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Rapid binding of β_2-microglobulin to renal brush-border membranes

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^{125}I-labelled human β_2-microglobulin binding to rat renal brush-border membranes was assessed by an in vitro assay under near physiological incubation conditions (i.e. low content of albumin). Binding rate was 55 pmol/min per mg protein in the presence of 200 nM of β_2-microglobulin and degradation rate was negligible versus binding rate. The binding rate was in reasonable agreement with the in vivo reabsorption rate, supporting the hypothesis of proteins binding to the luminal membrane during the process of reabsorption. Mild solubilizing treatment (Triton 0.1%) of brush border after β_2-microglobulin binding yielded the labelled molecule associated with a high-molecular-weight component. Aminopeptidase activity and binding ability were to a certain extent co-purified during the course of the brush-border preparation, suggesting that most of the β_2-microglobulin binding sites were localized in the brush-border membranes.

Introduction

Low-molecular-weight proteins are poorly retained by the kidney glomerulus and thus have a short plasma half-life [1–4]. They are readily taken up by the renal tubular cells, probably in the proximal part [5,6] and accumulate in the kidney [7,2]. They are subsequently degraded into their constitutive aminoacids which return to the plasma compartment [7,2]. The protein content of the tubular fluid in the rat may be estimated to be 200–300 μg/ml [8,9], an equivalent of about 2 or 3 mM of their constitutive amino acids. Thus renal uptake of proteins appears to be quantitatively as essential as amino acid reabsorption for the equilibrium of the body amino acid pool. In spite of this, very little is known about the precise mechanism of protein reabsorption.

Histological studies have shown that filtered proteins are found in endocytotic vesicles close to the brush-border membrane of tubular cell [10–12]. A binding process prior to internalization has been postulated by Maack and co-workers [2], considering that total reabsorption of proteins cannot be carried out by simple engulfment of tubular fluid (fluid phase endocytosis). However, data are lacking about the rate of binding to tubular cell membrane. Moreover, previous binding studies on in vitro protein binding to kidney brush border [13] have been carried out under incubation conditions differing from in vivo status, therefore preventing a comparison with in vivo studies.

In the present work we have measured the in vitro binding rate of a small protein, β_2-micro-

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globulin to renal brush-border membrane under near physiological conditions. We checked the validity of the experimental procedure and we compared the data with in vivo measured reabsorption. The degradation rate was also measured. β_2-Microglobulin is a 11 800 Da protein devoid of biological activity. Its in vivo renal handling has been previously described [14,15,4].

Methods

Preparation of brush-border membranes

Brush-border membranes were obtained by a $MgCl_2$ precipitation method according to Booth and Kenny [16] and Biber et al. [17] with a few modifications. Kidneys were dissected from three non-fasted male Wistar rats anesthetized with sodium pentobarbital (Nembutal, Abott, Chicago). Kidney cortex was minced, then homogenized in 10 mM mannitol, 2 mM Tris-HCl (pH 7.1) in a Virtis grinding mill run at speed 50 during 3×20 s. Buffer weight was 10-times that of cortical tissue. $MgCl_2$ (10 mM final concn.) was added and the homogenate stirred for 15 min. After centrifugation during 12 min at $3500 \times g$ (Sorvall RC3 with HS 4 rotor), supernatant was homogenized and filtered on a 100 μm pore size gauze-cloth. Filtrate was centrifuged at $22\,500 \times g$ for 12 min (Sorvall RC 2B or MSE HS 18, with a MSE 69 181 rotor). The pale upper layer of the pellet was carefully removed and discarded whereas the lower layer was resuspended in mannitol buffer (5-times the weight of cortical tissue) to which $MgCl_2$ was added up to 10 mM final and the mixture stirred for 15 min. After 12 min centrifugation at $4300 \times g$, supernatant was removed and run at $22\,500 \times g$ for 12 min. The pellet was then homogenized in 2 ml of NaCl-Hepes buffer used for further experiments. The last step was a washing in the latter buffer followed by centrifugation for 20 min at $30\,000 \times g$. All steps were performed at $+4°$C. The final membrane suspension was stored in aliquots in liquid nitrogen until utilization.

