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INTRINSIC PROTEINS OF THE INTESTINAL MICROVILLUS MEMBRANE

IODONAPHTHYLAZIDE LABELING STUDIES

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Summary

Isolated brush border membranes of the intestinal epithelial cell were labeled with a hydrophobic photoactive compound [^{125}I]iodonaphthylazide. High incorporation of the radioactive naphthylazide was noted for molecular weight bands of 99 000, 86 000, 65 000, 54 000 and 30 000. Minimal labeling occurred in the higher bands of 300 000, 135 000, 125 000 and 17 000. The iodonaphthylazide label was not removed by extensive papain digestion whereas chloramine T iodinated membranes released radioactivity under the same conditions. Neither enzymatic nor transport activities were inhibited by the presence of iodonaphthylazide or the irradiation process. On the basis of the presented data it is concluded that the iodonaphthylazide unspecifically labels those portions of membrane proteins which are inserted into the lipid bilayer matrix.

Introduction

The brush border membrane of the small intestinal epithelial cell is one of the most complex, albeit interesting, plasma membranes currently under investigation. Multiple transport [1—4] as well as enzymatic [5] activities have been well documented to reside in isolated microvillus membranes. While varied labeling techniques have been developed to classify those portions of membrane-bound proteins exposed to the aqueous environment (for a review of the subject see ref. 6), one of the major problems presently facing protein biochemists is the absence of suitable techniques to define protein moities embedded in the bilayer lipid matrix. Recently, through the use of apolar nitrene-generating compounds, photoactive covalent labeling of membrane components has been made possible [7]. The technique entails use of a hydrophobic azide compound which is allowed to dissolve into the membranes in the absence of

light. Light is then flashed, converting the azido groups into nitrenes which react with proteins within the lipid core. The present work describes the labeling of microvillus membrane proteins by one such label, [^{125}I]iodonaphthylazide.

Methods and Materials

Using Charles River rats (200 g), brush border membranes were isolated by the method of Hopfer et al. [1]. Protein was determined according to Lowry et al. [8].

Labeling experiments

5-[^{125}I]Iodonaphthyl-1-azide was prepared according to Gitler, C., Bercovici, T. and Bromberg, A. (in preparation) and stored in ethanol at −20° C. The purity of the azide preparation was routinely checked by thin layer chromatography utilizing hexane as developing solvent. Labling was carried out essentially as reported by Klip and Gitler [7]. Membranes (1—2 mg/ml) were suspended in 100 mM mannitol buffer containing 0.1 mM $MgSO_4$ and 1 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid) adjusted to pH 7.5 with Tris hydroxide. All operations when azide was present before irradiation were performed in subdued light. Iodonaphthylazide ($5 \cdot 10^6$ cpm/mg protein, specific activity > 50 Ci/mol), final concentration of alcohol in membrane preparation 0.1%, was added to the brush border membrane. Vesicles were irradiated for 2 min at 37° C. A Corning 7-60 filter was present to screen light wavelengths below 305 nm. Specifications of the photoactivation apparatus and iodonaphthylazide are described by Gitler et al. (see above). Upon termination of irradiation the membranes were recovered by centrifugation at 31 000 × *g* for 30 min. After irradiation all operations involving the membranes were carried out at 4° C unless otherwise specified. The vesicle preparation was then washed with the mannitol/HEPES/$MgSO_4$ buffer containing bovine serum albumin (15 mg/ml). Membranes were pelleted as previously. The washing step was repeated usually three times or until minimal radioactivity was present in the supernatant. The brush border vesicle preparation was then washed three additional times with mannitol/HEPES/$MgSO_4$ buffer containing no albumin.

Brush border membranes were additionally labeled with ^{125}I by the method of Hunter and Greenwood [9]. Membranes were washed once with 100 mM phosphate buffer pH 7.3 and pelleted by centrifugation at 31 000 × *g* for 30 min. Phosphate buffer was utilized to maintain a stable pH of 7.3 during the iodination. The recovered membranes (approx. 2 mg) were suspended in 0.65 ml phosphate buffer. Chloramine T (0.005 ml, 5 mg/ml) and $Na^{125}I$ ($5 \cdot 10^6$ cpm/mg protein) were simultaneously added to the membrane preparation. After 10 min the incubation was terminated by the addition of sodium metabisulfite (0.05 ml, 5 mg/ml). The iodinated membranes were washed two to three times with mannitol/HEPES/$MgSO_4$ buffer to remove noncovalently bound radioactivity.

