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Affinity identification of organic anion transporters in brush-border membrane vesicles from rat kidney

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Abstract

The inhibitory properties of bromoacetyl-*p*-aminohippuric acid as the affinity probe of the organic anion transport system were studied. Bromoacetylated *p*-aminohippurate was shown to be able to inhibit irreversibly the *p*-aminohippurate (PAH) uptake in brush-border membrane vesicles. The inhibition depends on both the time of treatment and the affinity probe concentration. The treatment of brush-border membrane with 1 mM bromoacetyl-*p*-aminohippurate for 1.5 h results in 100% irreversible inhibition of PAH transport but no changes were observed in the activity of alkaline phosphatase, γ-glutamyltranspeptidase or maltase. The affinity labelling of the organic anion transporters was performed with bromoacetyl-*p*-amino[^{3}H]hippuric acid. It was shown, by means of SDS-polyacrylamide gel electrophoresis, that the probe bound covalently to the brush-border membrane proteins with molecular masses of 28 kDa, 63 kDa, 98 kDa, and > 150 kDa. The data obtained with SITS and probenecide as the organic anion transport inhibitors indicate that brush-border membrane proteins of 28 kDa, 63 kDa, 98 kDa may correspond to the organic anion transport system.

Key words: Anion uptake; Membrane; Transport protein; Vesicle transport; (Rat kidney)

1. Introduction

The kidney transport system is concerned with the secretion of many endogeneous metabolites, drugs and natural toxins [1–4]. *p*-Aminohippuric acid is a substrate which has been used most frequently in studies of this system during the last two decades.

The investigations of the vesicular membrane preparations have made a substantial contribution to the present understanding of the mechanism of organic anion transport, which is reflected in a number of recent papers and reviews [1,5–12]. Although they provide a great amount of experimental data, there is no consensus among the researchers on the mechanisms and driving forces concerned with PAH transport. It is due, presumably, not only to the complication and certain disadvantages of membrane vesicles as a model for investigation of directed fluxes [7,13]. Recently, the question of different pathways for organic anion transport, to be realized under different physiological conditions was actively discussed [6,8–10,14]. In other words, the existence of different pathways of PAH transport is connected with the existence of different systems showing overlapping substrate specificity or with energy dependence on the common ion gradient. Such points of view inevitably pose the question that, in general terms, can be formulated as follows: are different pathways of PAH transport realized by means of different transporters or by the same, which mechanism of action (or interaction) is changed as a result of the given physiological conditions?

The answer to this question can be obtained only in the course of studies on the structural organization of the organic anion transport system.

The first attempt to solve this problem was made by Goldinger et al. [15]. They carried out the identification of the proteins attributed to the PAH transport systems of basolateral and brush-border membranes of rabbit kidney by means of a photoaffinity reagent (NAP-taurine). This reagent was shown to bind cova-

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lently to four proteins of the basolateral membrane, while no protein of the brush-border membrane was labelled.

In the present investigation we attempted to identify the proteins providing the PAH transport through the brush-border membrane of rat kidney cortex. For this purpose we used the affinity label – bromoacetyl-*p*-aminohippuric acid (BrAcPAH). The following reasons determined our choice [16]: (a) bromoacetylated affinity label can alkylate practically all nucleophilic protein groups; (b) BrAcPAH is a structural analog of PAH; (c) the structure of bromoacetylated affinity probes alone, due to their low reactivity becomes a determining factor in the reaction of covalent bond formation.

2. Materials and methods

The experiments were carried out on white mongrel rats. The animals were killed by decapitation, their kidneys were removed quickly, decapsulated and placed in the Ringer solution at 0–4°C. The isolation of the brush-border and basolateral membrane vesicles from kidney cortex was performed by the method of Sheikh et al. [17]. The final fractions were resuspended in the uptake medium containing the following ingredients (in mmol/l): mannitol 100, Tris-Hepes 20, $MgCl_2$ 1 (pH 7.4). The protein concentration in both brush-border and basolateral membrane fractions was 10–13 mg/ml. The transport and binding experiments were carried out on the next day after isolation.