Assessment of brush-border preparation

Protein content was determined according to the method of Lowry and coll. [18]. The activity of aminopeptidase M (EC 3.4.11.2) was assayed as described by Louvard et al. [19]. Alkaline phosphatase was determined according to Walter and Schutt [20], and acid phosphatase activity determination was modified from Biber et al [17]. Glucosaminidase (EC 3.5.1.33) was determined by the method of Scalera et al. [21]. β-Glucuronidase (EC 3.2.1.31) assay was modified from Fishman [22]. $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) content was determined according to Heller et al. [23]. Succinate–cytochrome-*c* reductase (EC 1.3.99.1) and NADPH–cytochrome-*c* reductase (EC 1.6.2.4) were assayed following Sottocasa et al. [24].

Mean enrichment in aminopeptidase was 9.5, yield was 15–20%. The preparation was essentially free of NADPH–cytochrome-*c* reductase (microsomes), succinate–cytochrome-*c* reductase (mitochondria) and of lysosomal contamination as assessed by glucuronidase and glucosaminidase activity. The main contaminants were acid phosphatase activity (enrichment 1.9 versus homogenate) as observed in the original paper [17] and $(Na^+ + K^+)$-ATPase (enrichment 1.1). Though acid phosphatase activity is a broadly used lysosomal marker, it probably originated in our preparation from a Golgi-apparented structure [25].

β_2-Microglobulin labelling

β_2-microglobulin was obtained from the pooled urine of several renal transplanted patients and isolated according to the procedure of Vincent and Revillard [26]. The protein was labelled with ^{125}I by the lactoperoxidase method of Thorell and Johansson [27]. Specific activity averaged 0.75 μCi/μg (9.9. 10^5 cpm/μg), which represents about one molecule of ^{125}I per one hundred molecules of β_2-microglobulin. Purity and homogeneity of the labelled protein was verified as described before [15]. The final labelled β_2-microglobulin preparation (30 to 40 μM) contained 10 μg bovine serum albumin (Fraction V, Sigma, purified by chromatography on ACA 44 gel 100 cm $\times$ 2.5 in the presence of 30 mM Tris-HCl buffer (pH 7.8) and 0.1 mM NaCl, then dialysed against the preparation buffer), per μg of β_2-microglobulin. The final preparation was dialyzed against 0.15 M NaCl, 0.066 M phosphate buffer (pH 7.4) and stored frozen.

Assay of ^{125}I-labelled β_2-microglobulin binding and degradation

^{125}I-labelled β_2-microglobulin binding on

brush-border membranes was determined by the rapid filtration technique. Composition of the incubation medium is indicated under each figure. All media were filtered through a 0.2 μm or 0.3 μm filter (Sartorius). Standard assay contained 20 μl of the membrane suspension (5 to 10 μg protien per assay) preincubated for 10 min at 37 °C in 80 μl incubation buffer. Binding was initiated by addition of 20 μl of labelled β_2-microglobulin. Standard assay contained 200 nM β_2-microglobulin diluted from labelled stock solution in incubation medium. Final content of incubation medium in albumin and phosphate brought with labelled β_2-microglobulin was 23 μg albumin/ml and 0.3 mM phosphate. When necessary 400 nM β_2-microglobulin was made up by addition of unlabelled β_2-microglobulin. Standard incubation time was 1 or 5 min. Incubation temperature was 37 °C. Each assay was carried out in triplicate. At the end of the incubation time a 20 μl aliquot was pipetted out into 480 μl cold stop solution (150 mM choline chloride, 10 mM Hepes (pH 7.4), fraction V bovine albumin (Sigma or Boehringer), 20 g/l) and saved for further degradation determination. 2 ml cold stop solution were then added to the remaining incubation medium and the totality rapidly filtered through a 0.2 μm cellulose acetate filter (Sartorius or Schleicher and Schüll) presoaked in stop solution. Correction for non specific binding of β_2-microglobulin to filter was made by substracting from each value that of the blank obtained by filtration of the incubation medium without membrane suspension (total binding). Correction for non specific binding of β_2-microglobulin to membranes was made by substracting from total binding data the value of an assay in which excess of unlabelled β_2-microglobulin (140-times the labelled β_2-microglobulin concentration) was added to the incubation medium. Radioactivity bound to filters was counted in a Packard gamma scintillation spectrometer.

