Histol Histopathol (2001) 16: 53-55

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Human lung fructose-1,6-bisphosphatase is localized in pneumocytes II

A. Gizak[1], D. Rakus[1], J. Kolodziej[2], M. Zabel[3], A. Ogorzalek[4] and A. Dzugaj[1]

[1]Department of Animal Physiology, Institute of Zoology, Wroclaw University, Wroclaw, Poland,

[2]Lower Silesian Pulmonary Center, Department of Thoracic Surgery, Wroclaw, Poland,

[3]Department of Histology, Wroclaw University of Medicine, Wroclaw, [4]Department of Histology, Poznan University of Medicine, Poznan, Poland and [5]Department of General Zoology, Institute of Zoology, Wroclaw University, Wroclaw, Poland

Summary. The localization of fructose-1,6-bisphosphatase (Fru-1,6-Pase EC 3.1.3.11) in human alveolar epithelium was determined immunohistochemically using a polyclonal antibody raised against the enzyme purified from human liver. The immunohistochemical analysis revealed that the Fru-1,6-Pase was localized in pneumocytes II and was absent in pneumocytes I. Hypothetically Fru-1,6-Pase participating in glucose-6-phosphate synthesis from noncarbohydrate precursors increases NADPH level which is used for surfactant synthesis and for glutathione redox cycle.

Key words: Fructose-1,6-bisphosphatase, Immunocytochemistry, Lung, Pneumocytes

Introduction

Fructose-1,6-bisphosphatase (Fru-1,6-Pase EC 3.1.3.11) catalyzes hydrolysis of fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate in the presence of such divalent cations like magnesium, manganese, zinc or cobalt. Monovalent cations are needed for the enzyme to achieve its maximum activity (Benkovic and DeMaine, 1982; Tejwani, 1983). The enzyme is inhibited competitively by fructose-2,6-bisphosphate and allosterically by AMP (Pilkis et al., 1981; Van Schaftingen et al., 1980). Brain, muscle and liver Fru-1,6-Pase isozymes have been found in mammalian tissue (Horecker et al., 1975; Tejwani, 1983; Liu and Fromm, 1988). The physiological role of brain isozyme is unknown. Muscle isozyme participates in glycogen synthesis from lactate (McLane and Holloszy, 1979; Hermansen and Vaage, 1979) and is involved in glycolysis regulation via futile cycle (Newsholme and Start, 1976). Liver isozyme also found in kidney, intestine and monocytes are recognized as a regulatory enzyme of gluconeogenesis (Mizunuma and Tashima, 1978; Tejwani, 1983). It has been suggested that besides liver and kidney lung is also a gluconeogenic organ. Latres et al. (1992) have observed glucose production by rat lung in a double cannulation system. Recently we have reported on purification of Fru-1,6-Pase from human lung (Skalecki et al., 1999). Based upon Western blot analysis, determined kinetic properties, as well as cDNA coding region, the lung Fru-1,6-Pase has been found to be identical with the liver isozyme. Since lung tissue is rather a heterogeneous population of cells (Crystal et al., 1997) we have attempted to locate Fru-1,6-Pase in lung cells. Previously, immunocytochemical investigation has been performed in order to map the cellular distribution of Fru-1,6-Pase in rat kidney and liver (Saez et al., 1996). Immunofluorescence studies of rat brain sections have demonstrated that Fru-1,6-Pase is an astrocyte-specific enzyme (Schmoll et al., 1995). Our paper is the first report on the localization of Fru-1,6-Pase in lung cells. The evidence presented here indicates that human lung Fru-1,6-Pase is localized in pneumocytes II. The physiological meaning of this finding is discussed.