The purity of the membrane preparations was assessed by assaying the specific activity of the following marker enzymes according to previously published procedures: alkaline phosphatase (EC 3.1.3.1) for brush-border membranes [18], Na^+/K^+-ATPase (EC 3.6.1.3) for basolateral membranes [19], glucose-6-phosphatase (EC 3.1.3.9) and succinate dehydrogenase (EC 3.1.99.1) for endoplasmic reticulum and mitochondria, respectively [20]. The activity of maltase (EC 3.2.1.48) and γ-glutamyltranspeptidase (EC 2.3.2.2) was assayed by methods previously described [20]. The protein concentration was determined by the method of Lowry et al. [21]. Compared to the initial homogenate, the fractions of brush-border membrane (BBM) vesicles were enriched 10–12 times in alkaline phosphatase with a yield of 10–15%. The fractions of basolateral membrane (BLM) vesicles were enriched 8–10 times in Na^+/K^+-ATPase with a yield of 8–10%. In both cases the enrichment factor for glucose-6-phosphatase and succinate dehydrogenase did not exceed 0.8.

The transport assay was determined by the rapid filtration method according to Mamelok and co-workers [22] with some modifications. The incubation medium consisted of (in mmol/l): mannitol 100, Hepes 20, pH = 7.4 at 37°C, *p*-amino[^{3}H]hippuric acid 0.15 with a specific activity of 3700 MBq/mmol. The reaction was initiated by mixing 10 μl membrane fraction and 50 μl incubation medium. The uptake of solute was determined at appropriate time intervals by diluting the incubation mixture with 2 ml ice-cold stop buffer that had the same composition as the incubation medium, but without substrate. The sample was immediately filtered under vacuum through a 0.45 μm cellulose nitrate filter (Chemapol) and was washed with 5 ml ice-cold stop buffer. The radioactivity remaining on the filters was counted using standard liquid scintillation techniques on an SL-30 scintillation meter (France). The background radioactivity was determined in the presence of vesicles when radioactive substrate and stop solution were added simultaneously. The experimental data were expressed as means ± S.E. and statistical analyses were performed with Student's *t*-test with $P < 0.1$ as the significance level.

The electrophoresis of the proteins of the brush-border and basolateral membranes was carried out in 9.4% SDS-polyacrylamide gel [23] with 6.4% urea [24]. Membrane fractions were treated before electrophoresis with the chloroform/methanol (2:1, v/v) mixture (0.2 ml membrane fraction per 6 ml of mixture). The probes were centrifuged and the pellet was dried on the rotary evaporator and resuspended in 100 μl uptake buffer with 2% SDS. Approximately 50–70 μg of protein were applied to the gel. Molecular mass standards used were phosphorylase B (94 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa). Gel tracts of interest were cut out from the rest of the gel and were sliced in 2-mm sections. Each slice was placed in 0.2 ml of 1% Triton X-100 solution, homogenized and placed at 37°C overnight. The radioactivity of the probe (100 μl) was determined by the standard liquid scintillation technique.

The isolation and quantitative analysis of the lipids were performed as described by Hise et al. [25].

The synthesis of the bromoacetyl-*p*-aminohippuric acid was carried out as follows. Dry dicyclohexylcarbodiimide (20 mmol) was added to the solution of bromoacetic acid (40 mmol) in 50 ml of dry dioxane at 12–15°C. After 20 min of incubation the reaction mixture was kept at 25°C for 10 min. The dicyclohexylurea was removed from the mixture by filtration. The filtrate was added in small portions to 20 ml ice-cold aqueous solution of *p*-aminohippurate (5 mmol) with 0.1 M $NaHCO_3$. The pH of the reaction mixture was maintained at 8–9 by 0.5 M NaOH. After the reaction was completed the mixture was evaporated to dryness and the solid residue was dissolved in water (100 ml). The red precipitate, formed after the acidification of the water solution to pH 3–3.5, was removed by filtration. The filtrate was evaporated once again to the volume of 20 ml. The white precipitate was collected,

washed and recrystallized twice from water. The yield of the bromoacetyl-*p*-aminohippuric acid was 55–65%.