Protein content of the incubation medium was low (about 0.003 g/100 ml). In order to restrict non-specific binding of β_2-microglobulin to the inner surface of incubation tubes, assays were carried out in albumin-coated glass tubes (glass tubes washed with 10 g/l fraction V bovine albumin, then rinsed three times in distilled water). Under these conditions, non-specific binding of β_2-microglobulin to incubation tubes was about 2.5% of total β_2-microglobulin. Degradation was determined in the 20 μl aliquot by trichloroacetic acid precipitation in the following way: 500 μl of trichloroacetic acid (20% (w/v)) were added to 500 μl of a mixture of aliquot and stop solution; after centrifugation 500 μl supernatant were counted for radioactivity. β_2-Microglobulin degradation in the absence of membrane was determined in the above-cited blank assay.

The validity of the binding measurements was checked by using the following controls: (1) Addition of 200 nM sodium iodide to the incubation medium (10 000-times over free radioactive iodide introduced with labelled β_2-microglobulin did not decrease radioactivity bound to filters, excluding the possibility of a significant binding of residual free iodide to membranes. (2) Dilution of the incubation medium by addition of 2 ml cold stop solution containing bovine serum albumin (20 g/l) partially removed membrane bound radioactivity (Fig. 1). In the case of standard filtration conditions (i.e. lag time of 5 s between stopping and filtration) β_2-microglobulin removal was estimated to be 10%.

Solubilization and analysis of membrane-bound radioactivity

In order to check that membrane bound radioactivity was labelled β_2-microglobulin, ten standard assays containing preincubated brush-border membranes (0.047 mg protein/ml), 200 nM β_2-microglobulin, 150 mM NaCl, 10 mM Hepes (pH 7.4) were incubated for 5 min at 37 °C then filtered on cellulose acetate filters as described above. Filters were layed by series of five in one ml cold NaCl, Hepes buffer containing Triton X-100 (0.1% or 0.25%). After 3 h at 4 °C, 300 μl of supernatant were applied on a S-300 Sephacryl gel column (0.7 × 80 cm) in the presence of 30 mM Tris-HCl buffer (pH 7.8), 0.1 M NaCl, 0.1% Tween 20. The remaining 700 μl of supernatant were dried overnight under vacuum in the presence of silica gel, then dissolved in 70 μl 0.0625 M Tris-HCl (pH 6.8) containing 2% sodium dodecyl sulphate, 10% glycerol, 0.1 M dithioerythritol and 0.03% Bromophenol blue. After 3 min at 100 °C, sample was applied to 15% polyacrylamide gel-sodium

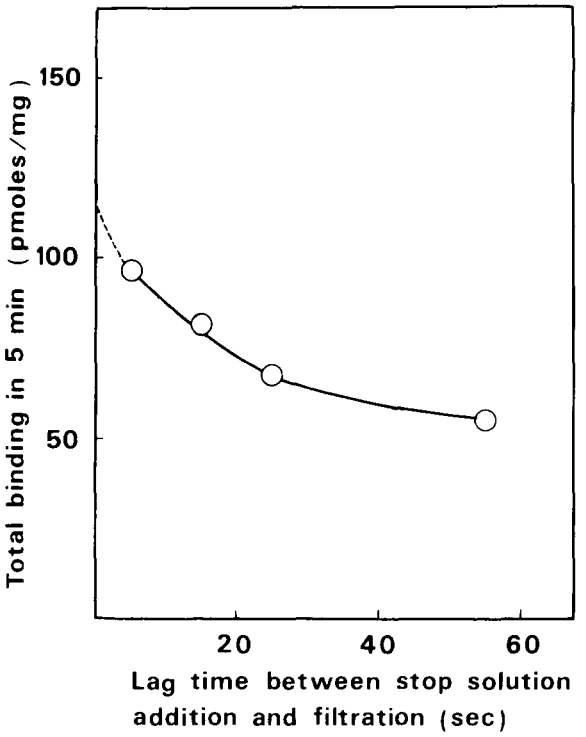

Fig. 1. Removal of bound β_2-microglobulin by stop solution. The assays were carried out in the standard conditions of preincubation and incubation in 150 mM NaCl, 10 mm Hepes (pH 7.4). 200 nM β_2-microglobulin, brush-border suspension 0.047 mg protein/ml. At the end of incubation time, the minimal lag time between addition of cold stop solution (150 mM choline chloride, 10 mM Hepes (pH 7.4), Fraction V bovine albumin, 20 g/l) and filtration process was 5 s. In this experiment several tubes were left aside at room temperature after addition of stop solution in order to obtain the total-lag time indicated in abscissa. The remaining of the filtration process was carried on as usual.

dodecyl sulphate, 0.1% and fractionated by electrophoresis according to the procedure of Laemmli [28]. The electrophoresis plate was then stained with Coomassie blue, dried and autoradiographed at −70°C on XO MAT R KODAK film with fluorescent screen (Chronex-Lighting Plus (Du Pont)).