Offprint requests to: Dr. Andrzej Dzugaj, Department of Animal Physiology, Institute of Zoology, Wroclaw University, Cybulskiego 30, Wroclaw, Poland. Fax: (48 71) 322-28-17. e-mail: dzugajan@biol.uni.wroc.pl

Materials and methods

Human lung tissue samples were removed surgically from 7 patients of the Lower Silesian Pulmonary Center. They were men, aged 49 to 60 years (mean 58 years) with histologically proven diagnosis of squamous cell lung cancer. The tumor was located in hillus and for this reason pneumonectomy was performed. Lung tissues were taken from the distal part of bronchus without pleura. The samples were taken in agreement with the rules of The Scientific Research Ethical Committee.

Large Volume Peroxidase Kit, Antibody diluent with background reducing components and DAB chromogen

were purchased from DAKO (USA), Canada balsam and Paraffin wax were from Fluka (Fuchs, Switzerland) and xylene, CH_3OH, NaCl, H_2O_3 were obtained from POCh (Gliwice, Poland). All other reagents were from Sigma (St. Louis, USA). All of the reagents were of the highest purity commercially available.

Antibody production

Antiserum against the human liver Fru-1,6-Pase was raised in rabbit by intracutaneous injection of the purified enzyme (4 mg; isolated from human liver according to Dzugaj and Kochman (1980)) and Freund's complete adjuvant. Immuneserum was partially purified using acetone powder from human liver. Reactivity of anti-Fru-1,6-Pase serum was estimated using double diffusion method (Clausen, 1988). Immunodiffusion resulted in a strong reaction between the antiserum and the pure enzyme.

Immunocytochemistry

Human lung tissue samples were fixed in Bouin solution for 24 hours at room temperature. After the fixation they were dehydrated and embedded in paraffin. 5 μm sections were cut from the blocks of tissues and mounted on slides. Before immunostaining, sections were deparaffinized and treated with 1% H_2O_2 for 30 minutes to inhibit endogeneous peroxidase. Then they were incubated with normal goat serum (1:20), with rabbit polyclonal antibodies against human liver Fru-1,6-Pase (1:2500) overnight at 4 °C and at room temperature for 1 hour, with biotin-labelled goat antirabbit IgG for 15 minutes and with streptavidin-peroxidase complex for 15 minutes (SAB – modified ABC method where streptavidin was substituted for avidin). Each incubation was followed by three washings with PBS. Finally the peroxidase reaction was developed using 3,3'-diaminobenzidine (DAB) (Hsu et al., 1981) and examined by light microscopy.

Controls

In control reactions either the primary antibody was omitted or nonimmune rabbit serum was used as a first layer.

Results

Anti-Fru-1,6-Pase antibodies reacted with cuboidal cells on the surface of lung alveoli (Fig. 1). No reaction of anti-Fru-1,6-Pase antibodies with lining alveoli flat cells nor with intercellular barrier was observed. This indicates the presence of Fru-1,6-Pase in pneumocytes II. No macrophages were observed on the surface of alveoli which might interact with anti-Fru1,6-Pase antibodies impeding pneumocytes II distinction.

When the anti-Fru-1,6-Pase serum was omitted or nonimmune serum was used peroxidase reaction was not observed.

Discussion

The alveolar epithelium is composed of squamous pneumocytes I and cuboidal pneumocytes II. Pneumocytes I constitute ca 8%, pneumocytes II - 15% of the lung cells. Pneumocytes I are highly evolved to function as a thin gas permeable membrane.

