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Properties of a Synthetic Plasma Membrane Marker: Fluorescent-Mercury-Dextran

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Summary. Aminoethyl-Dextran T 10 (mol wt approx. 10,000) was conjugated with *p*-Chloromercuribenzoic acid (*p*CMB) and labeled with fluorescein isothiocyanate (FITC). This coupling procedure does not affect the mercurial function of *p*CMB moiety of Fluorescent Mercury Dextran T 10 (FMD) since on the basis of mercury content its K_i-value for the $(Na^+–K^+)$-ATPase from rat kidney plasma membranes is identical with the K_i-value of unconjugated *p*CMB (3×10^{-6} M). FMD binds to plasma membranes if applied in vivo, which could be shown in experiments in which rat kidneys were perfused with FMD and the plasma membranes isolated after the perfusion. The membrane-FMD complex is stable during common isolation steps such as differential centrifugation, sucrose density gradient centrifugation and free-flow electrophoresis. This was shown by in vitro binding studies of FMD with isolated plasma membranes from rat kidney cortex. FMD may be removed from the plasma membranes by the addition of 1×10^{-4} M dithiotreitol. Since F-aminoethyl-Dextran T 10 (without *p*CMB) does not interact with the plasma membranes, it is suggested that the binding of FMD to plasma membranes may involve a Hg–SH reaction. FMD does not penetrate into rat kidney cells in contrast to *p*CMB, but can cross capillaries. Thus, FMD seems to be suitable (a) to label luminal and contraluminal surfaces of plasma membranes of epithelial structures, and (b) to be used as a fluorescent marker for plasma membranes during succeeding isolation procedures.

Epithelia from organs with transcellular transport processes possess a marked polarity in their morphological appearance and in the properties of their plasma membranes. To study the chemistry of the membrane-bound transport mechanisms in epithelia, the preparative isolation of membranes lining the luminal space and those in contact with the interstitium is of special value. For this purpose, specific properties of the membranes such as marker enzymes can be utilized. If this is not possible, artificial markers

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which can be applied to the plasma membrane from one side of the cell only may be helpful.

The synthesis of such a marker consisting of a Dextran molecule with a molecular weight of 10,000 to which *p*CMB and fluorescein are attached is described in this work. The interaction of this compound with plasma membranes of rat kidney cortex was studied in vivo and in vitro. After perfusion of kidneys with FMD the binding of FMD to the plasma membranes can be demonstrated. Isolated plasma membranes also react with FMD in vitro. By comparing the distribution of the fluorescence and the membrane marker enzymes, alkaline phosphatase and $(Na^+–K^+)$-ATPase, during different isolation procedures, it could be shown that both brush border membranes and membranes of the basal infoldings react with FMD. The membrane-FMD complex is not dissociated by differential centrifugation, sucrose gradient centrifugation and free-flow electrophoresis. Since FMD may be removed from the membranes with high concentrations of dithiotreitol, it is possible to study the function and composition of the plasma membranes after isolation in a relatively unaltered state.

Materials and Methods

*Synthesis of p*CMB-*Dextran* T 10

Aminoethyl-Dextran T 10 (AED T 10) was prepared from Dextran T 10 (mol wt approx. 10,000) with 2-aminoethyl-hydrogensulphate according to Eldjarn and Jellum [6]. The coupling of AED T 10 with *p*-Chloromercuribenzoic acid (*p*CMB) was performed basically according to Ohta *et al.* [20] with the following modifications: Equimolar concentrations of *p*-Chloromercuribenzoic acid and 1,1′-Carbonyldiimidazole, based on the molar amino content of AED T 10, were dissolved in 30 ml of anhydrous dimethylsulfoxide. To this mixture, 2 g of AED were added and rapidly stirred at room temperature for about 6 hr. The reaction mixture was diluted with 10 ml of distilled water and washed three times with 150 ml diethylether. The aqueous phase was diluted to 150 ml and filtrated. After the ether had been volatilized, the solution was washed with a 50-fold volume of distilled water by ultrafiltration in a Diaflo ultrafiltration cell (Model 50) equipped with UM-2 membrane (Amicon Corp.). The substance was further purified on a Sephadex G 25 column (2.5 × 36 cm, elution fluid: 0.01 M NaCl) and again concentrated to 20 ml by ultrafiltration.