Results

1. Binding measurements

β_2-Microglobulin binding was linear versus brush-border membrane content in the incubation medium up to 0.10–0.20 mg protein/ml (Fig. 2).

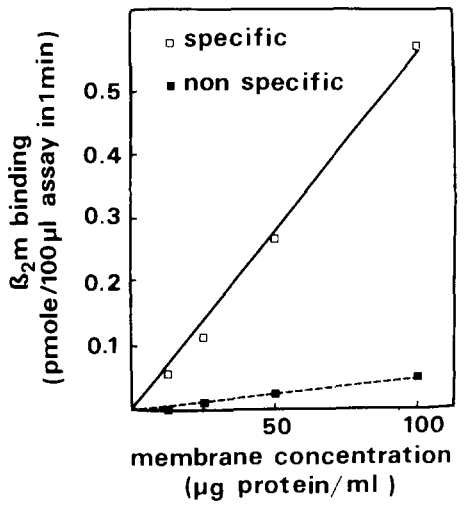

Fig. 2. Linearity of β_2-microglobulin (β_2m) binding versus membrane protein content. Brush-border membranes in indicated concentrations were preincubated at 37°C in 150 mM NaCl, 10 mM Hepes (pH 7.4). Labelled β_2m (200 nM) was then added in presence (non-specific binding, ■) or in absence (total binding) of 140-times excess of unlabelled β_2-microglobulin. Incubation was stopped after 1 min and the assay was filtered as described in Methods. Specific binding (□) = total binding − non-specific binding.

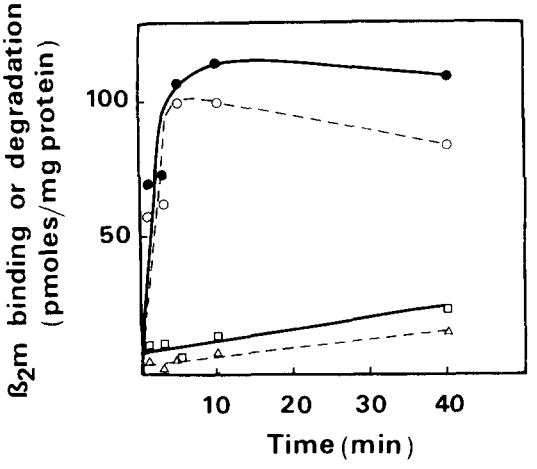

Fig. 3. Time course of β_2-microglobulin (β_2m) binding or degradation by brush-border membranes. Brush-border membranes (0.062 mg protein/ml) were equilibrated 10 min at 37°C in 150 mM NaCl, 10 mM Hepes (pH 7.4). Labelled β_2-microglobulin (200 nM) was then added in the presence (non-specific binding, □) or in absence (total binding, ●) of 70-times excess of unlabelled β_2-microglobulin. Incubation was stopped at the times indicated, binding and degradation (△) were determined as described in Methods. Specific binding (○) = total binding − non-specific binding. Binding data are representative of three experiments with three different preparations.

At higher membrane concentrations, binding plateaued whatever was the type of filters used (cellulose acetate 0.2 or 0.5 μm pore size or cellulose nitrate 0.65 μm pore size). Cellulose acetate was chosen for further experiments because of its lower adsorptive capacity for free labelled β_2-microglobulin. Non-specific β_2-microglobulin binding to brush-border membranes was also linear in this range of concentration but accounted only for a small fraction of total β_2-microglobulin binding (2.5%).

Time course measurements (Fig. 3) showed that specific binding was rapid since maximal binding was reached after 5 to 10 min. Non specific binding was slow and linear until 40 min. Similary degradation was slow, linear and negligible during that time and at that brush border concentration. Taken together, these data show that the best measurement conditions for β_2-microglobulin binding were a low brush-border membrane concentration and a short incubation time: in these conditions specific binding was high whereas non specific binding and degradation were low. Binding was influenced by temperature (Table I) since specific binding was three times higher at 37°C than at 20°C and even higher at 45°C. Non specific binding remained negligible whatever was the temperature.