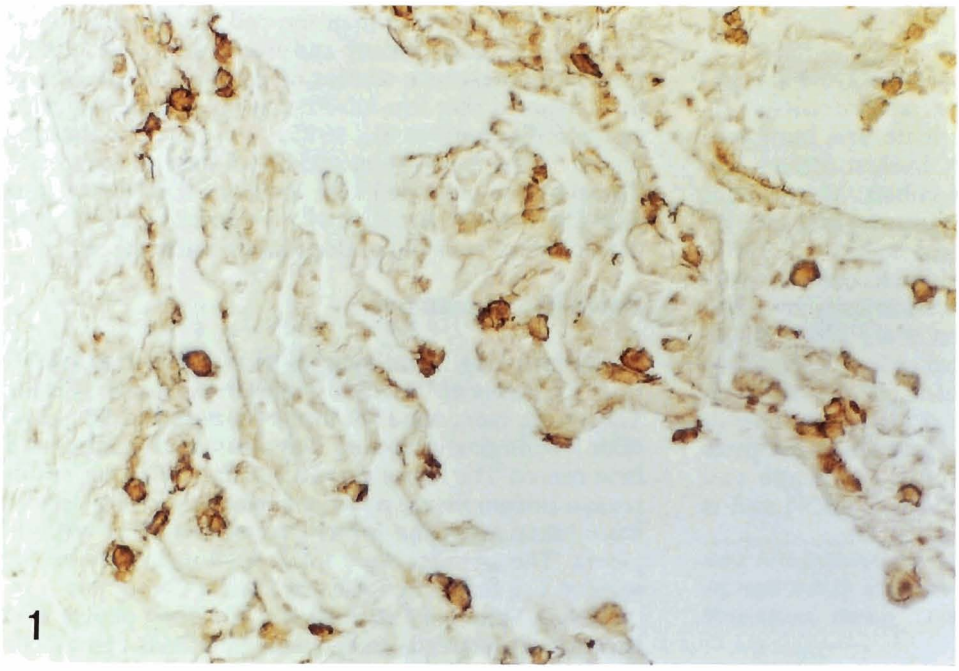

Fig. 1. Human lung sections stained with anti-Fru-1,6-Pase antibodies and SAB method. Brown colour indicates positive staining (DAB reaction). Positive immunoreaction is noted in pneumocytes II but not in pneumocytes I. x 400

Pneumocytes II are recognized as the cells that synthesize and secret pulmonary surface-active material - the surfactant. Numbers of papers have been published on surfactant synthesis; many fewer publications deal with lung carbohydrate metabolism and still fewer with lung gluconeogenesis.

Recently we found liver Fru-1,6-Pase isozyme in human lung tissue. Liver Fru-1,6-Pase is a key enzyme of gluconeogenesis. Its activity is regulated by fructose-2,6-bisphosphate whose concentration is under hormonal control. Fru-1,6-Pase participates in synthesis of glucose-6-phosphate which is hydrolyzed by glucose-6-phosphatase and released to the blood stream. Activity of glucose-6-phosphatase in lung is much lower than in liver (Skalecki et al., 1999). In lung the excess of glucose-6-phosphate might be used for glycogen synthesis or be oxidized by glucose-6-phosphate dehydrogenase. The oxidation of glucose-6-phosphate results in NADPH synthesis, which is a substrate for surfactant synthesis. NADPH also plays an important role in protection of cellular proteins and lipids against oxidizing effect of oxygen radicals.

We hypothesized that Fru-1,6-Pase participating in synthesis of glucose-6-phosphate was involved in a protection of lung cells against toxic effect of oxygen as well as in a surfactant production. Although the main source of glucose-6-phosphate is glucose from blood, an additional supply of glucose-6-phosphate seemed to be advantageous. Pneumocytes I, the very large flat cells that line more than 90% of the alveolar surface are exposed on increased partial pressure of oxygen. On the other hand, surfactant synthesis is localized in pneumocytes II. Therefore, we expected that Fru-1,6-Pase should be localized in both types of pneumocytes. Contrary to our expectations Fru-1,6-Pase was detected only in pneumocytes II. Its presence in surfactant synthesizing cells supports our hypothesis that this enzyme participates in synthesis of glucose-6-phosphate from noncarbohydrate precursors facilitates surfactant synthesis. The absence of Fru-1,6-Pase in pneumocytes I does not apparently support our hypothesis on its participation in protection of lung cells against toxic effect of oxygen. It has been reported that pneumocytes I do not contain catalase (Crystal et al., 1997) and this may indirectly indicate that these cells are less sensitive to oxygen radicals. Pneumocytes II, which supposedly are very sensitive to oxygen radicals, contain all sets of antioxidant enzymes and nonenzymatic oxygen radical scavengers (Crystal et al., 1997). In other words Fru-1,6-Pase is present in pneumocytes II, since it increases total production of NADPH, which is used in surfactant synthesis and for glutathione redox cycle.

