Journal of Cellular Biochemistry 78:62–72 (2000)

Subcellular Localization of Aldolase B

Doris E. Sáez and Juan C. Slebe*

Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

Abstract The localization of the aldolase B isozyme was determined immunohistochemically in rat kidney and liver using a polyclonal antibody. Aldolase B was preferentially localized in a nuclear region of hepatocytes from the periportal region and was absent in those from the perivenous region. Aldolase B was also preferentially localized in the proximal tubules and was absent in other structures of the renal cortex as well as in the renal medulla. Using reflection confocal microscopy, the enzyme was preferentially localized in a nuclear position in liver and renal cells, which was similar to the cellular and intracellular location found for the gluconeogenic enzyme fructose-1,6-bisphosphatase (Sáez et al. [1996] J. Cell. Biochem. 63:453–462). Subcellular fractionation studies followed by enzyme activity assays revealed that aldolase activity was associated with subcellular particulate structures. Overall, the data suggest that different aldolase isoenzymes are needed in the glycolytic and gluconeogenic pathways. J. Cell. Biochem. 78:62–72, 2000.

Key words: fructose 1,6-bisphosphate aldolase; gluconeogenesis; immunolocalization; nuclear association; confocal microscopy

Fructose-1,6-bisphosphate aldolase (aldolase; fructose 1,6-bisphosphate D-glyceraldehyde 3-phosphate lyase; EC 4.1.2.13), a key enzyme of the gluconeogenic and glycolytic pathways, catalyzes the reversible conversion of fructose-1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxy-acetone phosphate [Morse and Horecker, 1968]. In mammalian tissues there are three aldolase isozymes, commonly referred as A, B, and C [Rajkumar et al., 1966; Lebherz and Rutter, 1969; Penhoet and Rutter, 1975]. The A and C forms of the enzyme (found in muscle and brain, respectively) have a markedly higher catalytic preference for fructose 1,6-bisphosphate over fructose 1-phosphate, whereas the B form of the enzyme, found in liver and kidney, shows no particular preference for either substrate [Eagles and Iqbal, 1973]. The aldolase isozymes are tetramers composed of identical subunits of molecular weight around 39,500. Each subunit has an active center lysine residue that forms a covalent Schiff base intermediate during the catalytic cycle [Littlechild and Watson, 1993]. The regulation of the transcription of the aldolase gene has been studied in liver [Gregori et al, 1993., Ito et al, 1998], kidney [Vallet et al., 1995], and tumor cells [Schaffer et al., 1997].

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 enzymes do not exist in a soluble form only; they also may be reversibly associated with cellular structures [Masters et al., 1987; Srere, 1987]. Moreover, glycolytic enzymes are supposed form clusters that allow channeling of substrate [Stephan et al., 1986; Neuzil et al., 1990; Tompa and Batke, 1990; Ovadi, 1995]. Interestingly, we have found that the gluconoegenic enzyme FBPase is located at the nuclear periphery in rat liver and kidney cells [Sáez et al., 1996]. Since aldolase and FBPase catalyzes sequential reactions, it seems reasonable to consider the idea

Abbreviations: FBPase, fructose-1,6-bisphosphatase; fru-1,6-P_2, fructose-1,6-bisphosphate; aldolase, fructose-1,6-bisphosphate aldolase; EDTA, ethylene diamine tetraacetic acid; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin; PAP, peroxidase-anti-peroxidase.

Grant sponsor: FONDECYT; Grant numbers: 1951215, 1981001, 2930044; Grant sponsor: Dirección de Investigación, Universidad Austral de Chile; Grant number: S-95-41.

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

Received 3 September 1999; Accepted 28 December 1999

This article published online in Wiley InterScience, April 2000.

that they form part of a multienzyme complex. Such "enzyme clusters" could provide a suitable device in the control of metabolic crossroads by increasing the efficiency of the sequential catalytic steps throughout the decrease of transit time for the passage of metabolites between the enzymes. We recently demonstrated by using equilibrium chromatography that specific interactions between FBPase and aldolase B from pig kidney occur, and also that these interactions are modulated by the substrate [Sáez and Slebe, 1999, unpublished data]. The results support the notion that both enzymes would have a similar intracellular localization. Since there are no data on the intracellular localization of aldolase B in liver and kidney, it was important to determine the localization of this enzyme.

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 renal cortex heterogeneity, with gluconeogenesis being located in the proximal and glycolysis in the distal tubules [Schmidt et al., 1975]. We observed [Sáez et al., 1996] that FBPase was preferentially localized in hepatocytes of the periportal region of the liver and was absent from the perivenous region. The enzyme was also preferentially localized in the cortex of the kidney proximal tubules.

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

MATERIALS AND METHODS

Antibody Production

A rabbit antiserum against purified pig kidney aldolase B [Alarcón et al., 1971] was prepared. White rabbits received two subcutaneous injections at 1-week intervals of an emulsified mixture of purified aldolase B (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 aldolase B 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 Laemli [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-aldolase B serum (1:100,000), and the antibody-antigen interactions were detected using peroxidase coupled to goat anti-rabbit IgG. The use of higher concentrations of antibody (1:50,000) improved the strength of the signal without increasing the nonspecific staining in immunodots (data not shown).