The synthesis of bromoacetyl-*p*-amino[^{3}H]hippuric acid was carried out starting from *p*-amino[^{3}H]hippuric acid (37 MBq with specific activity 14.8 GBq/mmol) by the method described above but on the small scale mode (1:500). The purification of the bromoacetyl-*p*-amino[^{3}H]hippuric acid was performed immediately after the condensation step by paper chromatography in a mixture of n-butanol/acetic acid/water (4:1:5, v/v).

The treatment of the membrane fractions with bromoacetyl-*p*-aminohippuric acid was performed at 37°C according to the following procedure. The aliquot of the affinity reagent was added to 0.1 ml of the membrane suspension in the uptake medium (the protein concentration was 10–12 mg/ml). The final concentrations of BrAcPAH and vesicle treatment time are given in the legends to the figures. The reaction was terminated at a pre-set time by diluting the incubation mixture with 4 ml uptake buffer and the probe was kept 15 min at 37°C. Then the sample was centrifuged at 30000x*g* for 30 min. The pellet was resuspended in 4 ml of the uptake medium and the incubation and centrifugation steps were repeated. The pellet obtained after the second centrifugation was collected and resuspended in the uptake buffer (modified membrane fraction).

The chemical reagents used in this study were as follows: *p*-aminohippuric acid (PAH), probenecid, bromoacetic acid, and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) were from Serva; dicyclohexylacarbodiimide was obtained from Aldrich; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), and 2-amino-2-hydroxymethylpropane-1,3-diol (Tris) were from Sigma; *p*-amino[^{3}H]hippuric acid was from Amersham.

3. Results

A typical dynamics of the [^{3}H]PAH total uptake (specific plus nonspecific components) for the brush-border membranes vesicles is illustrated in Fig. 1: curve 1, unmodified vesicles; curve 2, vesicles modified by 0.5 mM BrAcPAH for 1 h. It can be seen that the treatment of BBMV with affinity reagent leads to a significant decrease of the initial rate of [^{3}H]PAH uptake as compared to that of unmodified vesicles. In both cases the addition of 2 mM SITS to the incubation medium inhibits considerably the [^{3}H]PAH uptake (Fig. 1, curves 3 and 4). It should be noted that, both in the presence and in the absence of SITS in the incubation medium, the values of total uptake at the short incubation time (up to the 3 s) are the same for modified and unmodified membrane fractions. These total uptake

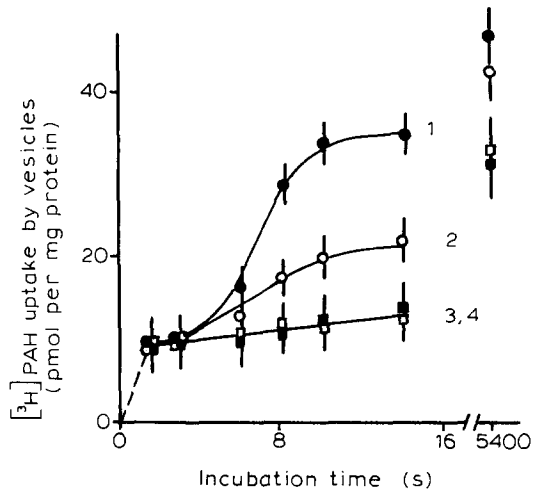

Fig. 1. Time dependence of [^{3}H]PAH total uptake for the unmodified (closed symbols) and by BrAcPAH-modified (open symbols) BBM vesicles; 1 and 2, without SITS; 3 and 4, with 2 mM SITS in incubation medium.

values practically coincide with the background level (9 ± 5 pmol/mg protein) measured by the standard procedure described in Materials and methods. Therefore, the uptake value at 3 s of incubation was used further as the value of the nonspecific component of the total [^{3}H]PAH uptake.