Dextran content was determined by the anthrone method [5] and the number of amino groups with 2, 4, 6-trinitrobenzene sulfonic acid by the method of Habeeb [8]. The mercury content was measured by the dithizone method according to the method of the Trade Hygienical Institute of the State of Norway, performed by Dr. Herrmann, analytical laboratory of the Farbwerke Hoechst, Frankfurt, Germany. The *p*CMB content in *p*CMB-Dextran T 10 could also be measured by the typical *p*CMB spectrum curve with a maximum at 242 nm. The results obtained showed the product to contain Dextran, amino groups and mercury in the following proportions: 1:3:1.5. The yield of the coupling procedure was about 60% referred to Dextran. *p*-CMB-Dextran T 10, stored at 4 °C, is stable for approximately 6 to 8 weeks.

*Conjugation of p*CMB-*Dextran* T 10 *with Fluorescein Isothiocyanate* (FITC)

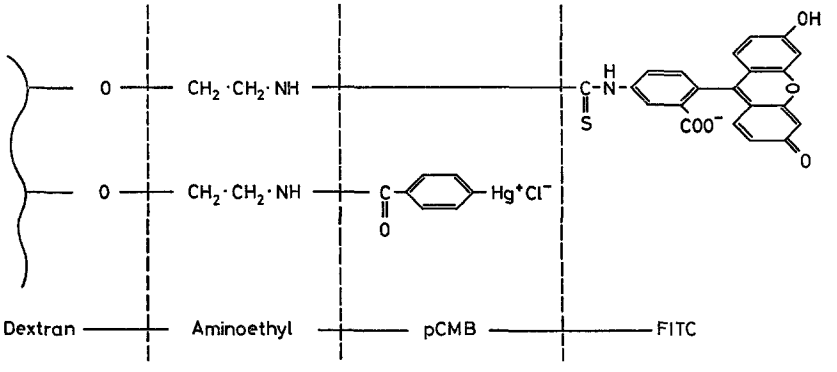
Equimolar concentrations of fluorescein isothiocyanate (FITC on celite) and *p*CMB-Dextran T 10, based on the nitrogen content of *p*CMB-Dextran T 10, where dissolved in 0.5 M HCO_3^-/CO_3^{2-} buffer (pH 9.0 at 20 °C) [22]. The reaction mixture was stirred for 1 hr at 20 °C. The product was purified by gel filtration using a Sephadex G 75 column followed by washing the eluate by ultrafiltration. In the final product Dextran T 10 [5], mercury and fluorescence were determined. FMD (*see* Scheme 1) can be separated from nonfluorescent *p*CMB-Dextran T 10 by column chromatography on DEAE-cellulose. The final product contains FITC/Hg/Dextran in a ratio 1:1.5:1. The fluorescence measurements were made with a Farrand spectrophotometer (Farrand Optical Co.) using FITC-solution as standard. Emission and excitation spectra were recorded for all mixtures. All data presented were measured at a wavelength of 495 nm for excitation and 530 nm for emission. For binding studies, only fresh conjugated solutions of FITC–*p*CMB-Dextran = Fluorescent Mercury Dextran (FMD) were used. When FMD was used to label plasma membranes, the unspecific "fluorescence" of the membrane proteins was measured under identical conditions and this value was subtracted from the total fluorescence (*see* Fig. 1).

Isolation of Plasma Membranes from Rat Kidney Cortex

Cell membranes of rat kidney cortex were isolated by differential centrifugation in an isotonic sucrose medium as previously described [13]: ST-buffer (250 mM sucrose, 10 mM triethanolamine, pH 7.6 at 20° C) was used during the homogenization, centrifugation and labeling procedures. The enrichment of plasma membrane bound enzymes, alkaline phosphatase and $(Na^+–K^+)$-ATPase, compared to the starting material was 8 and 5, respectively. The plasma membranes obtained by this procedure were used for labeling studies with FMD.

Labeling of Renal Plasma Membranes with FMD *in vivo*[1]

To study the binding of FMD to renal plasma membranes in vivo, kidneys of anesthesized rats were perfused in the following manner: A polyethylene catheter was in-