Working in these near physiological conditions (37°C, 150 mM NaCl (pH 7.4), low content of albumin) the following quantitative data were obtained: binding rate at 200 nM β_2-microglobulin was 54.8 ± 11.7 pmol/min per mg protein (mean ± S.D. of eight experiments with three different preparations). Binding rate at 400 nM β_2-microglobulin was 94.2 pmol/min per mg protein (mean of two experiments with two different preparations). Binding at 10 min (i.e. in the plateau region) was 71 pmol/mg protein mean of three experiments with two preparations with 200 nM β_2-microglobulin and 100 pmol/mg protein (mean of two experiments with one preparation) with 400 nM β_2-microglobulin.

TABLE I

TEMPERATURE EFFECT ON β_2-MICROGLOBULIN BINDING

Brush-border membranes (0.05 mg protein/ml) were preincubated 10 min at the indicated temperature in 150 mM NaCl, 10 mM Hepes (pH 7.4). Labelled β_2-microglobulin (200 nM) was then added in presence (non-specific binding) or in absence (total binding) of 140-times excess of unlabelled β_2-microglobulin. Incubation was stopped after 1 min and the assay was filtered as described in chapter Methods. Specific binding = total binding − non specific binding.

Temperature (°C)	β_2-Microglobulin binding in 1 min (pmol/mg protein)	
	specific	non specific
0	15	3
20	26	3
37	79	10
45	98	11

2. Relationship between specific binding of β_2-microglobulin and aminopeptidase activity

In an attempt to locate binding sites within the cell, specific binding of β_2-microglobulin was measured in aliquots of different fractions obtained in the course on the brush-border preparation, and

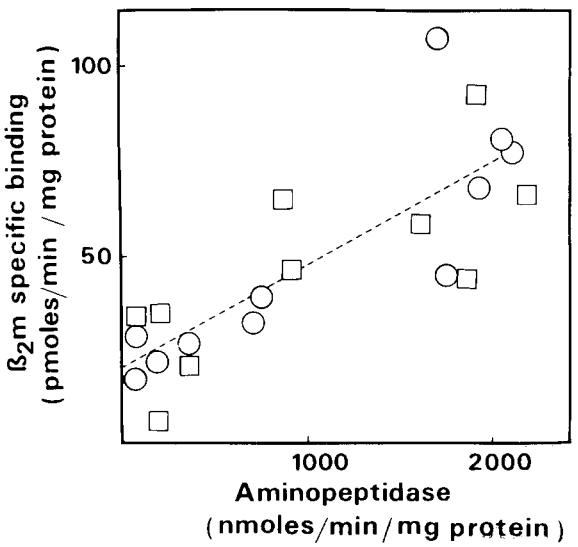

Fig. 4. Relationship between specific binding of β_2-microglobulin (β_2m) and aminopeptidase activity in different fractions obtained in the course of a brush-border preparation. 20-μl aliquots of different fractions obtained in the course of a brush-border preparation (supernatant or resuspended pellet) were incubated and filtered in the standard assay conditions (see Fig. 3). The protein content ranged between 0.037 and 0.063 mg/ml. Incubation time was 1 min. Presented data are drawn from two different preparations (○ and □, respectively. The broken line represents the correlation line obtained by the least-squares method.

plotted versus aminopeptidase activity (Fig. 4). Gaussian analysis of correlation is not possible in this type of sampling where a some samples are subfractions of others (i.e. data are not independent from each other). Nevertheless some conclusions may be drawn from the observation of Fig. 4. Aminopeptidase activity and specific binding activity are to some extent co-purified since fractions with the highest aminopeptidase content are also those with the highest binding ability. However, the ordinate of the regression line (mean straight line obtained by the least-squares method) is far above zero, indicating that some fraction with low aminopeptidase content can also bind β_2-microglobulin. The relationship between binding and alkaline phosphatase (not shown) was similar to that of binding and aminopeptidase. Besides measurements of non-specific binding indicated that it represents a greater fraction of total binding in homogenate than in final brush-border fraction (homogenate: 40%; brush-border fracton 10%, mean of two experiments).

3. Solubilization of membrane-bound radioactivity

In order to characterize radioactivity bound to membranes after filtration, binding assays and filtration were carried out as described above, then filters treated by Triton X-100, a neutral detergent.