Tissue Immunocytochemistry

Renal and hepatic tissue obtained from adult Holtzman rats (body weight, 300–350 g) was 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; Sáez et al., 1996]. 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-HCl, 0.15 M NaCl, pH 7.8, each. Anti-aldolase B antibodies were used. Dilution 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, Denmark) at a dilution of 1:80 and the PAP complex (Dako) at 1:100 were applied for 30 min each. Peroxidase activity was visualized by incubating the sections in 0.1% (w/v) 0f 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 nonimmune rabbit serum at the same dilution. Alternatively, the first antibody was preabsorbed overnight at 4°C with an excess of the same aldolase B used for immunizations, and then centrifuged at 10,000*g* to remove the immune precipitates. In further controls, we used an antibody prepared against pig kidney FBPase [Sáez et al., 1996] and 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 argon ion laser (488 nm) illumination at the Centro de Equipo Mayor (CEM) of the Facultad the 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 was 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].

Aldolase B activity was assayed spectrofotometrically at 30°C by following the rate of NADH consumption at 340 nm in the presence of an excess of both gliceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase [Rajkumar et al, 1966; Alarcón et al, 1971]. The assay system of 0.5 ml contained 35 mM Tris-HCl buffer, pH 7.5, 0.1 mM EDTA, 3.3 mM 2-mercaptoethanol, 0.6 mM fructose 1,6-bisphosphate, 0.1 mM NADH, 1.5 μg of aldolase, 8.7 U of triose phosphate isomerase, and 3 U of gliceraldehyde-3-phosphate dehydrogenase, and Triton X-100 as indicated in Table I. The reaction was initiated by the addition of aldolase. One unit of enzyme activity is defined as the amount of aldolase B that catalyzed the formation of 1 μmol of NAD^+ per min under the conditions described. The concentration of aldolase B was determined by absorbance at 280 nm using a $\epsilon^{1\ mg/ml}$ value of 0.89 [Penhoet et al., 1969].

TABLE I. Subcellular Distribution of Aldolase From Rat Liver and Kidney[a]

	Enzyme activity (U/g tissue)			
	Liver		Kidney	
Fraction	A	B	A	B
Initial homogenate (H)	5.3	2.7	4.4	2.0
Citoplasmic extract (E)	4.5	2.9	3.1	1.95
Nuclear fraction (N)	0.08	0.02	0.66	0.13
Heavy mitochondria (M)	0.25	0.12	0.34	0.07
Light mitochondria (L)	0.25	0.18	0.48	0.20
Microsomal fraction (P)	0.15	0.08	0.19	0.05
High-speed supernatant (S)	3.30	2.80	1.30	1.04

[a]A, aldolase activity in fractions; B, fractions were preincubated with 0.1% Triton X-100 at 20°C, for 20 min. Aldolase activity was assayed in the presence of 0.004% Triton X-100.

RESULTS

Characterization of the Anti-Aldolase B Antibody

The specificity of the anti-aldolase B antibody was assesed by immunobloting. The anti-aldolase B antibody immunostained a single band in rat kidney extracts, with an apparent subunit molecular weight of approximately 39,500 (Fig. 1, lane 3). The immunoreactive band migrated identically to purified aldolase B from pig kidney (Fig. 1, lane 4). Parallel Coomassie blue staining revealed the presence of more than 40 different protein bands in the rat kidney sample (Fig. 1, lane 1), indicating the elevated specificity of the anti-aldolase B antibody. Only one protein band was in the lane containing the purified aldolase B from pig kidney (Fig. 1, lane 2), indicating the high degree of purity of the sample utilized to elicit the anti-aldolase B antibody. Confirming the specificity of the anti-aldolase B antibody, no

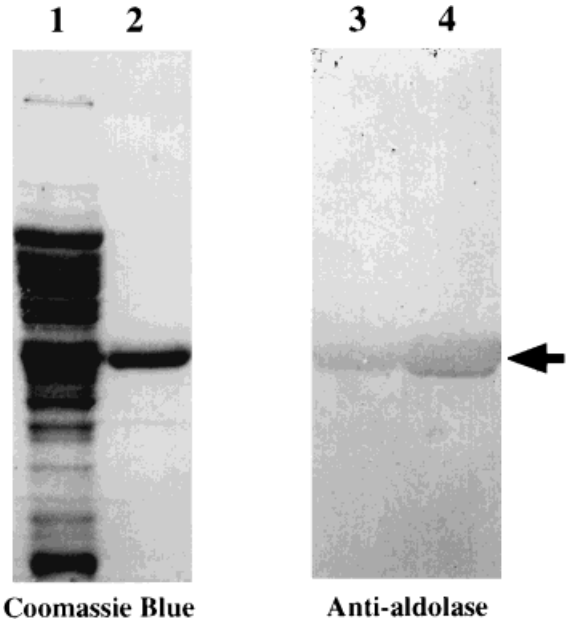

Fig. 1. Characterization of the anti-aldolase B-antibody. **Left:** Coomassie blue-stained acrylamide gel after SDS-PAGE. **Lane 1,** 40 μg of a partially purified preparation of aldolase. **Lane 2,** 2.5 μg of pure adolase. **Right:** Samples were fractionated by SDS-PAGE, transferred to nitrocellulose membranes, and reacted with the anti-aldolase antibody followed by a peroxidase-labeled secondary antibody as described in Materials and Methods. **Lane 3,** partially purified sample of aldolase; **lane 4,** pure aldolase.

reactivity was observed when the blots were incubated with a preabsorbed antibody or preimmune serum (data not shown).

Immunolocalization of Aldolase B in Rat Kidney and Liver Cells

Rat kidney sections were highly immunoreactive with the anti-aldolase B antibody (Fig. 2A,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 the renal medulla (data not shown). In addition to the immunoreactive material associated with the cytoplasm and the plasma membrane, aldolase B immunoreactive cells showed a clear staining associated with the entire nucleus (Fig. 2A,B). Prominent nuclear staining was also seen in hepatocytes from periportal regions (Fig. 2D,E), although immunoreactive material was also present in the cytoplasm and associated with the cell membrane. No immunoreactive 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-aldolase B 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 aldolase, in rat kidney and liver cells. As a control for a nuclear localization of aldolase, sections from rat liver were reacted with an antibody for the glucose transporter GLUT 2, which is specifically expressed in liver [De Vos 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 GLUT 2.