Scheme 1. Structure of FMD

1 These experiments were performed together with G. Rumrich whose skillful assistance is gratefully acknowledged.

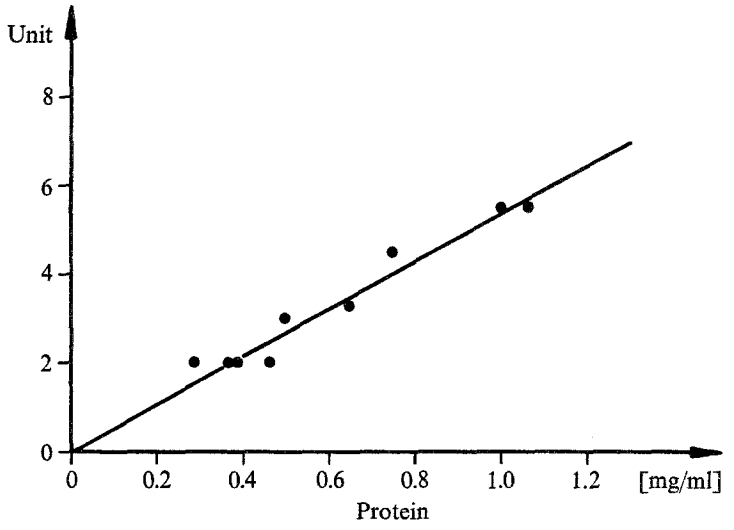


Fig. 1. "Fluorescence" of unlabeled membrane proteins in isotonic sucrose medium (250 mM sucrose, 10 mM triethanolamine-HCl, pH 7.6 at 20 °C), measured at 495 nm and 530 nm. One unit corresponds to the fluorescence of 1.25 pmoles fluorescein isothiocyanate (in ST-buffer, pH 7.6 at 20 °C)

serted into the aorta in the region of the arteria renalis. Then the aorta was ligated and the kidneys were allowed to drain by opening the vena renalis. The perfusate, a modified Ringer's solution which was oxygenated and at body temperature, was introduced at a rate of 30 ml/min. A hydrostatic pressure of 145 cm of water was maintained throughout the perfusion. Control animals were perfused for 5 min. Animals receiving FMD were perfused by the following scheme: The kidneys were first perfused with the modified Ringer's solution for a period of 1 min, then the same solution containing FMD at a concentration of 1×10^{-4} M based on mercury content, was introduced at a flow rate of 16 to 30 ml/min for 30 sec. The perfusion was then stopped for 1 min and this procedure was repeated once. Finally, the kidneys were perfused with the modified Ringer's solution again for 1 min to remove any unbound FMD.

From both kinds of kidneys plasma membranes were isolated as previously described and the fluorescence (495 and 530 nm) and the protein content of the plasma membrane fractions were determined.

Labeling of Plasma Membranes with FMD *and Studies on the Stability of the* FMD-*Membrane Complex*

Continuous Sucrose Gradient. 0.5 ml of the plasma membrane fraction containing about 7 mg of membrane protein was incubated with 0.1 ml FMD-solution (mercury content 6×10^{-5} M) in ST-buffer for 5 min at room temperature. This membrane solution was placed on top of a continuous sucrose gradient which was prepared from 5 ml 31 % and 5 ml 41 % sucrose in triethanolamine-buffer (10 mM, pH 7.6 at 20 °C) using the method of Martin and Ames [19]. In some experiments Fluorescein-Aminoethyl-Dextran T 10 containing no *p*CMB was used instead of FMD. To study the reversibility of the binding of FMD to the membranes, dithiotreitol (2×10^{-4} M) was added to labeled

membranes prior to the centrifugation. Centrifugation (90 min or 18 hr at 105,000 × *g*) was done in "Strohmaier cells" which permit the separation of 11 successive fractions, number 1 corresponding to the top of the gradient. In each fraction the content of protein, alkaline phosphatase, (Na^+–K^+)-ATPase and the fluorescence were measured.

Free-Flow Electrophoresis. Plasma membranes were subdivided by preparative free-flow electrophoresis into brush border membranes and membranes of the basal infoldings as previously described [9]. The membrane fractions were incubated with 1×10^{-5} M FMD for 5 min at room temperature. The membrane suspension was then diluted 10-fold with ST-buffer and centrifuged at 35,000 × *g* for 20 min. The sediments were taken up in chamber-buffer (250 mM sucrose, 10 mM triethanolamine, 10 mM acetate, pH 7.4 at 20 °C) and reelectrophorized separately. In the fractions obtained by the electrophoresis, protein content, alkaline phosphatase and (Na^+–K^+)-ATPase activities and fluorescence were determined. In control experiments Fluorescein-Aminoethyl-Dextran T 10 was used instead of FMD.

Enzyme Assays

(a) Alkaline phosphatase (E. C. 3.1.3.1) activity was determined by the Merckotest® method in the presence of 0.5% bovine albumin.

(b) The activities of Mg^{2+}-ATPase and (Na^+–K^+)-ATPase (E. C. 3.6.1.3) were measured as described by Kinne *et al.* [14]. The fractions obtained from the continuous sucrose gradient and from the electrophoresis experiments were treated with desoxycholate and ethylene-diamintetraacetate-sodium salt [12]. *p*CMB, *p*CMB-Dextran T 10 as well as FMD were added to the reaction mixture containing the pretreated membranes immediately before the incubation started. The inorganic phosphate liberated was determined by a modification of the methods described by Bartlett [1] and Fiske and Subbarow [7].