Electrophoresis

Sodium dodecyl sulfate gel electrophoresis was carried out according to

Weber and Osborn [10]. The electrophoresis was performed in 0.1% sodium dodecyl sulfate, 0.05 M sodium phosphate buffer pH 7.3 using, with one noted exception, 7.5% polyacylamide gels. Samples, at 1 mg/ml, were heated at 100°C for 10 min with 10 mM phosphate buffer pH 7.3, 1% sodium dodecyl sulfate and 10% mercaptoethanol before electrophoresis. When nonreducing conditions were desired samples were dissolved in 0.1—1% sodium dodecyl sulfate in the absence of mercaptoethanol and electrophoresed without prior heating. Gels were stained with Coomassie Brillant Blue and destained with 7% acetic acid. The mobilities of protein bands were calculated relative to bromophenol blue.

Determination of the position of sucrase activity in polyacrylamide gels after electrophoresis was carried out as described by Maestracci et al. [11]. Brush border membrane samples were dissolved in 1% sodium dodecyl sulfate and applied to 5% polyacrylamide gels without heating or addition of mercaptoethanol. Immediately after completion of the electrophoresis the gels were removed, sliced and enzymatic activity determined.

Gels of iodinated membranes were first stained to visualize protein banding patterns and then cut into 0.7 mm slices. The slices were counted in a gamma counter for radioactive content.

Papain digestion

A 4.3 ml solution of 100 mM mannitol, 1 mM HEPES/Tris pH 7.5, 20 mM cysteine and 2 mM EDTA adjusted to pH 7.5 was saturated with nitrogen for 10 min prior to addition of 0.01 ml papain suspension. Nitrogen was bubbled through the papain-buffer mixture for an additional 30 min to activate the papain. From the activated papain solution 0.5 ml was removed and added to the labeled brush border membranes (0.1 unit/mg membrane protein) initiating the 37°C digestion. Addition of 0.2 ml membrane sample to 0.2 ml 20 mM iodoacetic acid adjusted to pH 7.5 terminated the incubation. Membrane samples were immediately centrifuged at 32 000 × *g* for 10 min.

Transport studies

Transport experiments were carried out as reported elsewhere [4]. Briefly, the isolated membranes were added to a 20°C medium containing (final concentration): 100 mM mannitol, 1 mM HEPES/Tris, pH 7.5, 0.1 mM $MgSO_4$ buffer, 100 mM NaSCN and either 1 mM D-[6-^{3}H]glucose or L-[1-^{3}H(N)]glucose. The uptake of glucose was terminated by the removal of an aliquot (40—100 μg of membrane protein) from the incubation medium and rapid 50-fold dilution with ice-cold buffer. The dilution buffer contained 200 mM NaCl, 100 mM mannitol, 10 mM HEPES/Tris, pH 7.5 and 10 mM $MgSO_4$. Additionally, the dilution buffer contained 0.4 μCi D-[1-^{14}C]sorbitol per ml. The diluted aliquot was filtered immediately through a Satorius filter (No. 11305, 0.6 μm) and the collected membranes were rinsed once with 4 ml of dilution buffer not containing isotope. The isotope served as a correction for unspecific retention of radioactivity due to insufficient washing. The filters were dissolved and counted in liquid scintillation fluid.

Enzymatic assays

Sucrase (sucrose α-glucohydrolase, EC 3.2.1.48) was measured by the

method of Messer and Dahlqvist [12] with a modification reported earlier [1]. Aminopeptidase (EC 3.4.1.2) was assayed according to Wacker et al. [13] using L-leucine-*p*-nitroanilide as substrate. Alkaline phosphatase (EC 3.1.3.1) activity was measured as reported in ref. 14.