Nuclear Localization of Aldolase B in Rat Kidney and Liver Cells

The immunostaining pattern with the anti-aldolase B antibody seen with conventional light microscopy (Fig. 2) suggested the association of aldolase B with the nuclei of rat liver and kidney cells. Further analysis using confocal microscopy clearly demonstrated a nuclear localization of aldolase B in both liver and kidney cells (Figs. 3–5). A composite figure, obtained by superimposing a confocal microscope image (viewed in reflection contrast mode, green) on the conventional light microscopy image, showed the nuclear staining with the anti-aldolase B antibody in liver cells of the periportal region (Fig. 3B). When the same field was viewed only in the reflection contrast mode (Fig. 3A) and a set of confocal images was taken at 0.25-μm intervals, the antibody staining was confined to the perinuclear region (Fig. 4). It must be noted that although aldolase showed perinuclear localization, there also was nuclear staining. When kidney samples were analyzed by using the same technique (Figs. 3C,D, 5), an association of aldolase B with the nuclei was obtained; nevertheless, some perinuclear staining also was revealed.

Subcellular Distribution of Aldolase

The results of rat liver and kidney fractionation are shown in Table I. In two experiments, 1–1.2 g of tissue were fractionated by differential centrifugation as described in Materials

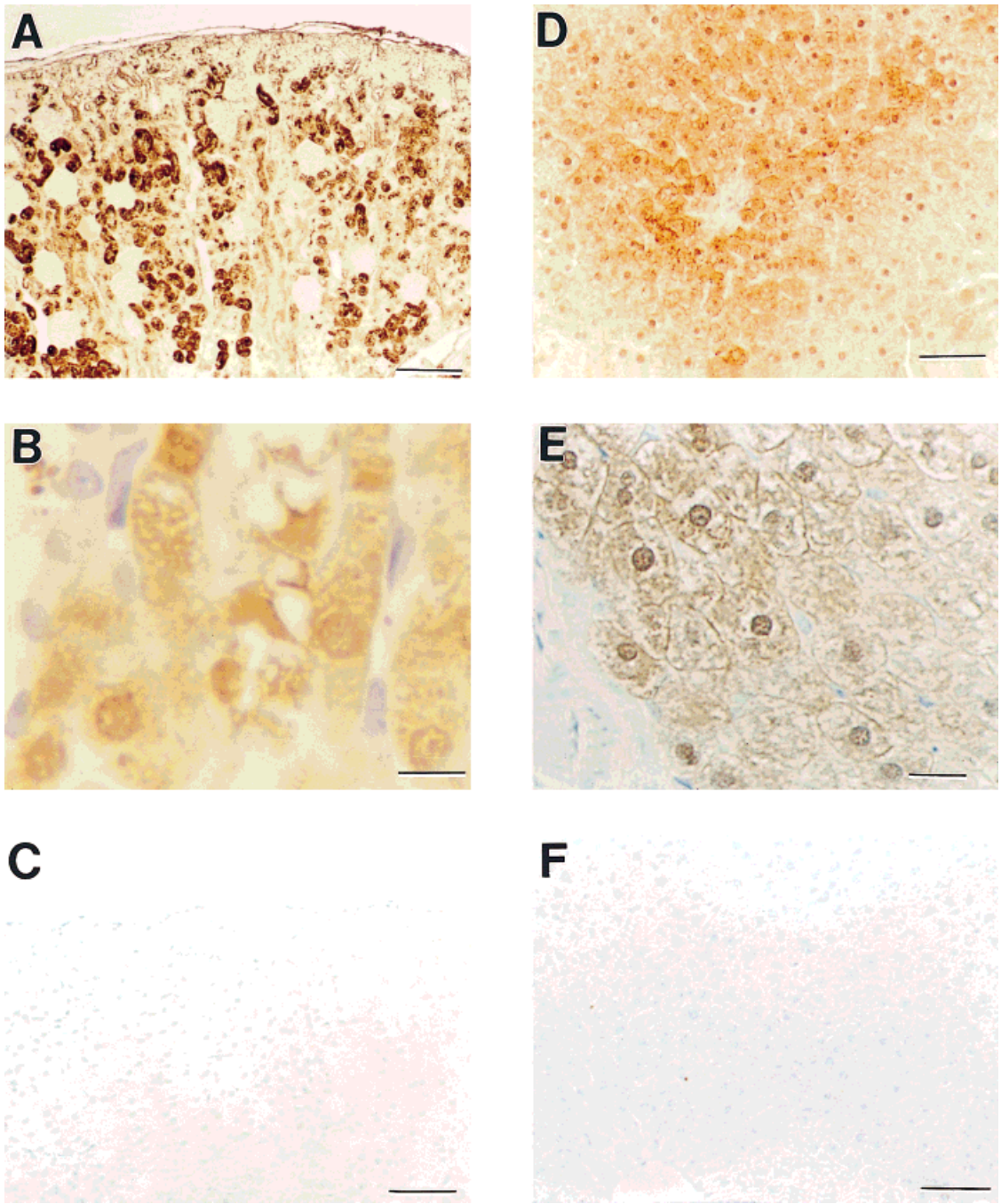

Fig. 2. Localization of aldolase B in rat kidney and liver by conventional light microscopy. **A:** Nuclear staining of kidney proximal tubule cells reacted with the anti-aldolase B antibody. Bar, 60 μm. **B:** Higher magnification of a kidney sample, showing the nuclear staining with the anti-aldolase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

and Methods, and the subcellular compartments were identified by their corresponding marker enzymes. In both tissues, the aldolase activity was clearly present in all the fractions (Table I). The data of the aldolase activity indicate that no activity was apparently lost during the fractionation procedure. Unexpectedly, when the different fractions were treated with