The protein content in the fractions was measured after precipitation of the protein by 10% TCA in the cold and dissolution of the precipitate in 1 N NaOH according to Lowry *et al.* [17].

Materials

p-Chloromercuribenzoic acid and 1,1'-Carbonyldiimidazol were obtained from Fluka, Frankfurt-Eschborn. Anhydrous dimethylsulfoxide (stored over molecular sieve 4 Å, Merck, Darmstadt), diethylether and 2-aminoethyl-hydrogensulphate were purchased from Merck, Darmstadt. Dextran T 10, Sephadex G 25 fine, and Sephadex G 75 were obtained from Pharmacia, Frankfurt and FITC on celite from Calbiochem, Switzerland.

Results

Chemical Properties of FMD

Solubility. FMD is soluble up to concentrations of 10 mmoles/liter; the aqueous solution has a pH of 7.0 and the substance is stable in solution for at least 10 weeks. This could be determined by comparing the mercury content of the solution immediately after synthesis and passage over Sephadex and the mercury content after storage for 10 weeks at 4 °C and

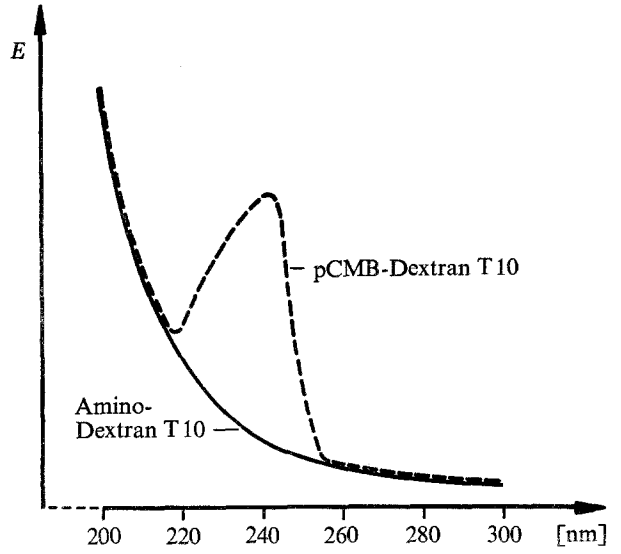


Fig. 2. UV-spectrum of Aminoethyl-Dextran T 10 (——) and *p*CMB-Dextran T 10 (-----) in 0.010 M phosphate buffer (pH 7.0 at 20 °C)

passage over Sephadex again. The values obtained were 1.45 mercury/1 Dextran, for the newly synthesized FMD and 1.33 mercury/1 Dextran, for the stored product.

UV-Spectrum. As shown in Fig. 2, the UV spectrum of *p*CMB-Aminoethyl-Dextran T 10 as compared to the spectrum of Aminoethyl-Dextran T 10 exhibits a new peak which corresponds to the extinction maximum of free *p*CMB. The height of this peak correlates with the amount of *p*CMB bound as determined by direct mercury analysis.

Fluorescence. The maximum of excitation and emission are 495 and 530 nm for the bound fluorescein molecule. This is slightly different from the fluorescence of the free FITC compound (490 and 530 nm).

Biological Activity of FMD

Action on SH-*Group Containing Enzymes.* As an example of an SH-group containing plasma membrane enzyme, (Na^+-K^+)-ATPase was chosen. It has been shown by Skou and other authors [15, 24] that this enzyme can be inhibited effectively with SH-reagents. As shown in Fig. 3, *p*CMB and FMD inhibit the (Na^+-K^+)-ATPase to the same extent if compared on the basis of their mercury content. At 1×10^{-4} M mercury the activity of the enzyme is completely abolished; the mercury concen-