In the next series of experiments the ability of BBM vesicles for PAH uptake was studied after the treatment of membrane fractions with the affinity probe at different concentrations and incubation times. Fig. 2 shows the dependence of PAH uptake by modified BBM vesicles in the course of BrAcPAH treatment. It can be seen that the total PAH uptake (10 s incubation) decreased uniformly with time of treatment (Fig. 2, curve 1). The PAH uptake at a short incubation time (3 s) at first increases slightly and then decreases

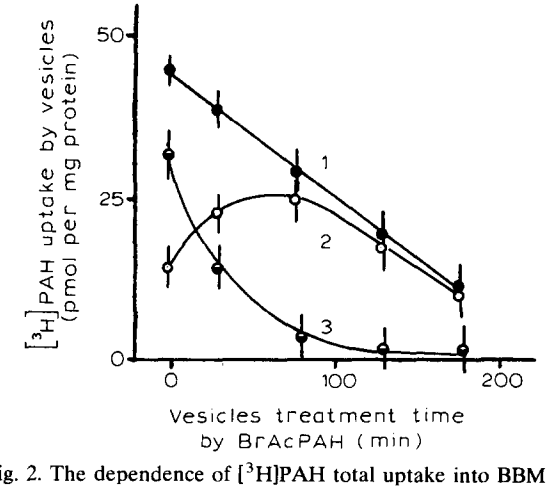

Fig. 2. The dependence of [^{3}H]PAH total uptake into BBM vesicles as a function of time of vesicle treatment with BrAcPAH; 1, 10 s of [^{3}H]PAH uptake; 2, 3 s of [^{3}H]PAH uptake; 3, differential curve between 1 and 2. BrAcPAH concentration: 1 mM.

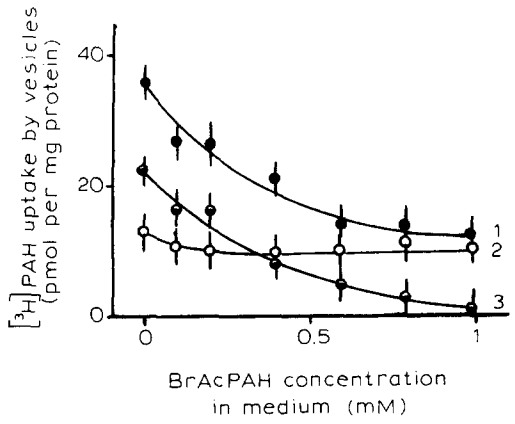

Fig. 3. The dependence of [^{3}H]PAH total uptake into BBM vesicles as a function of BrAcPAH concentration in the treatment medium; 1, 10 s of [^{3}H]PAH uptake; 2, 3 s of [^{3}H]PAH uptake; 3, differential curve between 1 and 2. BrAcPAH treatment time: 90 min.

uniformly with time of vesicle treatment with the affinity reagent (Fig. 2, curve 2). When the time of treatment is more than 90 min, the values of [^{3}H]PAH uptake at 10 s and 3 s of incubation are the same.

The dependence of PAH uptake by BBM vesicles as a function of the affinity probe concentration is shown in Fig. 3. It is seen that the PAH uptake at 3 s incubation remains constant over the range of BrAcPAH concentrations (Fig. 2, curve 2), while the curve of 10 s PAH uptake decreases exponentially with the increasing affinity reagent concentration (Fig. 3, curve 1).

The modification procedure of the membrane fractions with the affinity probe may, in principle, lead to essential changes in membrane structure, which in turn may influence the intrinsic membrane functions. We have studied such a possibility, comparing the activity of the membrane enzymes in the native brush-border membrane fraction and that modified by 1 mM BrAcPAH for 1.5 h. No differences were found in the activity of alkaline phosphatase, maltase and γ-glutamyltranspeptidase for the initial and the modified membrane fraction. Table 1 presents the data on the distribution of affinity reagent among the protein and lipid parts of brush-border membranes. The membrane fraction modified by 1 mM BrAc[^{3}H]PAH for 1.5 h was fractionated into proteins and individual lipids. The amount of covalently bound BrAc[^{3}H]PAH in separate lipid fractions was determined after their extraction from silica gel. The results of Table 1 show that the main part of the covalently bound affinity probe (85%) is associated with proteins and only 15% with lipids. It should be noted that there is no predominant covalent binding of BrAcPAH with any individual lipid.