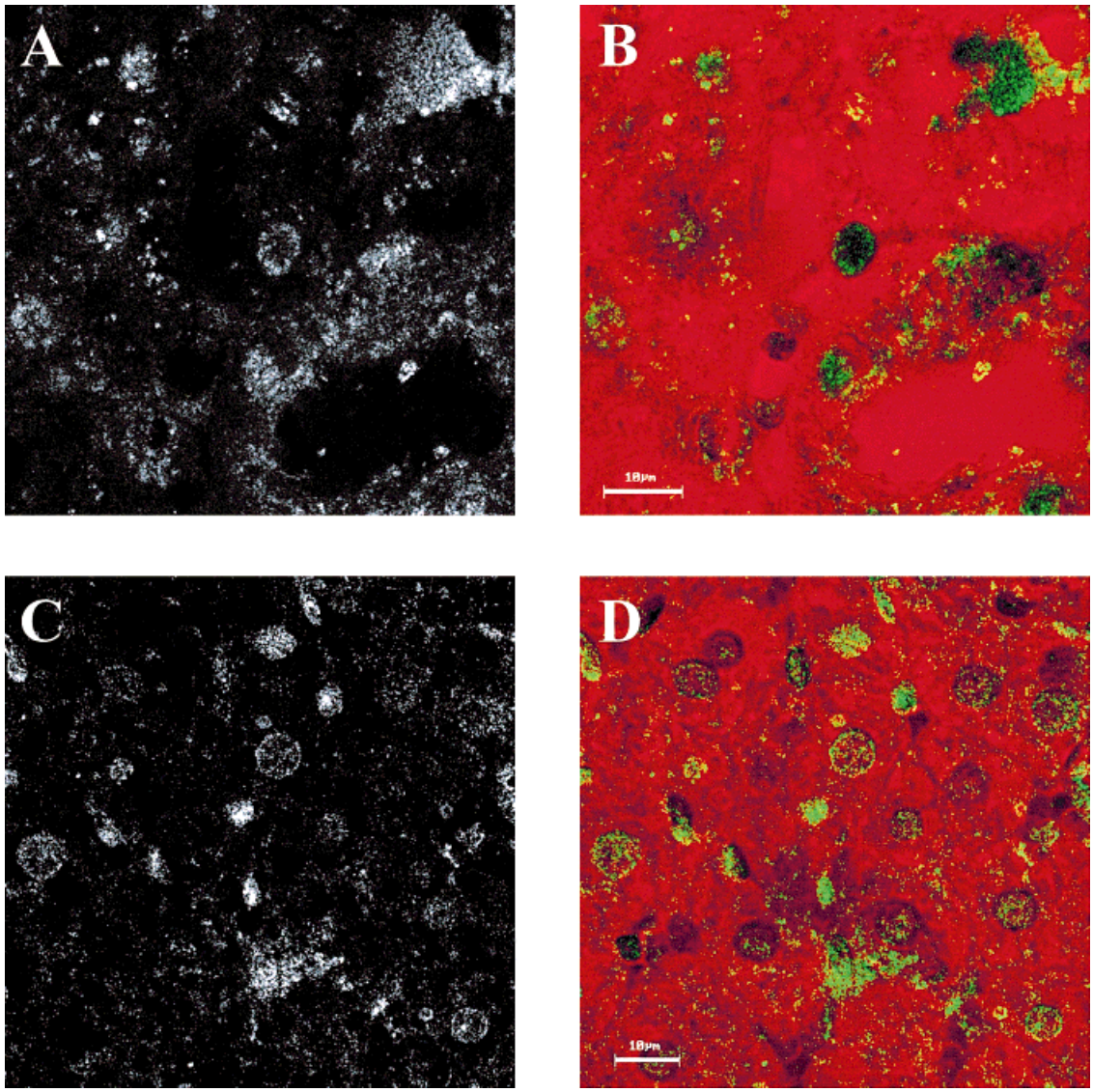

Fig. 3. Localization of aldolase B in liver and kidney cells by confocal microscopy. **A and C:** Nuclear staining for aldolase B in reflection contrast mode in proximal tubules (A) or in hepatocytes (C) from the periportal region. Bar, 10 μm. **B and D:** Same field as in A and C, viewed in a composite figure obtained by superimposing a reflection contrast image (green) on the conventional light microscopy image (red), showing nuclear staining for aldolase B.

Triton X-100 prior to measuring aldolase activity, we observed a clear decrease in the amount of aldolase activity present in the fractions from both liver and kidney. On the other hand, Triton X-100 did not have the same effect on the amount of aldolase activity detected in the high-speed supernatant. In liver and kidney cells, there was approximately a 2.0–4.0-fold decrease in the amount of the aldolase activity in the fractions. Control experiments indicated that the aldolase 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 aldolase activity present in the high-speed supernatant. Overall, the data are compatible with an activation of aldolase when the enzyme is associated with particulate elements.