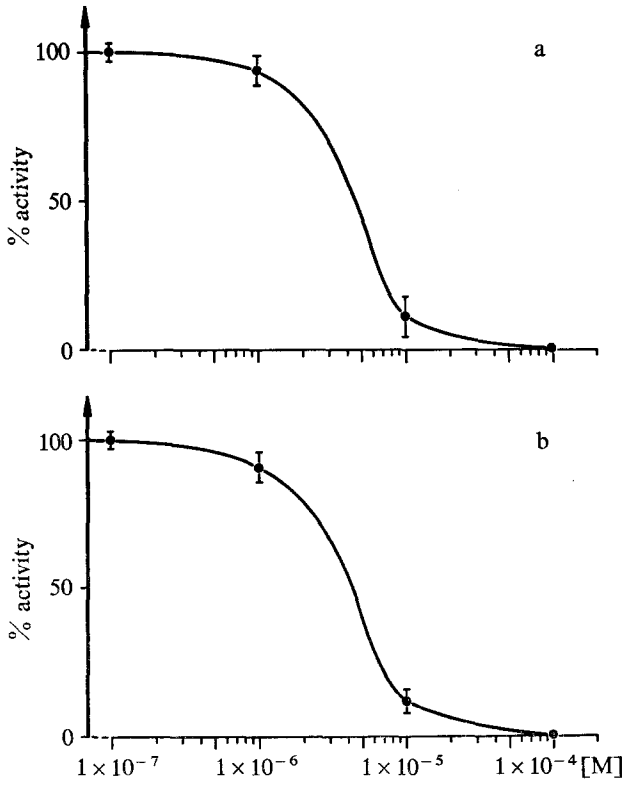


Fig. 3. Inhibition of plasma membrane (Na^+–K^+)-ATPase from rat kidney cortex: (a) by *p*CMB, and (b) by *p*CMB-Dextran T 10. The concentration given refers to the organic mercury content of the solution. Activities are given in percent of control, each point representing the mean (± SE of mean) of 4 determinations

tration for half maximal inhibition is 3×10^{-6} M which corresponds well with the values given by the above-mentioned authors [15, 24]. In control experiments neither Dextran T 10 nor Aminoethyl-Dextran T 10 showed any inhibitory effect on the (Na^+–K^+)-ATPase activity. The inhibition can be reversed by cysteine, indicating a mercury-SH-group interaction.

Binding of FMD *to Plasma Membranes in vivo.* To demonstrate the binding of FMD to plasma membranes in vivo, the fluorescence of the membranes prepared from the kidneys perfused with the modified Ringer's solution (control) and those obtained from FMD-perfused kidneys were compared. It was found that the plasma membranes of the FMD-perfused kidneys contained 11 *p*moles FMD/mg protein, if the fluorescence was measured at the optimal wavelength of 495 nm for excitation and of 530 nm for emission and corrected for the light-scattering and fluorescence on the

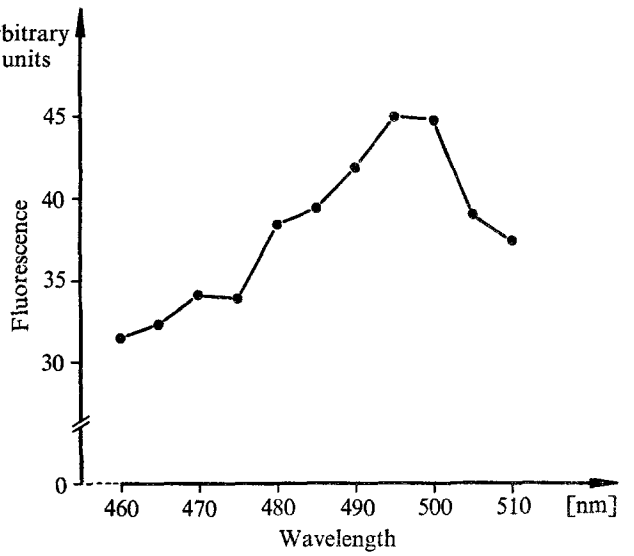


Fig. 4. Fluorescence of plasma membranes isolated from rat kidneys which were perfused with FMD in vivo. At a constant analyzing wavelength of 535 nm the excitation spectrum was recorded and corrected for the unspecific protein fluorescence. The curve shows the typical fluorescence spectrum of FMD standard solution. 45 arbitrary units correspond at 495/535 nm, 11 pmoles FITC/mg protein

protein basis of the control membranes. Since at this low intensity level of fluorescence the correction was about 50% of the fluorescence measured, the spectra of the FMD-treated and control membranes at equal protein levels were determined. Using a wavelength of 535 nm for the analyzing system, the typical excitation spectrum of FMD could be obtained (*see* Fig. 4) in contrast to the control membranes.

Binding of FMD *to Isolated Plasma Membranes.* To demonstrate the binding of FMD to isolated plasma membranes, membranes were incubated with FMD and then separated from the uncoupled FMD by density gradient centrifugation. As shown in Fig. 5, alkaline phosphatase, $(Na^+–K^+)$-ATPase and protein show the same distribution which also coincides with the peak of the fluorescence. The first fraction of the gradient (not shown in Fig. 5) contains a large amount of FMD either in a free form or bound to soluble proteins. The binding of FMD to plasma membranes withstands long periods of centrifugation. After centrifugation for 16 hr instead of 90 min, the fluorescence of the plasma membrane peak decreases only by 25%. Control experiments with FITC-Aminoethyl-Dextran T 10 (without *p*CMB) show no fluorescence in the plasma membrane peak of the gradient (*see* Fig. 5); all fluorescence remains on top of the gradient.