To identify the transporters of the organic anion transport system the membrane fractions were treated with 0.3 mM BrAc[^{3}H]PAH for 30 min. Three types of

Table 1
The distribution of BrAc[^{3}H]PAH between brush-border membrane proteins and individual lipids

Membrane components	BrAc[^{3}H]PAH binding (pmol/mg protein)
Proteins	46 ±4
Lipoproteins	8 ±2
Lipids	10 ±2
Sphingomyelin	1.6±0.4
Phosphatidylserine	2.8±0.6
Phosphatidylcholine	2.0±0.4
Phosphatidylethanolamine	1.6±0.3
Cholesterol and minor lipids	1.8±0.4

Data presented are means ± S.E.

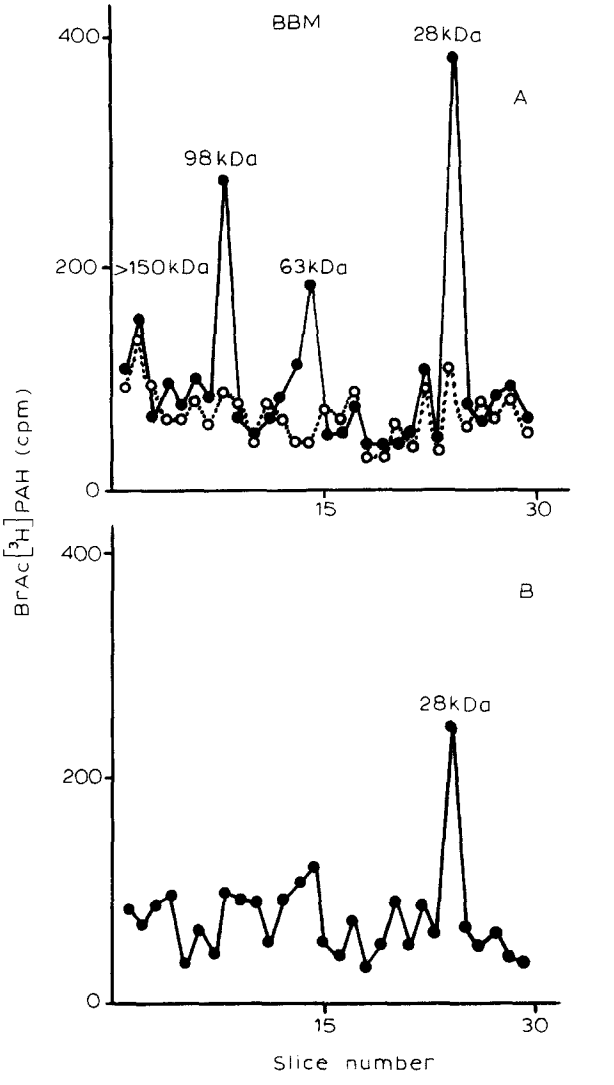

Fig. 4. The SDS-gel electrophoresis assay of BrAc[^{3}H]PAH incorporation into BBM proteins; (A) without (closed symbols) and with (open symbols) 2 mM SITS in the treatment medium; (B) after the preliminary nonradioactive BrAcPAH protection of the membrane in the presence of 5 mM probenecide.

affinity modification procedures were used in the present work. In the first one the initial membrane fractions were treated with the radioactive affinity probe. In the second, the treatment conditions were the same but with addition of 2 mM SITS in the incubation medium. In the third variant the membrane fraction was initially treated with 0.3 mM nonradioactive BrAcPAH for 30 min in the presence of 5 mM probenecide and, after the removal of affinity reagent, the radioactive affinity treatment was repeated under the same conditions but without probenecide. After each labelling procedure the membrane proteins were separated by SDS-polyacrylamide gel electrophoresis and assayed for covalently bound BrAc[^{3}H]PAH as described in Materials and methods.

