Alkaline Phosphatase: A Marker Enzyme for Brush Border Membrane?*

Investigators who are engaged in subcellular fractionation of renal or intestinal tissue use alkaline phophatase (a. P'tase = orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) as a marker enzyme for the identification of the brush border membrane fraction. Results of studies performed with the aid of electron microscopic techniques are conflicting with regard to the subcellular localization of a. P'tase. One group of investigators described the localization of a. P'tase as being only in the microvilli of the renal proximal tubular cell[1,2]; another group found the enzymatic reaction product to be localized in the whole cell surface membrane including basal infoldings and in the membrane bordering intercellular space[3,4]. A special technical analysis revealed the subcellular distribution of a. P'tase in the renal proximal tubular cell depending on the particular fixation technique of specimens for the electron microscope[5].

We therefore reinvestigated this topic of subcellular distribution of a. P'tase, using an ultramicrotechnique for quantitation of the activity in the basal and luminal area of the epithelial cell of rat renal proximal tubule.

Methods. 5 male Wistar rats weighing 200 g were nephrectomized. A kidney cone mounted upright on a tissue-holder was frozen in liquid nitrogen and 16 µm thick serial sections were cut in a cryostat. The first section was lyophilized over 12 h (vacuum 5×10^{-4} mm Hg), the second was stained with PAS. The stained section served as a guide for identification of the proximal convoluted portion (PTC) and the proximal straight portion (PTR) in the lyophilized section.

Single PTC and PTR were dissected out freehand from the lyophilized section under a stereomicroscope ($\times 100$) in a room of constant temperature (18 °C) and humidity (43%). At a 100-fold magnification the diameter of PTC and PTR is large enough to separate the luminal and basal areas from the proximal tubular epithelial cell. The dry tissue fragments were weighed on a quartz fiber balance. The dry weights of the luminal and basal areas ranged from 1–6 ng, of single PTC from 10–20 ng, of PTR from 10–25 ng. The dry weight served as reference for enzyme activity. The following substrate medium was used for measuring a. P'tase activity[6]: 2-amino-2-methyl 1.3 propanediol 0.5 *M* pH 10.0; *p*-nitro-phenyl phosphate 8 m*M*; $MgCl_2$ 2 m*M*; bovine serum albumin 0.05%. To determine the cyanide-sensitive activity fraction, 10 m*M* cyanide (CN) were added to the substrate medium. 700 nl substrate medium were placed at the bottom of wells in an ice-cooled teflon block; single dry tissue pieces were put into the medium droplet and covered with oil droplets to prevent evaporation. After incubation for 30 min at 37 °C in a water bath, the enzyme reaction was stopped by heating the teflon block for 5 min at 100 °C; the teflon block was afterwards cooled in an ice bath. 600 nl of the incubation medium were pipetted into 50 µl 0.15 *N* NaOH and kept at room temperature for 20 min. The reaction product *p*-nitrophenol was measured in a microcuvette at 410 nm.

The enzyme activity was expressed in moles *p*-nitrophenol/kg dry weight/h at 37 °C.

Results and discussion. In the epithelial cell of the proximal tubule, the basal labyrinth is limited to the basal area whereas the brush border represents the luminal area. Basal infoldings do not reach the luminal surface. Therefore it is relatively easy to separate from the proximal tubular cell the basal area, which contains exclusively basal labyrinth membranes beside mitochondria, and the luminal area including brush border membranes[7].

Table I shows that a. P'tase is not only found in brush border, but also in basal area fragments of PTC and PTR. The activity in basal area fragments is relatively strong compared with the activity of the total segment. Therefore a P'tase does not reveal an activity asymmetry for the subcellular distribution as shown for Na K ATPase in the proximal tubular cell[8].