(a) After solubilization with 0.1% Triton, Triton extract was chromatographed in the presence of 0.1% Tween-20. Fig. 5A shows that after 5 min of incubation, solubilized membrane bound radioactivity was found mainly in a peak of intermediate molecular weight between albumin and IgG. Control chromatography of pure β_2-microglobulin treated with 0.1% Triton (not shown) showed that in the same experimental conditions β_2-microglobulin did not form aggregates and did not bind to bovine serum albumin. Besides, an aliquot of the solubilized extract was treated by 0.1 M dithioerythritol (reducing cystinyl bonds) and 2% SDS (dissociating interchains bonds) for 3 min at 100 °C, then underwent a polycrylamide gel electrophoresis in the presence of SDS. Autoradiography of the electrophoresis (Fig. 6) showed that after dissociating treatment solubilized membrane bound radioactivity was found exclusively in the β_2-microglobulin position (12000 Da). Thus the high molecular weight radioactivity peak observed in chromatography (Fig. 5) was made of native β_2-microglobulin associated to a membrane structure.

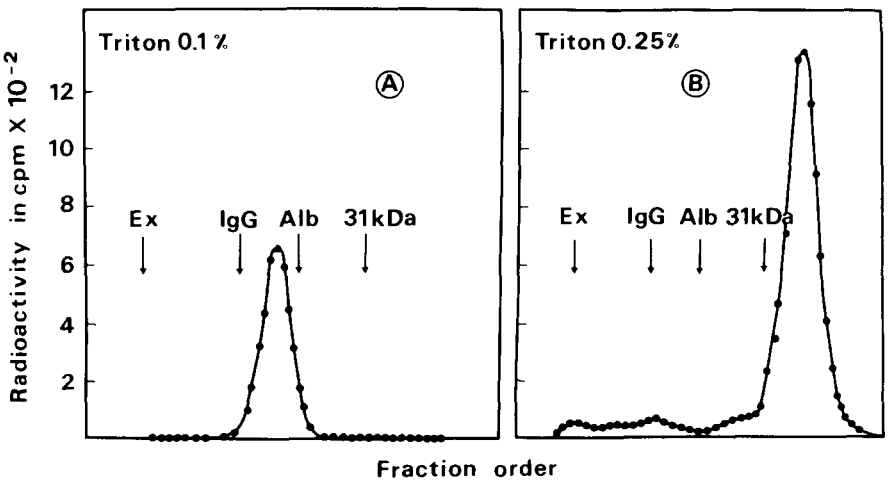

Fig. 5. Chromatography of solubilized bound radioactivity. Ten assays containing brush-border membranes (0.047 mg protein/ml) and labelled β_2-microglobulin (200 nM) were incubated for 5 min and then filtered on cellulose acetate filters (0.2 μm pore size) in standard procedure conditions (see Fig. 3). The filters were treated by Triton X-100 0.1% (A) or 0.25% (B) in 2 ml incubation buffer at 4 °C. 3 hours later 300 μl solubilization buffer were chromatographed on S-300 Sephacryl gel in the presence of Tris-HCl (30 mM) (pH 7.8), NaCl (0.1 M), Tween 20, 0.1%. The column was previously calibrated with the indicated molecular weight markers; Ex, excluded volume. 31 kDa was α_1-microglobulin.

