muscle aldolase on both sides of the Z-line. Biochem Biophys Res Commun 311:294–299.
Rakus D, Pasek M, Krotkiewski H, Dzugaj A. 2003b. Muscle FBPase in a complex with muscle aldolase is insensitive to AMP inhibition. FEBS Lett 547: 11–14.
Reyes A, Burgos ME, Hubert E, Slebe JC. 1987. Selective thiol group modification renders fructose-1,6-bisphosphatase insensitive to fructose 2,6-bisphosphate inhibition. J Biol Chem 262:8451–8454.
Rottmann WH, Tolan DR, Penhoet EE. 1984. Complete amino acid sequence for human aldolase B derived from cDNA and genomic clones. Proc Natl Acad Sci USA 81:2738–2742.
Saez DE, Slebe JC. 2000. Subcellular localization of aldolase B. J Cell Biochem 78:62–72.
Saez DE, Figueroa CD, Concha II, Slebe JC. 1996. Localization of the fructose 1,6-bisphosphatase at the nuclear periphery. J Cell Biochem 63:453–462.
Schmid H, Scholz M, Mall A, Schmidt U, Guder WG, Dubach UC. 1977. Carbohydrate metabolism in rat kidney: Heterogeneous distribution of glycolytic and gluconeogenic key enzymes. Curr Probl Clin Biochem 8:282–289.
Schmidt U, Dubach UC, Guder WG, Funk B, Paris K. 1975. Metabolic patterns in various structures of the rat nephron. The distribution of enzymes of carbohydrate metabolism. Curr Probl Clin Biochem 4:22–32.
Shearwin K, Nanhua C, Masters C. 1990. Interactions between glycolytic enzymes and cytoskeletal structure—The influence of ionic strength and molecular crowding. Biochem Int 21:53–60.
Spivey HO, Ovadi J. 1999. Substrate channeling. Methods 19:306–321.
Srere PA, Ovadi J. 1990. Enzyme–enzyme interactions and their metabolic role. FEBS Lett 268:360–364.
Stumvoll M, Meyer C, Mitrakou A, Nadkarni V, Gerich JE. 1997. Renal glucose production and utilization: New aspects in humans. Diabetologia 40:749–757.
Tejwani GA. 1983. Regulation of fructose-bisphosphatase activity. Adv Enzymol Relat Areas Mol Biol 54:121–194.
Vandewalle A, Wirthensohn G, Heidrich HG, Guder WG. 1981. Distribution of hexokinase and phosphoenolpyruvate carboxykinase along the rabbit nephron. Am J Physiol 240:F492–F500.
Wojtas K, Slepecky N, von Kalm L, Sullivan D. 1997. Flight muscle function in *Drosophila* requires colocalization of glycolytic enzymes. Mol Biol Cell 8:1665–1675.
Yañez AJ, Bertinat R, Concha II, Slebe JC. 2003a. Nuclear localization of liver FBPase isoenzyme in kidney and liver. FEBS Lett 550:35–40.
Yañez AJ, Nualart F, Droppelmann C, Bertinat R, Brito M, Concha II, Slebe JC. 2003b. Broad expression of fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase provide evidence for gluconeogenesis in human tissues other than liver and kidney. J Cell Physiol 197:189–197.
Zimmer DB, Magnuson MA. 1990. Immunohistochemical localization of phosphoenolpyruvate carboxykinase in adult and developing mouse tissues. J Histochem Cytochem 38:171–178.