The results raise two questions: 1. Does the hydrolytic activity in the basal area concern a real a. P'tase activity? 2. What subcellular structure of the cell base is the activity bound to? As to the first point: Table II demonstrates a CN-sensitive a. P'tase activity within the basal area of PTC and PTR. Cyanide (CN) stands for a specific inhibitor of a. P'tase in a number of tissues including kidney[9,10]. The relatively strong CN-insensitive hydrolytic fraction in the basal area of 50% (PTC) and 30% (PTR) – compared to luminal area – might represent ATPase activity, e.g. Mg ATPase. At pH 10.0, where the a. P'tase is fully active, Na K ATPase will be inactivated, whereas ouabain insensitive Mg ATPase demonstrates still an optimal activity[11]. However, CN in a 10 m molar concentration has no influence on both ATPases[11]. Therefore the CN-sensitive activity fraction (Table II) stands for a. P'tase.

As to the second point: In the basal area of PTR, there is only $^1/_3$ of CN-sensitive a. P'tase compared to the identical area of PTC (Table II). Morphometric data reveal that the amount of basal labyrinth membrane and mitochondria in the cell of PTR is only $^1/_3$ of that of PTC[12].

Table I. Subcellular distribution of alkaline phosphatase activity in epithelia of rat renal proximal tubular segments

Structure	One single portion	Basal area	Luminal area
PTC	36.6±11.9[a] (9)	41.9±11.1 (15)	137.8±79.2 (12)
PTR	19.0± 7.3 (8)	11.7± 5.5 (7)	171.0±70.5 (10)

PTC, proximal tubular convoluted portion; PTR, proximal tubular straight portion. [a] MKH = moles *p*-nitrophenol/kg dry weight/h at 37°C±SDM. Number of analyses in brackets.

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[1] E. Reale, Expl. Cell Res. *26*, 210 (1962).
[2] S. Goldfischer, E. Essner and A. B. Novikoff, J. Histochem. Cytothem. *12*, 72 (1964).
[3] E. Mölbert, F. Duspiva and O. Deimling, J. biophys. biochem. Cytol. *7*, 387 (1960 a).
[4] E. Mölbert, F. Duspiva, O. v. Deimling, Histochemie *2*, 5 (1960 b).
[5] E. Reale and L. Luciano, Histochemie *8*, 302 (1967).
[6] O. H. Lowry, N. R. Roberts, M. L. Wu, W. S. Hixon and E. J. Crawford, J. biol. Chem. *207*, 19 (1954).
[7] J. Rhodin, Int. Rev. Cytol. *7*, 485 (1958).
[8] U. Schmidt, U. C. Dubach, I. Bieder and B. Funk, Pflügers Arch. ges. Physiol., *330*, 265 (1972).
[9] D. R. Harkness, Arch. Biochem. Biophys. *126*, 513 (1968).
[10] U. Schmidt and U. C. Dubach, Ergebn. inn. Med. Kinderheilk. *32*. 83 (1971).
[11] U. Schmidt and U. C. Dubach, Progr. Histochem. Cytochem. *2*, 185 (1971).
[12] H. Sitte: in *Sekretion und Exkretion* (Ed. K. E. Wolfarth-Bottermann; Springer Verlag Berlin, Heidelberg, New York 1965), p. 343.

Table II. Cyanide (CN) sensitive alkaline phosphatase activity in the basal and luminal area of rat renal proximal tubular segments

Structure	Without CN	With 10 m*M* CN	CN sensitive phosphatase	CN-sensitive activity total activity
Basal area from PTC	41.9±11.1[a] (15)	20.4±14.8 (7)	21.5	0.51
from PTR	11.7± 5.5 (7)	3.8± 1.4 (6)	7.9	0.68
Luminal area from PTC	137.8±79.2 (12)	3.6± 2.8 (5)	134.2	0.97
from PTR	171.0±70.5 (10)	4.2± 2.5 (6)	166.8	0.97

PTC, proximal tubular convoluted portion; PTR, proximal tubular straight portion. [a] MKH = moles *p*-nitrophenol/kg dry weight/h at 37°C ± SDM. Number of analyses in brackets.

As yet it is not clear whether mitochondrial membrane contains a. P'tase[13]. Another intracellular site of a. P'tase is the Golgi apparatus. Morphometric data in rat renal tubule reveal a much higher amount of Golgi membranes in the PTR than in the PTC epithelia[14]. However, a. P'tase in basal area demonstrates an inverse activity pattern (Table I). The conclusion can be drawn that a. P'tase activity (Table I and II) in the basal area of PTC and PTR belongs to the basal labyrinth membrane.