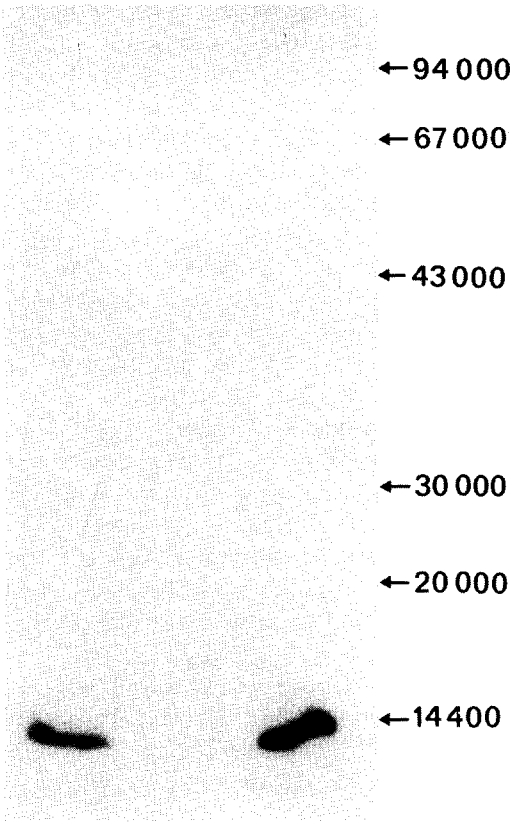

Fig. 6. Electrophoresis gel autoradiography of a membrane Triton extract treated by SDS and dithioerythritol. Brush-border membranes were incubated in the presence of labelled β_2-microglobulin and filtered as indicated in Fig. 5. Filters were then similary treated by 1% Triton as indicated in Fig. 5. The Triton extract was further treated by 2% SDS and 0.1 M dithioerythritol for 3 min at 100 °C, then it underwent an electrophoresis on 15% polyacrylamide gel containing 0.1% SDS. The electrophoresis plate was autoradiographed as indicated in Methods. The places of molecular weight markers on electrophoesis plate as revealed by Coomassie blue are indicated by arrow in the margin. The two lanes originated from duplicate layers.

(b) After membrane solubilization by 0.25% Triton, the extract was chromatographed in the presence of 0.1% Tween-20. A single peak of radioactivity was seen in the 12000 position (Fig. 5B). This result indicates that 0.25% Triton dissociates the complex β_2-microglobulin/β_2-microglobulin-binding membrane component, whereas 0.1% Triton does not. Moreover this corroborates the conclusion of autoradiography that is membrane bound radioactivity is made of intact labelled β_2-microglobulin. Total amount of radioactivity solubilized by 0.25% Triton was far above that solubilized by 0.1% Triton.

Discussion

Until now, few data were available on measurement of protein binding to renal brush-border membranes [29–31,13]. This is probably due to technical difficulties of handling low protein concentrations in vitro. The high adsorptive capacity of proteins on incubation tubes, pipettes, cone tips and filters led most investigators to incubate studied proteins in presence of large excess of bovine serum albumin (generally 0.1 to 1%, i.e. 1 to 10 mg/ml). This is far above the normal protein content of tubular fluid and since pilot studies showed that albumin might interfere with β_2-microglobulin binding (see also Fig. 1 and Methods) we aimed at minimizing the amount of albumin in our binding experiments. However, small amounts of albumin were still required to prevent the auto-irradiation process which occurs in the pure labelled β_2-microglobulin stock solution. Final albumin content in our binding experiments was 24 μg/ml, that is close to the estimated concentration of albumin in tubular fluid the β_2-microglobulin/albumin ratio in our experiments (1/10) was also close to that of normal tubular fluid. As Selenke and Foulkes [30] first noticed, we observed that pre-equilibration of brush borders with buffer at 37 °C was necessary for optimal binding. At low membrane concentrations, binding was linearly correlated with membrane content, thus insuring good conditions for binding measurement (Fig. 2). However, despite an excess of free labelled ligand, β_2-microglobulin binding plateaued at very low membrane concentrations if compared to those generally used in transport studies [32]. A possible explanation could be that brush-border membranes are by themselves competitors for β_2-microglobulin binding, possibly by means of their surface proteins. Competition would not occur in vivo where brush-border membranes are held on by a rigid cytoskeleton. It is by no means possible to compare membrane concentration in a soluble system to protein content in an in vivo system. However, the other parameters of our standard incubation con-

ditions were not very far from in vivo physiologic conditions: 200 nM β_2-microglobulin (normal content of rat tubular fluid in rat 400 nM β_2-microglobulin), 0.15 M NaCl (pH 7.4), temperature 37°C and low content in serum albumin. The main disparities were absence of divalent cations, ion transmembranal gradients and other low-molecular-weight proteins. In our simplified conditions binding rate was 55 pmol/min per mg protein, that is far above values obtained for insulin binding in previous studies made in the presence of albumin [29,31].

In order to test the likelihood of our in vitro binding data versus in vivo reabsorption data in the same biological model [15,4], we may estimate the binding rate of β_2-microglobulin in the living animal taking aminopeptidase enrichment factor as an index for quantitating brush border amount in whole homogenate.

(1) Initial rate of binding was about 95 pmol/min per mg protein at physiological β_2-microglobulin level.

(2) Mean aminopeptidase enrichment factor was 9.5. Thus the fraction of renal cortical homogenate proteins made of the considered brush border proteins was 1/9.5 (assuming that aminopeptidase is an ideal marker). Given that renal cortical homogenate proteins from one rat of 200 g averaged 140 mg (mean of seven preparations), mean brush border protein content in a rat of 200 g must be 140/9.5 = 14.7 mg.