A typical radioactivity profile of brush-border membrane proteins after the electrophoretic separation is presented in Fig. 4. It can be seen that the treatment of vesicles by BrAc[^{3}H]PAH according to the first procedure gives rise to four major radioactivity peaks

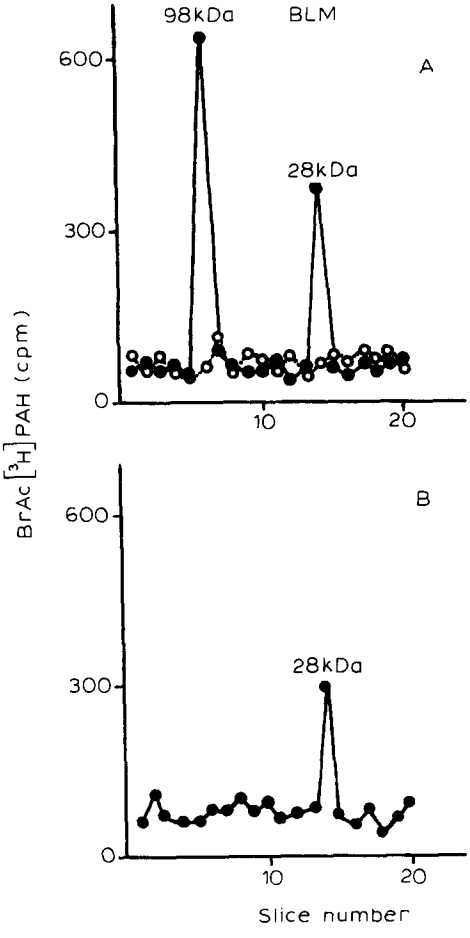

Fig. 5. The SDS-gel electrophoresis assay of BrAc[^{3}H]PAH incorporation into BLM proteins; (A) without (closed symbols) and with (open symbols) 2 mM SITS in the treatment medium; (B) after the preliminary nonradioactive BrAcPAH protection of the membrane in the presence 5 mM probenecide.

Table 2
The frequency of the events of BrAc[^{3}H]PAH incorporation into brush-border and basolateral membrane proteins

Membrane	Number of experiments	Molecular mass of proteins			
		28 kDa	63 kDa	98 kDa	> 150 kDa
BBM	8	8	2	8	4
BLM	4	4	–	3	–

which belong to the proteins with molecular masses of 28 kDa, 63 kDa, 98 kDa, and > 150 kDa (Fig. 4A, solid line). After the addition of 2 mM SITS to the incubation medium during the labelling procedure the only radioactive peak that remains corresponds to the protein with molecular mass > 150 kDa (Fig. 4A, dotted line). The third type of modification by BrAc[^{3}H]PAH of the brush-border membrane proteins leads to the disappearance of the peaks corresponding to the proteins with molecular masses of 63 kDa, 98 kDa and > 150 kDa, and the reduction of the peak associated with the 28 kDa protein (Fig. 4B). The same experimental scheme of affinity identification of transporters was applied to the organic anion transport system of the basolateral membrane. The typical results of a separate experiment are presented in Fig. 5. The affinity treatment of the basolateral membrane proteins in accordance with the first procedure gives two major radioactivity peaks associated with the proteins of 28 kDa and 98 kDa (Fig. 5A, solid line). The addition of SITS to the incubation medium during the affinity treatment leads to total disappearance of the peaks (Fig. 5A, dotted line). The basolateral membrane proteins treatment with BrAc[^{3}H]PAH using the third variant results in a decrease of the radioactive peak of the 28 kDa protein and the disappearance of the peak associated with the 98 kDa protein.

The frequency of the events of affinity probe incorporation into the separate membrane proteins in accordance with the first procedure are summarized in Table 2. One can see that for both the brush-border and the basolateral membrane the covalent binding of the affinity probe to the proteins with molecular masses of 98 kDa and 28 kDa occurs more often.