DISCUSSION

The most striking observation of this study is that the glycolytic and gluconeogenic enzyme aldolase B has perinuclear and nuclear localization in rat liver and kidney cells. These finding were substantiated by immunocytochemistry at light microscopy and were confirmed

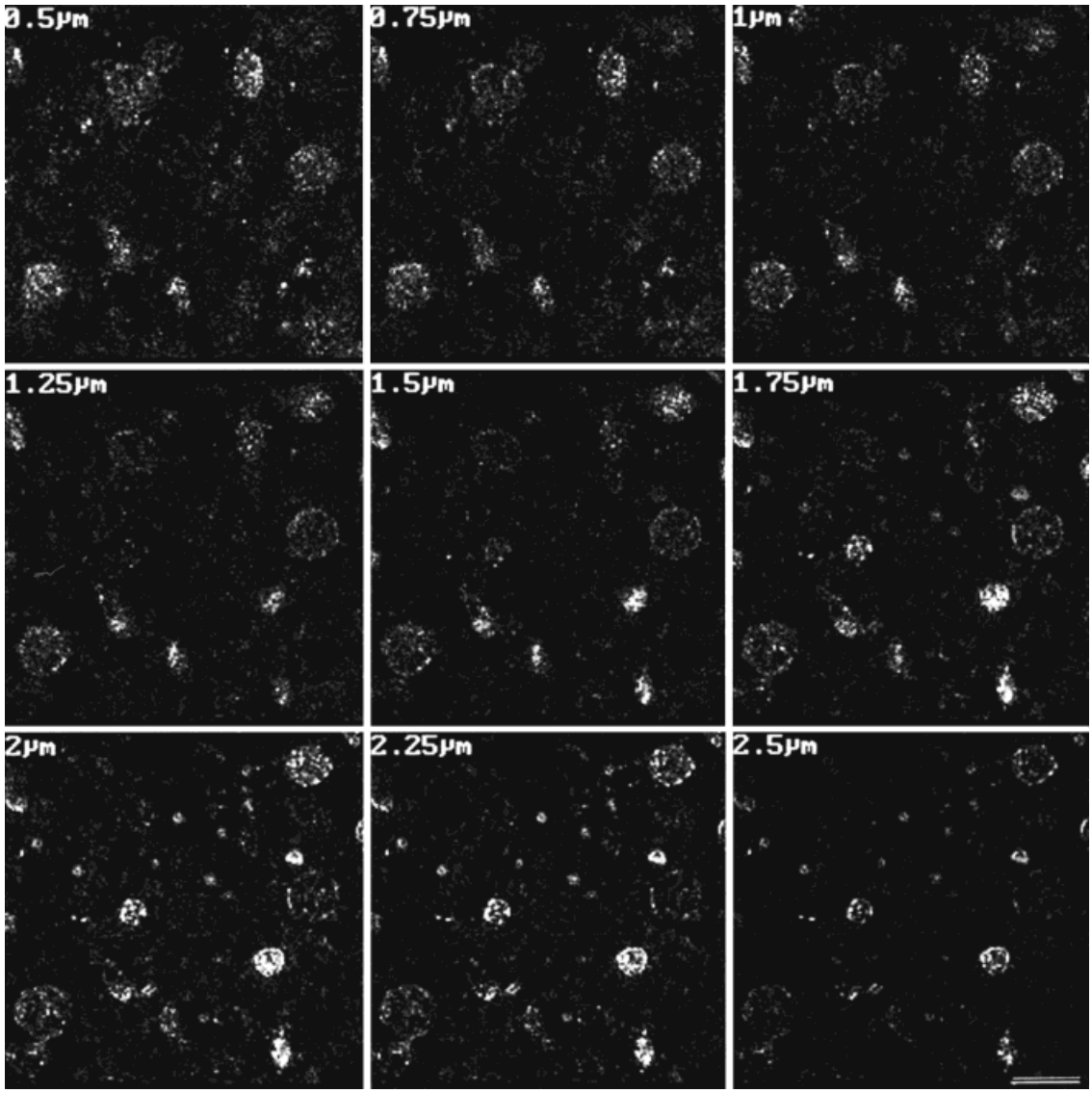

Fig. 4. Localization of aldolase B at the nuclear periphery in liver cells by confocal microscopy: Series of confocal images, at 0.25-μm intervals, through the nuclei from Figure 3, bottom row. Bar, 10 μm.

with the use of reflection confocal microscopy. The localization of aldolase, which is similar to that found for FBPase [Sáez et al., 1996], contrasts with the traditional notion that these enzymes, as well as the majority of the glycolytic enzymes, are located in the soluble portion in 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.

It has been shown that certain proteins, like gliceraldehyde-3-phosphate dehydrogenase and others [Singh and Green, 1993] which can be found in the nucleus and in the cytoplasm of the cell, are involved in other cellular activities unrelated to their primary function. In fact, there are examples of proteins whose combined functions are believed to link the energy metabolism to certain activities related to the regulation of gene expression [Singh and Green, 1993]. Interestingly, it has been reported that human muscle glycogen synthase may possess some additional functions not yet described [Ferrer et al., 1997], which involve its shuttling between the nucleus and the cytoplasm. Conversely, the accumulation of rat liver glucokinase in the nucleus, and its translocation to cytoplasm in response to glucose, may only con-