Two thirds of a. P'tase in rat renal tubular epithelia consist of an insoluble desmoenzyme and $^1/_3$ of a soluble lyoenzyme[15]. The lyoenzyme is localized in the basal labyrinth membrane as shown by electron microscopic technique[15]. This might explain the lack of a. P'tase in basal labyrinth membrane fractions[16].

Our results show that a. P'tase is localized in the whole surface membrane of the renal proximal tubular cell, including the basal infoldings. This fact excludes a. P'tase to serve as a marker in differentiating brush border from basal labyrinth. As a proof for the purity of the brush border fraction, we propose the lack of Na K ATPase activity as such a marker. Na K ATPase is localized only in the basal labyrinth membrane and is missing in the brush border[8].

Zusammenfassung. Mit Hilfe einer Ultramikrotechnik konnte an isolierten gewundenen und geraden Hauptstücken der Rattenniere gezeigt werden, dass die alkalische Phosphatase nicht nur in der Bürstensaum–, sondern auch in der basalen Labyrinthmembran lokalisiert ist. Das Enzym ist infolgedessen kein geeignetes Leit-Enzym zur Erkennung des Bürstensaumes aus einem Plasmamembrangemisch. Es wird vorgeschlagen, das Fehlen der Na K ATPase-Aktivität als Mass für die Reinheit der Bürstensaumfraktion zu benutzen.

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[13] D. B. RODYN, in *The Biological Basis in Medicine* (Eds. E. E. BITTAR and N. BITTAR, Academic Press, London, New York 1969), Vol. 1, p. 123.
[14] TORHORST, H. P. ROHR and F. GLOOR, Verh. dt. Ges. Path. *53*, 391 (1969).
[15] M. M. NACHLAS, W. PRINN and A. M. SELIGMAN, J. biophys. biochem. Cytol. *2*, 487 (1956).
[16] R. KINNE, J. E. SCHNITZ and E. KINNE-SAFFRAN, Pflügers Arch. ges. Physiol. *329*, 191 (1971).
[17] Grants of SNF Nr. 3.207.69 and DFG.

Reaction of DNA with Phosphoric Acid Esters: Gasoline Additive and Insecticides

Trimethyl phosphate (TMP), a gasoline additive and the insecticides dichlorvos (DDVP, 0, 0-dimethyl-2, 2-dichlorovinyl phosphate) and dipterex (dylox, 0, 0-dimethyl-2, 2, 2-trichloro-l-hydroxyethyl phosphonate) have recently been reported to exhibit 'suspicious' biologic properties suggesting that they may have adverse effects on health. Thus TMP is mutagenic in mice[1] and DDVP in *E. coli*[2]. Dipterex has been stated to be weakly carcinogenic for rats[3] and DDVP to cause chromosome aberrations in onion root tip cells[4]. In terms of present concepts, all of these phenomena presumably result from an effect of these agents on cellular DNA. Indeed the alkylation of DNA by DDVP has been reported[5]. In this report the effect of DDVP on DNA is confirmed, moreover, it is shown that TMP and dipterex also alter DNA. This may then provide a chemical basis for the observed biological effects of these substances.

Exposure of DNA to these phosphoric acid esters[6] resulted in noticeable diminutions in the sedimentation coefficient of this biopolymer (Table). Presumably these changes reflect alkylation of DNA followed by some de-

[1] S. S. EPSTEIN, W. BASS, E. ARNOLD and J. BISHOP, Science *168*, 584 (1970).
[2] G. LÖFROTH, C. KIM, and S. HUSSAIN, Environ. Mutagen Soc. Newsletter *2*, 21 (1969).
[3] R. PREUSSMANN, Food Cosmet. Toxicol. *6*, 576 (1968).
[4] K. SAX and H. J. SAX, Jap. J. Genetics *43*, 89 (1968).
[5] G. LÖFROTH, Naturwissenschaften *57*, 393 (1970).
[6] Trimethyl phosphate was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Dipterex (Dylox) was received from Chemagro Corp., Kansas City, Mo., and dichlorvos (DDVP, Vapona) from Shell Chemical Co., San Ramon, Calif.