(3) According to the above estimates, in a living animal of 200 g, the binding rate should be 95 × 14.7 = 1400 pmol/min.

(4) Given that glomerular filtration rate of a 200 g rat is about 2 ml/min and that β_2-microglobulin is almost completely taken up by the kidney, normal tubular uptake is about 400 nM × 2 ml/min = 800 pmol/min.

The above estimation must be taken with care because membrane content in living animal is only a gross approximation. Furthermore our in vitro assay was only a simplified model of in vivo conditions. Nevertheless the measured binding rate seems to be sufficiently high to account for a biological phenomenon implicated in the reabsorption process. Parallel increase of binding ability with aminopeptidase activity in several fraction of the membrane preparations as well as decrease of non-specific binding ratio in brush border versus homogenate suggest that a specific binding structure is at least partly associated with brush border. Taken together these results indicate that the in vitro measured binding resemble the in vivo process as it was predicted from whole organ studies.

Binding plateaued at 5 or 10 min and further slightly decreased, probably because of degradation of bound β_2-microglobulin. Degradation was a very slow process in our in vitro conditions (Fig. 3) whereas other investigators reported conditions where insulin degradation by brush-border membranes was much faster than binding [29]. This must be due in part to the much faster binding observed in our albumin near-free assay, and in another part to the very different membrane/ligand ratio we used. Since binding rate is of the same magnitude as reabsorption rate our results suggest that brush-border proteases play a minor role in degradation of proteins as was previously stated [2,15].

Since binding data provide an estimate of the amount of β_2-microglobulin to brush-border membranes in equilibrium conditions, i.e. at 10 min, we may try to calculate the amount of membrane that would be internalized if β_2-microglobulin was endocytosed with the totality of the membrane to which it binds: reabsorption rate being 800 pmol/mg per min, and the amount of bound β_2-microglobulin being about 100 pmol/mg in vitro with physiological β_2-microglobulin content, 8 mg membrane protein would have to be internalized each min. Assuming the above estimated whole animal brush-border content, this evaluation attributes to the entire luminal membrane a turnover rate of about 2 min. This is well above the so far known values for membrane turnover during endocytosis: macrophages and BHK cells internalize the totality of their membrane within 30 min [33,34], fluid phase endocytosis rate of cultured Madin Darby canine kidney cells is slower than that of macrophages [35]. Therefore it would seem unlikely that simple endocytosis could account for the reabsorption rate of β_2-microglobulin. Several studies on other cell types have shown that endocytosed proteins might laterally move on the cell surface and cluster in coated pits before internalization. Clustering accelerates internalization of proteins and is probably mediated by

a membrane receptor [25]. Data shown in Fig. 4 suggest that a cellular structure other than brush border might bind β_2-microglobulin, raising the question of internalization of the β_2-microglobulin binding structure. Such a binding and internalization process was already stated in other cell types for several proteins on the basis of histological and biochemical studies [33,25]. It was also suggested by histological studies in kidney brush border after injection of labelled proteins [11,12].

The second part of our study shows that a β_2-microglobulin binding component was demonstrable in the brush-border membrane fraction. When membranes were solubilized by 0.1% Triton X-100, bound radioctivity was shown to be native labelled β_2-microglobulin, and β_2-microglobulin was associated with a Triton-soluble membrane component. In fact, kidney brush border membrane was shown to contain at least five different peptidases [36,37] that are probably able to bind β_2-microglobulin. However, chromatography of bound radioactivity revealed a unique peak: this is explained by the very low protease activity in our binding conditions. Besides, β_2-microglobulin binding to other membrane components more labile in 0.1% Triton cannot be excluded. The occurrence of a protein binding membrane structure in kidney brush border has already been postulated before [2,33]. It was also suggested by the high β_2-microglobulin-binding rate (see above) and by the co-purification of aminopeptidase and β_2-microglobulin binding ability (Fig. 4). Receptors for native or modified albumin were identified in liver [38] and in kidney basolateral membrane [39]. However, this is to our knowledge, the first direct evidence for a protein binding brush-border component in kidney.

In conclusion the presented work shows that kidney brush-border membranes are able to rapidly bind β_2-microglobulin on a membrane component. It provides a method to determine binding rate with experimental conditions close to in vivo status. The binding is proportional to membrane content and thus affinity can be measured with accuracy. Affinity and specificity of the binding will be described in another report.

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