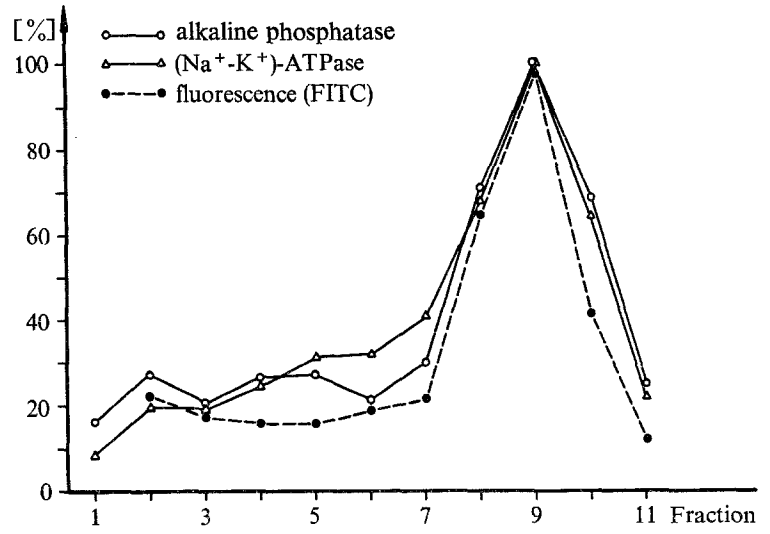


Fig. 5. Distribution of plasma membrane enzymes alkaline phosphatase and (Na^+–K^+)-ATPase and fluorescence (495 nm/530 nm) in the continuous sucrose gradient (31 to 41 %) 0.5 ml of plasma membranes (corresponding to about 7 mg protein) were incubated with 0.1 ml 6×10^{-5} M FMD (based on the mercury content) for 5 min at room temperature. Thereafter the incubation mixture was layered on top of the gradient and centrifuged for 90 min at 105,000 × *g*. Data are expressed as percentages of the maximal enzyme activities or of the fluorescence measured in fraction 9 of this gradient. The fluorescence on top of the gradient representing both the amount of uncoupled FMD as well as of FMD bound to soluble proteins is not shown in this figure. The fluorescence is corrected for the fluorescence obtained with unlabeled membranes using the standard curve given in Fig. 1 (for 1 mg protein maximal 20% of the fluorescence measured). The means of 4 experiments are given

Fig. 6 demonstrates that the binding of FMD is reversible by dithiotreitol. If labeled membranes are treated with this thiol at a concentration 20 times higher than that of FMD prior to centrifugation, the fluorescence of the plasma membrane peak is reduced to 25% of the control with FMD alone. The amount of FMD released from the membranes is recovered on top of the gradient. The amount of FMD which, under these conditions, is not removed by dithiotreitol may either have a very high affinity to the membranes or may be enclosed in membrane vesicles formed during the incubation procedure.

The binding of FMD to plasma membranes could also be demonstrated in the free-flow electrophoresis. This technique makes it possible to study the interaction of FMD with a membrane derived from the luminal face of the cell – in the proximal tubule this is the brush border – and to investigate the binding of FMD to the membranes of the basal infoldings of

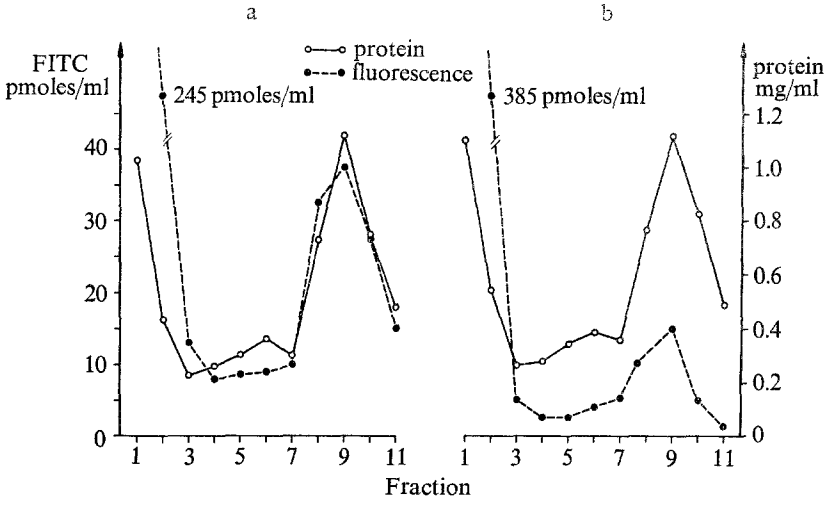


Fig. 6. Distribution of protein and fluorescence (495 nm/530 nm) in the continuous sucrose gradient (31 to 41%) after incubation of plasma membranes (*see* legend of Fig. 5) with FMD. In Fig. 6*b* dithiotreitol was added to the sample after incubation with FMD