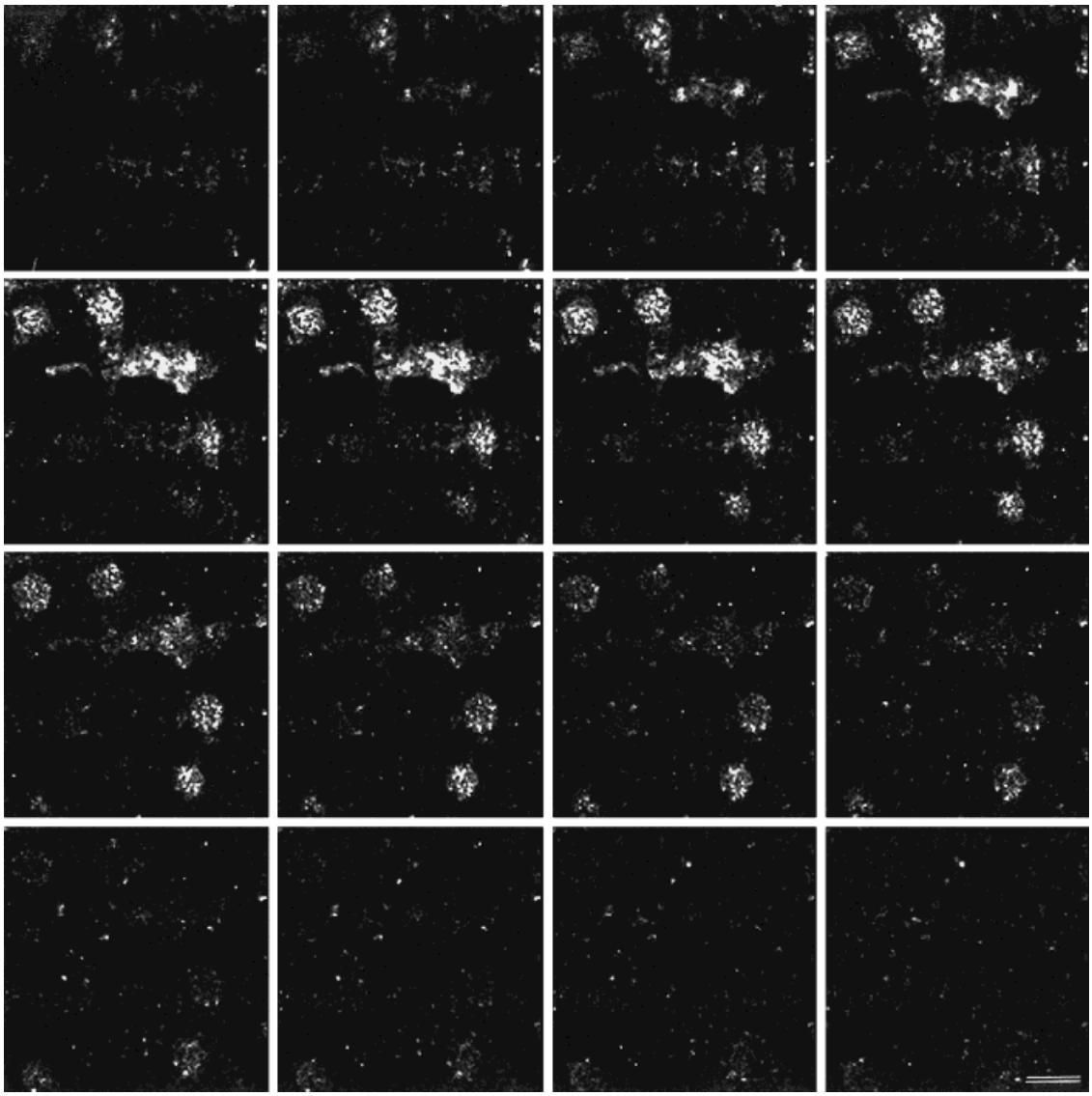

Fig. 5. Localization of aldolase B at the nuclear periphery in kidney cells by confocal microscopy. Series of confocal images, at 0.25-μm intervals, through the nuclei from Figure 3, top row. Bar, 10 μm.

stitute an additional regulatory effect of its activity [De la Iglesia et al., 1999]. Whether aldolase B has some additional functions, or its subcellular localization constitutes a regulatory mechanism of its activity, cannot be established with the present data. Nevertheless, an extra function to its enzymatic activity appears to be plausible, in view of the data which suggest that aldolase plays a critical role in the dynamic association of GLUT4 vesicles with the actin cytoskeleton [Kao et al., 1999], i.e., aldolase functions as a scaffolding protein for GLUT4.

There is evidence that certain enzymes of the glycolytic and gluconeogenic systems may be associated with specific subcellular particulate elements, and that the complexes of sequential metabolic enzymes are often bound to structural elements of the cell [Srere, 1987; Masters et al., 1987; Shearwin et al., 1990]. The glycolytic type III isozyme of hexokinase [Preller and Wilson, 1993] has been found associated with the nuclear periphery. Likewise, there are data indicating that aldolase in fibroblasts [Pagliaro and Taylor, 1988; Minaschek et al., 1992] and

glucose-6-phosphatase in muscle and endothelial cells [Watanabe et al., 1986; Borgers et al., 1971] are associated with structures in the perinuclear region.

There is also evidence for the existence of specific interactions between many "soluble" sequential enzymes of metabolic pathways [Srivastava and Bernhard, 1986; Srere, 1987]. The data indicate that there are few, if any, free enzymes within cells. It is noteworthy that FBPase and aldolase B not only have the same intracellular location, but also that there are specific interactions between both enzymes, which are modulated by the substrate [Sáez and Slebe, 1999, unpublished data]. The formation of a complex between these metabolically sequential enzymes could facilitate channeling of fructose-1,6-bisphosphate and/or alter the kinetic properties of the enzymes and thereby contribute to the gluconeogenic flux.