References

Benkovic S.J. and deMaine M. (1982). Mechanism of action of fructose 1,6-bisphosphatase. Adv. Enzymol. 53, 45-82.

Clausen J. (1988). Immunochemical techniques for the identification and estimation of macromolecules. Burdon R.H. and van Knippenberg P.H. (eds). Elsevier. Amsterdam, New York, Oxford. p 281.

Crystal R.G., West J.B., Weibel E.R. and Barnes P.J. (1997). The lung. Section III. 2nd ed. Crystal R.G., West J.B., Weibel E.R. and Barnes P.J. (eds). Lippincott - Raven. Philadelphia, New York. pp 445-981.

Dzugaj A. and Kochman M. (1980). Purification of human liver fructose-1,6-bisphosphatase. Biochim. Biophys. Acta 614, 407-412.

Hermansen L.and Vaage O. (1979). Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. Am. J. Physiol. 233, E422-429.

Horecker B.L., Melloni E. and Pontremoli S. (1975). Fructose 1,6-bisphosphatase: properties of the neutral enzyme and its modification by proteolytic enzymes. Adv. Enzymol. 42, 193-226.

Hsu S.M., Raine L. and Fanger H. (1981). Comparison between ABC and unlabelled antibody (PAP) procedures. J. Histochem. Cytochem. 29, 1079-1081.

Latres E., Alemany M. and Remesar X. (1992). In vivo glucose metabolism in the rat lung. Biochem. Arch. 8, 175-182.

Liu F. and Fromm H.J. (1988). Purification and characterisation of fructose-1,6-bisphosphatase from bovine brain. Arch. Biochem. Biophys. 260, 609-615.

McLane J.A. and Holloszy J.O. (1979). Glycogen synthesis from lactate in three types of skeletal muscle. J. Biol. Chem. 254, 6548-6553.

Mizunuma H. and Tashima Y. (1978). Fructose 1,6-bisphosphatase of the small intestine. J. Biochem. 84, 327-336.

Newsholme E.A. and Start C. (1976). Regulation in carbohydrate metabolism in muscle. In: Regulation in metabolism, John Wiley & Sons Ltd. London. pp 88-145.

Pilkis S.J., El-Maghrabi M.R., McGrane M.M., Pilkis J. and Claus T.H. (1981). The role of fructose 2,6-bisphosphate in regulation of fructose-1,6-bisphosphatase. J. Biol. Chem. 256, 3619-3622.

Saez D.E., Figueroa C.D., Concha I.I. and Slebe J.C. (1996). Localization of the fructose 1,6-bisphosphatase at the nuclear periphery. J. Cell. Biochem. 63, 453-462.

Schmoll D., Cesar M., Fuhrmann E. and Hamprecht B. (1995). Colocalization of fructose-1,6-bisphosphatase and glial fibrillary acidic protein in rat brain. Brain Res. 677, 341-349.

Skalecki K., Rakus D., Wisniewski J.R., Kolodziej J. and Dzugaj A. (1999). cDNA sequence and kinetic properties of human lung fructose(1,6)bisphosphatase. Arch. Biochem. Biophys. 365, 1-9.

Tejwani G.A. (1983). Regulation of fructose-bisphosphatase activity. Adv. Enzymol. Relat. Areas Mol. Biol. 54, 121-194.

Van Schaftingen E., Hue L. and Hers H.G. (1980). Fructose-2,6-bisphosphate, the probable structure of the glucose- and glucagon-sensitive stimulator of phosphofructokinase. Biochem. J. 192, 897-901.

Accepted July 10, 2000