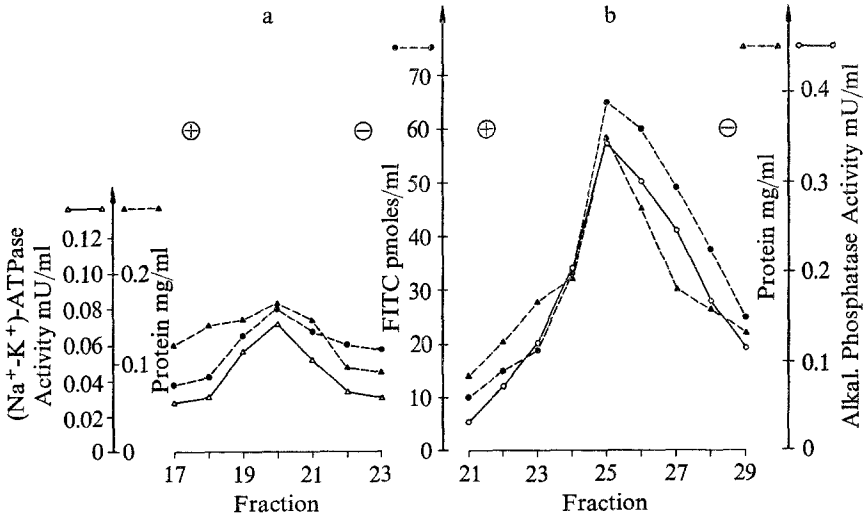


Fig. 7. Binding of FMD to luminal and contraluminal surface plasma membranes of rat kidney. The membrane fractions obtained by free-flow electrophoresis [9] were pooled separately, incubated with 1×10^{-5} M FMD for 5 min at room temperature, washed with ST-buffer and reelectrophorized, as described under Methods. (*a*) Distribution of (Na^+–K^+)-ATPase, protein and fluorescence (495 nm/530 nm) after the second run. (*b*) Distribution of alkaline phosphatase, protein and fluorescence after the second run

the proximal tubule facing the interstitium. As shown in Fig. 7, FMD binds to brush border membranes (marker enzyme alkaline phosphatase) as well as to membranes of the basal infoldings which contain the $(Na^+–K^+)$-ATPase. It is also worthwhile to mention that the concentration of free FMD which dissociates from the membranes during the incubation procedure used to measure the $(Na^+–K^+)$-ATPase activity in fractions obtained after sucrose gradient centrifugation or free-flow electrophoresis is too small to inhibit the enzyme. Addition of 1 mM cysteine to the incubation mixture does not increase the $(Na^+–K^+)$-ATPase activity of these fractions.

Discussion

The approach used in the presented study follows the lines elaborated by Maddy [18] to label externally exposed membrane components with small molecules. Compounds used for this purpose should have the following properties [18]: (1) be nonpermeant; (2) react under physiological conditions; (3) be detectable in small amounts.

In addition, Hoelzl-Wallach [11] added the requirement of no or minimal perturbation of membrane function by this label. According to these criteria, several reagents of small molecular size have been synthesized [2, 3, 4, 21].

The ambiguities inherent in the use of low-molecular-weight labels and the laboriousness of evaluating their permeabilities have fostered the development of techniques where suitable macromolecules are used as carriers covalently coupled to the labeling reagent [16, 29]. However, the use of polypeptides as such carrier molecules introduced the additional problems of polyfunctionality and size polydispersity.

The use of carbohydrates [10] such as Dextran as a carrier reduces markedly the number of functional groups capable of reacting with the membrane [11]. The polydispersity can be diminished by separation of the polymer on Sephadex column. In addition, Dextran is water soluble, stable in the physiological pH range and nontoxic for mammalian cells as demonstrated by its wide use as a plasma expander in medical care.