The immunohistochemical data indicating the perinuclear and nuclear localization of aldolase B in rat liver and kidney cells appear to be at odds with the homogeneization data indicating the presence of aldolase activity in the high-speed supernatant of these cells and a low enzymatic activity in the corresponding nuclear fraction. These observations can be reconciled if we consider the possibility that aldolase is associated with the nuclear structures through weak interactions that are easily broken during disruption of the ordered intracellular structure and environment that results from the homogenization process. 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 with the nuclear periphery with which aldolase is associated, without necessarily destroying the interaction between aldolase and these subcellular structures. Support for this hypothesis is provided by the results of fractionation studies (Table I) that showed that aldolase activity present in the initial homogenate and in the cytoplasmic extract fraction was decreased 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. A possible explanation for this detergent effect is that binding of aldolase to particulate structures generates a more active enzyme conformation state. Overall, our data indicate that the presence of a given enzymatic activity in a high-speed supernatant is not a definitive proof that such an enzyme corresponds to a "soluble enzyme."

The model of "metabolic zonation" proposes that gluconeogenesis and glycolysis are spatially separated in periportal and pericentral (perivenous) hepatocytes [Jungermann and Sasse, 1978; Jungermann and Katz, 1989], and also in 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 [Gumucio, 1989; Ross and Guder, 1982, respectively], and implies that the preferential location of metabolic pathways in separate distinct zones of liver and kidney is of mayor importance for organ function. Nevertheless, some of this data could also be interpreted as representing an artifact which occurred during the experimental procedure employed.

There are no data available on the localization of aldolase B in the liver, and there are only a few studies in the kidney. Electrophoretic analysis of samples dissected from rabbit kidney revealed both A-type and B-type aldolases in the zymogram of the cortex and aldolase A in the medulla [Lebherz and Rutter, 1969]. In addition, detection of enzyme activity in tissue sections of rat kidney have shown aldolase A mainly in the medulla and aldolase B in the cortex and glomeruli [Wachsmuth et al., 1975]. Nevertheless, data favoring particulate localization of these enzymes would make use of immunohistochemical techniques. Our immunohistochemical data, showing that aldolase B is exclusively localized in proximal tubules in the cortex of the kidney and preferentially in hepatocytes of the periportal region of the liver and is absent in those of the perivenous region, are consistent with the concept of metabolic zonation in both organs. The expression of the aldolase B gene in the proximal tubules by using RT/PCR was recently reported [Vallet et al., 1995]. The overall data strengthen the notion that different aldolase isoenzymes participate in glycolysis and gluconeogenesis: probably aldolase B in gluconeogenesis, and aldolase A in glycolysis. Interestingly, the work of Wojtas et al. [1997] confirms the idea that a catalytically competent enzyme content is not sufficient for proper cell function, and that enzyme location and specific enzyme

interactions are also critical for physiological function.

ACKNOWLEDGMENTS

We are grateful to Dr. Ilona I. Concha (Universidad Austral de Chile, Chile) for her interest and discussions during the preparation of the manuscript, and Patricio H. Rodriguez (Centro de Investigación Minera y Metalúrgica, Chile) for his skilled assistance with the processing of the confocal microscopy images.

REFERENCES

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

Borgers M, Scaper J, Schaper W. 1971. Localization of specific phosphatase activities in canine coronary blood vessels and heart muscle. J Histochem Cytochem 19: 526–539.

Bronfmann M, Inestrosa NC, Nervi FO, Leighton F. 1984. Acyl-CoA synthetase and the peroxisomal enzymes of oxidation in human liver. Biochem J 224:709–720.

Brooks SP, Storey KB. 1991. Where is the glycolytic complex? A critical evaluation of present data from muscle tissue. FEBS Lett 278:135–138.

de Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. 1955. Tissue fractionation studies. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem J 60:604–617.

De la Iglesia N, Veiga-da-Cunha M, Van Schaftingen E, Guinovart JJ, Ferrer JC. 1999. Glucokinase regulatory protein is essential for the proper subcellular localisation of liver glucokinase. FEBS Lett 456:332–338.

Deutsch NF. 1967. Preparation of inmunoglobulin concentrates. In: Williams CA, Chase MW, editors. Methods in immunology and immunochemistry, volume 1. New York: Academic Press. p 315–321.

De Vos A, Heimberg H, Quartier E, Huypens P, Bouwens L, Pipeleers D, Schuit F. 1995. Human and rat beta cells differ in glucose transport but not glucokinase gen expression. J Clin Invest 96:2489–2495.

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

Ferrer JC, Baqué S, Guinovart JJ. 1997. Muscle glycogen synthase translocates from the cell nucleus to the cytosol in response to glucose. FEBS Lett 415:249–252.

Figueroa CD, Caorsi C, Vio CP. 1984. Visualization of renal kallikrein in luminal and basolateral membranes: effect of the tissue processing method. J Histochem Cytochem 32:1238–1240.

Figueroa CD, McIver AG, MacKenzie JC, Bhoola KD. 1988. Localization of immunoreactive kininogen and tissue kallikreinin the human nephron Histochemistry 89:437–442.

Gregori C, Kahn A, Pichard AL. 1993. Competition between transcription factors HNF1 and HNF3, and alternative cell-specific activation by DBP and C/EBP contribute to the regulation of the liver-specific aldolase B promoter. Nucleic Acids Res 21:897–903.

Gumucio JJ. 1989. Hepatocyte heterogeneity: the coming of age from the description of a biological curiosity to a partial understanding of its physiological meaning and regulation. Hepatology 9:154–160.

Ito J-U, Kuzumaki T, Otsu K, Iuchi Y, Ishikawa K. 1998. Hormonal regulation of aldolase B gene expression in rat primary cultured hepatocytes. Arch Biochem Biophys 350:291–297.

Jungermann K, Katz N. 1989. Functional specialization of different hepatocyte populations. Physiol Rev 69:708–764.