4. Discussion

Many experimental data now confirm that PAH transport through the brush-border and basolateral membranes is carried out by means of specific transporters [6,10,26–29]. It is also well known that it can be *trans*-stimulated by the gradient of Cl^-, pH, PAH, HCO_3^-, uric acid and lactate [6,9,27,30] and *cis*-inhibited by probenecid, SITS, DIDS (4,4′-diiso-

thiocyanostilbene-2,2′-disulfonic acid) and other organic anions [1,8,31,32]. It was assumed on the basis of kinetic data that there are some physiologically conditioned pathways of PAH transport in brush-border membrane of kidney proximal tubules [8–10,14]. The same point of view was suggested earlier concerning other transport systems [33–35] and substrates [6].

In our work we have established that BrAcPAH inhibits the transport of PAH in brush-border membrane vesicles. As shown in Fig. 1, treatment of vesicles with 0.3 mM BrAcPAH (with its subsequent removal from the incubation medium) leads to a 4-fold drop in the initial transport rate. It should be noted that the dynamics of the total value of [^{3}H]PAH uptake by vesicles has two steps, the first being rapid and the second (at incubation times > 3 s) being slow. The jump of the uptake at the first step reflects the substrate sorption on the membrane surface [36,37], which in the present case is the same for BrAcPAH-modified and unmodified brush-border vesicles. In the most general case the value of the sorption component of the [^{3}H]PAH total uptake can depend on both the concentration and the time of treatment of the vesicles with the affinity reagent. Therefore, the PAH uptake in the inner volume of vesicles (the proper PAH transport) was calculated as the difference between the uptake at 10 s and 3 s of incubation under the given experimental conditions.

The treatment of brush-border membrane vesicles with different concentrations of BrAcPAH (with its subsequent removal from the incubation medium) leads to the progressive PAH transport decrease down to zero value (Fig. 3, curve 3). The affinity reagent concentration, with which the inhibition reaches 50% is equal to 0.3 mM (at the treatment time of 1.5 h). The variation of the time of treatment of brush-border membrane vesicles at a constant concentration of BrAcPAH (1 mM) also leads to a progressive decrease in PAH transport; 50% inhibition was reached in 30 min. At 90 min treatment time the PAH transport is depressed almost completely (Fig. 2, curve 3). Taken together, the data presented in Figs. 1, 2 and 3 show that irreversible inhibition of PAH transport by BrAcPAH depends on both the concentration of the reagent and on the time of treatment. To reach at least 50% inhibition the treatment time must be substantially longer than that during which the net PAH flux ($J_O = J_{in} - J_{out}$) reaches zero value. In other words, following the assumption of the existence of some structurally different pathways of PAH transport, one can say that, under the conditions examined, BrAcPAH inhibits that transport pathway, which is realized under the conditions of equilibrium exchange (when $J_{in} = J_{out}$). On the other hand, the fact of irreversible inhibition was established on the basis of the initial rate of [^{3}H]PAH transport, when its flux into vesicles exceeds the reverse one. Therefore, BrAcPAH inhibits that pathway of PAH transport which is realized under the conditions close to zero *trans*-uptake.

For the identification of proteins taking part in the PAH transport we used four criteria: (a) the value of radioactivity peaks on SDS-polyacrylamide gel after the treatment of the membrane fraction with BrAc[^{3}H]PAH; (b) the change of these values after treatment of the membrane with BrAc[^{3}H]PAH in the presence of SITS; (c) the change of protein radioactivity peaks of the membrane protected previously by nonradioactive BrAcPAH in the presence of probenecid; (d) the frequency of the peak appearance.