Since SH-groups in biological membranes have been shown to be very reactive with organic mercurial compounds [23], we have coupled the SH-reagent *p*CMB with Dextran according to Ohta *et al.* [20]. In contrast to these authors [20] who used Dextran of a large molecular size for studies on single erythrocyte cells, we wanted to synthesize a compound which could be applied via the capillary system from the interstitial site of the cell in organs such as kidney, pancreas, intestine and gallbladder, etc., without crossing the epithelial layer. Therefore, we reduced the molecular weight

of Dextran to approximately 10,000 which enables the molecule to penetrate the capillary wall as shown in the perfusion studies of FMD binding to plasma membranes in vivo. Such permeability could also be shown in experiments on the isolated perfused cat pancreas where *p*CMB-Dextran T 10 could inhibit the stimulatory action of secretin on electrolyte- and water secretion [28]. Secretin is known to function only from the interstitial surface.

Nevertheless, this molecule is large enough not to penetrate cell membrane of the kidney proximal tubules up to a contact time of 5 min. This is documented in microperfusion experiments of this organ in which *p*CMB-Dextran T 10 in contrast to *p*CMB at an equal concentration of mercury (2×10^{-4} M) does not inhibit the isotonic Na^+-transport [26]. This lack of inhibition cannot be due to the loss of the mercurial function of *p*CMB-Dextran T 10 since as shown in Fig. 3 the ability of both *p*CMB and *p*CMB-Dextran T 10 to inhibit the (Na^+–K^+)-ATPase is identical. To use *p*CMB-Dextran T 10 for labeling studies with plasma membranes, a fluorescent moiety was introduced into the compound to provide an easily detectable marker. Fluorescein was chosen because the coupling of this substance to *p*CMB-Dextran T 10 is easily achieved by the use of fluorescein isothiocyanate and the fluorescence characteristics of membrane protein and lipids do not interfere with the typical properties of the fluorescein molecule. Measuring the fluorescence of the membrane fractions' binding of FMD could be observed as well after incubation of isolated plasma membranes with FMD in vitro as after perfusion of kidneys with FMD-containing solutions. The amount bound under these two conditions differed markedly. In vitro, approximately 10^{-8} moles FMD/mg protein bind to plasma membranes which corresponds with the amount of N-ethylmaleimide (NEM) which is bound to the same membrane preparation [25]. This result is unexpected considering the difference in molecular size of FMD and NEM. The amount of FMD attached to the plasma membranes in vivo is much lower. Since the FMD complex is stable during common isolation procedures (*see* Results) due to the high affinity of Hg to SH-groups with dissociation constants of 10^{-8} to 10^{-11} M [27], the lower degree of labeling may reflect the different conditions present in vivo such as need of FMD to pass glomerula and peritubular capillaries which could reduce the effective concentration at the membranes. Furthermore, it may also be due to the fact that the number of SH-groups accessible in vivo from the outside is strikingly lower as similarly observed by Lengsfeld and Hasselbach [16].

The crypticity of SH-groups in the membranes may be one difficulty in using FMD as marker during isolation procedures for plasma mem-

branes. Another difficulty arises from the measurement of the fluorescence in the different fractions obtained during isolation. Despite the fact that fluorescein does not interfere with the fluorescence characteristics of membrane proteins and lipids, a high background due to light-scattering of the particle-rich solutions sometimes makes the detection of the specific fluorescence impossible. In our experiments an enrichment of the membranes by a factor of 5 to 8 compared to the starting material was obtained; this made it possible to measure the fluorescence at a background value of 50% of the total fluorescence found. The background can be reduced by several procedures. The one is a slight change in the analyzing wavelength in order to be able to record the fluorescence characteristics which otherwise fall in the region of light-scattering. Therefore, the excitation spectrum of FMD bound to membranes in vivo was recorded using the wavelength of 535 nm instead of 530 nm, thereby reducing the light-scattering to 10% of that value measured at 530 nm. Another possibility to reduce the unspecific background is to derive advantage from the reversibility of the FMD-binding by thiols. Thereby the fluorescence can be extracted from the membranes and be measured with very low interference of membrane particles.

A special problem for labeling plasma membranes from epithelial cell layers in vivo is to mark selectively either the luminal or the contraluminal membranes of the cell. In the case of the proximal tubule we could show that both the luminal (brush border membranes) as well as the contraluminal plasma membranes (membranes of the basal infoldings) do bind FMD. Therefore, the labeling of a specific cell membrane surface can be achieved by a selective application of FMD to one side of the epithelial layer.

In summary, FMD seems to be very suitable to label plasma membranes from the different areas in epithelial cells: It is not permeable, reacts under physiological conditions, carries a sensitive marker and causes a minimal perturbation of membrane function because of the reversibility of the reaction (in the presence of high concentration of thiols).

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