Jungermann K, Sasse D. 1978. Heterogeneity of liver parenchymal cells. Trends Biochem Sci 3:198–202.

Kao WK, Noda Y, Johnson JH, Pessin JE, Saltiel AR. 1999. Aldolase mediates the association of F-actin with the insulin-responsive glucose transporter GLUT4. J Biol Chem 274:17742–17747.

Laemli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680–685.

Lebherz HG, Rutter WJ. 1969. Distribution of fructose diphosphate aldolase variants in biological sistems. Biochemistry 8:109–121.

Littlechild JA, Watson HC. 1993. A data-based reaction mechanism for type I fructose bisphosphate aldolase. Trends Biochem Sci 18:36–39.

Maretzki D, Reiman B, Rapaport SM. 1989. A reappraisal of the binding of cytosolic enzymes to erythrocyte membranes. Trends Biochem Sci 14:93–96.

Masters CJ, Reid S, Don M. 1987. Glycolysis-new concepts in an old pathway. Mol Cell Biol 76:3–14.

McLean IW, Nakane PK. 1974. Periodate-Lysine-paraformaldehyde fixative: a new fixative for immuno electron microscopy. J Histochem Cytochem 22:1077–1081.

Minaschek G, Groeschel-Stewart U, Blum S, Bereither-Hahn J. 1992. Microcompartmentation of glycolytic enzymes in cultured cells. Eur J Cell Biol 58:418–428.

Morse DE, Horecker BL. 1968. The mechanism of action of aldolases. Adv Enzymol 31:125–181.

Neuzil J, Danielson H, Welch GR, Ovadi J. 1990. Cooperative effect of fructose-bisphosphate and gliceraldehyde-3-phosphate dehydrogenase on aldolase action. Biochim Biophys Acta 1037:307–312.

Ovadi J. 1995. Cell architecture and metabolic channeling. R.G. Landes Co., Austin TX.

Pagliaro L, Taylor DL. 1988. Aldolase exists in both the fluid and solid phases of cytoplasm. J Cell Biol 107:981–991.

Penhoet EE, Rutter WJ. 1975. Detection and isolation of mammalian fructose-diphosphate aldolases. Methods Enzymol 42:240–249.

Penhoet EE, Kochman M, Rutter W. 1969. Isolation of fructose diphosphate aldolases A, B, and C. Biochemistry 8:4391–4395.

Preller A, Wilson JE. 1993. Localization of the type III isozyme of hexokinase at the nuclear periphery. Arch Biochem Biophys 294:482–492.

Rajkumar TV, Woodfin BM, Rutter W. 1966. Aldolase B from (adult) rabbit liver. Methods Enzymol 9:491.

Ross BD, Guder WG. 1982. Heterogeneity and compartmentation in the kidney. In: Sies H, editor. Metabolic compartmentation. New York: Academic Press.

Sáez DE, Slebe JC. 1999. Subcellular localization and association between fructose 116-bisphosphatase and aldolase. FASEB J 13:A1451 (686).

Sáez 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.

Sasse D, Katz N, Jungermann K. 1975. Functional heterogeneity of rat liver parenchyma of isolated hepatocytes. FEBS Lett 57:83–87.

Schaffer D, Hamm-Kunzelmann B, Brand K. 1997. Glucose regulates the promoter activity of aldolase A and pyruvate kinase M_2 via dephosphorilation of SP1. FEBS Lett 417:325–328.

Schmidt U, Marosvari I, Dubach UC. 1975. Renal metabolism of glucose: anatomical sites of hexokinase activity in the rat nephron. FEBS Lett 53:26–28.

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.

Singh R, Green MR. 1993. Sequence-specific binding of transfer RNA by glyceraldehyde-3-phosphate dehydrogenase. Science 259:365–368.

Srere PA. 1987. Complexes of sequential metabolic enzymes. Annu Rev Biochem 56:89–124.

Srivastava DK, Bernhard SA. 1985. Enzyme-enzyme interactions and the regulation of metabolic reaction pathways. Curr Top Cell Regul 28:1–109.

Stephan P, Clarke F, Morton D. 1986. The indirect binding of triose phosphate isomerase to myfibrils to form a glycolytic enzyme minicomplex. Biochem Biophys Acta 873: 127–135.

Sternberg LH, Hardy PH Jr, Cuculis JJ, Meyer HG. 1970. The unlabeled antibody enzyme method of immunochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333.

Tompa P, Batke J. 1990. Fructose-1,6-bisphosphate aldolase preferentially associates to gllyceraldehyde-3-phosphate dehydrogenase in a mixture of cytosolic proteins as revealed by fluorescence energy transfer measurements. Biochem Int 20:487–494.

Tsang V, Peralta J, Simons A. 1983. Enzyme-linked immunoelectrotransferblot techniques (EITB) or studying the specificities of antigens and antibodies separated by gel electrophoresis. Methods Enzymol 92:377–391.

Vallet V, Bens M, Antoine B, Levrat F, Miquerol L, Kahn A, Vandewalle A. 1995. Transcription factors and aldolase B gene expression in microdissected renal proximal tubules and derived cell lines. Exp Cell Res 216:363–370.

Wachsmuth ED, Thoner M, Pfeireder G. 1975. The cellular distribution of aldolase isozymes in rat kidney and brain determined in tissue sections by the immunohistochemical method. Histochemistry 45:143–161.

Watanabe J, Kanamura S, Kanai K, Shugyi Y. 1986. Cytochemical and biochemical glucose 6-phosphatase activity in skeletal muscle cells of mice. Anat Rec 214:25–31.

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.