Materials

Proteins, their molecular weights and sources used for polyacrylamide gel calibration are: rabbit muscle phosphorylase A (tetramer, 370 000, Sigma), bovine liver catalase (tetramer, 232 000, Sigma), rabbit muscle phosphorylase A (dimer, 185 000, Sigma), bovine serum albumin (dimer, 136 000, Calbiochem), rabbit muscle phosphorylase A (92 500, Sigma), bovine serum albumin (68 000, Calbiochem), bovine liver catalase (58 000, Sigma), ovalbumin (43 000, Sigma), hog stomach mucosa pepsin (35 000, Sigma), papain (23,000 Worthington), egg white lyzozyme (14 300, Sigma) and bovine pancreatic insulin (5700, Sigma). Radioactive isotopes were obtained from New England Nuclear. Documentation for materials used in the synthesis of iodonaphthylazide is given by Gitler, C. et al. (in preparation).

Results

Electrophoretic fractionation of microvillus membrane proteins

The polyacrylamide gel system employed was standardized by protein markers with molecular weights from 6000 to 370 000. When the proteins' mobilities, relative to bromophenol blue, were plotted as a function of their molecular weight on a semilogarithmic scale a linear relationship between relative mobility and molecular weight existed over the range of 6000—136 000. Above 136 000 the curve was hyperbolic. Points above the highest marker utilized (phosphorylase A tetramer, 370 000) were drawn by extrapolation.

The typical electrophoretic protein pattern observed when brush border membranes were treated with 1% sodium dodecyl sulfate in the presence of 10% mercaptoethanol is shown in Fig. 1. The highly reproducible banding pattern is numbered from lower to higher mobilities. Corresponding apparent molecular weights of the numbered protein bands are also given in Fig. 1. Polypeptide heterogenity of this membrane system is immediately evident. The apparent molecular weights varied from 380 000 to 17 000. Prominent bands were located at 135 000, 130 000, 125 000, 110 000, 73 000 and 65 000. Directly below band 20 a diffuse band with approximately the same mobility as bromophenol blue could be observed in the gels. Below this point distinct Coomassie Blue staining bands were not evident, indicating the absence of lower molecular weight peptides.

The position of sucrase, a major brush border membrane protein, in 5% polyacrylamide/sodium dodecyl sulfate gels, was determined according to Maestracci et al. [11] as described in Methods. The apparent molecular weight for this protein was 260 000 in the system employed. The band containing sucrase activity was extracted, treated with 1% sodium dodecyl sulfate and mercaptoethanol. Electrophoresis in a 7.5% polyacrylamide/sodium dodecyl sulfate system yielded a Coomassie Blue staining band with an apparent M_r =