It is seen in Fig. 4A (data of a typical experiment) that there are four major radioactivity peaks corresponding to the BBM proteins with molecular masses of 28 kDa, 63 kDa, 98 kDa, and > 150 kDa. The presence of SITS in the incubation medium leads to the elimination of the radioactivity peaks of the proteins of 98 kDa, 63 kDa, and 28 kDa (Fig. 4A, dotted line), but the peak corresponding to the protein with molecular mass > 150 kDa remains. Strictly speaking, SITS is not a structural analog of PAH, although it is well known that it inhibits PAH transport [1,8,31,32]. In other words, SITS cannot have a noticeable influence on the covalent binding of BrAcPAH with amino-acid residues of proteins not participating in PAH transport and must decrease (or totally suppress) affinity reagent binding with the active site of PAH transporters. Hence, the probability is high enough that the proteins with molecular masses 98 kDa, 63 kDa, and 28 kDa are relevant to the system of organic anion transport in BBM, but that the protein with molecular mass > 150 kDa does not.

In the case when the membranes are protected, only one major peak of radioactivity remains on the SDS electrophoretogram corresponding to protein with molecular mass 28 kDa (Fig. 4B), but the peaks of proteins of 63 kDa and 98 kDa diminish to practically background level.

The ability or inability of probenecid to protect the organic anion transporters depends on the difference in affinity of BrAcPAH and probenecid to the active site of the transporter given. Therefore, the results presented in Fig. 4B can reflect the fact that 28 kDa proteins (in contrast to 98 kDa and 63 kDa proteins) have a higher affinity to probenecid than to BrAcPAH. It is to be noted here that 28 kDa and 98 kDa proteins were labelled in all cases (according to the data of eight experiments), but that 63 kDa protein was labelled in two cases only.

The whole complex of the existing data allows us to assume that 28 kDa protein does belong to the organic anion transport system of the brush-border membrane. It should be noted that this assumption is in agreement with the data of Kwon et al. [38] describing the expres-

sion of organic anion transport in oocytes. The size of the mRNA that induced PAH transport in *Xenopus* oocytes indicates that the expressed protein must be smaller than 47 kDa.

At the same time, the possibility that 98 kDa and 63 kDa proteins also are members of this system cannot be rejected. For instance, it is quite possible that the 98 kDa protein is the structural element of the probenecid-insensitive (or low-sensitive) pathway of PAH transport in contrast to the 63 kDa protein which may participate in a transport pathway realized under other physiological conditions. On the other hand, the difference in frequency of the radioactivity peak appearance of 28 kDa, 98 kDa and 63 kDa proteins can reflect the variation (from one experiment to the other) in content of basolateral membrane as an admixture component in the vesicle fraction of brush-border membrane. However, the experiments on the affinity identification of the proteins of the organic anion transport system of basolateral membrane (which were carried out under the same conditions) do not permit us to make such a conclusion: in both cases (Figs. 4 and 5) the radioactivity peak values on SDS-polyacrylamide gel are close to each other. Further, results on the covalent binding of BrAcPAH with proteins of the PAH transport system of basolateral and brush-border membranes agree well with one another.

In the case of basolateral membrane two major radioactivity peaks are observed on SDS-polyacrylamide gel corresponding to proteins with molecular masses of 98 kDa and 28 kDa (Fig. 5A, solid line). SITS inhibits completely covalent binding of BrAcPAH to these proteins (Fig. 5A, dotted line). The use of probenecid as protector gives the result coinciding with that for brush-border membrane (Fig. 5B).

In the literature there is only one paper dedicated to the identification of renal organic anion transporters [15]. Using the photoaffinity probe NAP-taurine which differs from BrAcPAH by the mechanism of covalent bond formation [16], Goldinger and co-authors identified four proteins of basolateral membrane with molecular masses of 108 kDa, 64 kDa, 52 kDa and 26 kDa, and could not reveal proteins responsible for PAH transport in brush-border membrane.

Comparison of our data with the data of Goldinger et al. shows an agreement with regard to the three proteins of 98 (108) kDa, 63 (64) kDa and 28 (26) kDa. It is likely that the small differences in molecular masses are simply due to different laboratory practices in their and our determinations by SDS electrophoresis. We would like to emphasise that the conditions of affinity identification of the transporters in the work of Goldinger et al. were very similar to ours. They used 5–10 min to obtain irreversible inhibition of PAH transport by means of NAP-taurine, which corresponds fairly well to the conditions of anion exchange.

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