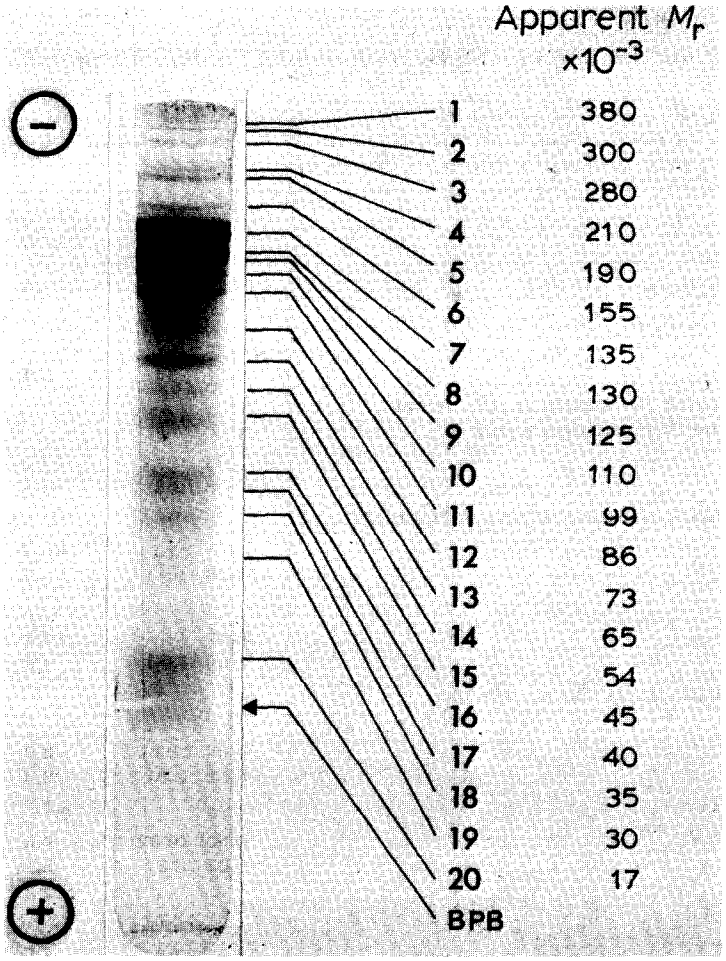

Fig. 1. Electrophoretic protein pattern of intestinal microvillus membrane. The membranes (40 μg protein) were treated with 10% mercaptoethanol and complexed with 1% sodium dodecyl sulfate before being subject to electrophoresis. Protein band numbering is from cathode (—) to anode (+). The position of bromophenol blue (BPB) is indicated by an arrow.

135 000. This band possessed an identical relative mobility to that of band 7 in Fig. 1. Many of the microvillus membrane hydrolases, including sucrase, have been identified as glycoproteins [15—17]. Molecular weights for these proteins should therefore be regarded as approximate, due to the reported anomalous behavior of glycoproteins in sodium dodecyl sulfate gels [18].

Iodonaphthylazide labeling of isolated brush border membranes

Intestinal brush border membrane was isolated and labeled with [^{125}I]iodonaphthylazide as described in Methods. The distribution of radioactivity and protein throughout the labeling and washing steps is shown in Table I. When [^{125}I]iodonaphthylazide was added to the microvillus membranes, 98% of the radioactivity partitioned into the membrane (step 2). After irradiation and addition of bovine serum albumin-containing buffer, the supernatant contained

TABLE I

RECOVERY OF [^{125}I]IODONAPHTHYLAZIDE AND PROTEIN DURING LABELING AND WASHING PROCEDURES

Details concerning the labeling and washing procedures are given in the Methods section. The buffer used throughout all procedures was 100 mM mannitol, 1 mM HEPES/Tris pH 7.5. Bovine serum albumin, when present, was 15 mg/ml. The percent of total radioactivity is indicated in parentheses.

Procedure	cpm X 10^{-6}	Protein (mg)
1. Membranes plus iodonaphthylazide	13.88 (100)	2.0
2. Centrifugation in dark		
Supernatant:	0.28 (2)	—
Pellet:	13.61 (98)	2.0
3. Suspension of pellet (2) in buffer and irradiation. Addition of buffer containing bovine serum albumin. Centrifugation		
Supernatant:	4.08 (29)	60.1
Pellet:	9.48 (68)	4.3
4. Suspension of pellet (3) in buffer containing albumin. Centrifugation		
Supernatant:	1.01 (7)	65.4
Pellet:	8.46 (61)	5.5
5. Repetition of step 4.		
Supernatant:	0.42 (3)	62.3
Pellet:	8.06 (58)	4.0
6. Suspension of pellet (5) in buffer containing no. albumin. Centrifugation		
Supernatant:	0.08 (0.6)	3.9
Pellet:	8.02 (58)	2.5
7. Repetition of step 6		
Supernatant:	0.003 (0.02)	0.5
Pellet:	8.02 (58)	2.1
8. Repetition of step 7		
Supernatant:	0.001 (0.01)	—
Pellet:	8.02 (58)	2.0

TABLE II

SPECIFIC ACTIVITIES OF MICROVILLUS MEMBRANE ENZYMES BEFORE AND AFTER IODONAPHTHYLAZIDE LABELING PROCEDURES

	Sucrase *	Alkaline phosphatase *	Leucine aminopeptidase *
Isolated membrane before treatment	3.29	3.18	5.49
Membrane after irradiation, with no azide present	3.30	3.17	5.49
Membrane after irradiation, with azide present	3.29	3.18	5.49
Membrane at step 8	3.32	3.20	5.50

* μmol substrate hydrolysed per min per mg protein.

29% of the total radioactivity while 68% of the ^{125}I-label was located in the pellet (step 3). Further washing with the bovine serum albumin-containing buffer removed 8% and then 3% of the total radioactivity (steps 4, 5). Additional washes with albumin-free buffer served to remove albumin from the membrane while very little radioactivity was present in the supernatant (steps 6, 7, 8). Remarkably, 58% incorporation of the iodonaphthylazide into the brush border membrane occurred (step 8). Bovine serum albumin served to remove non-

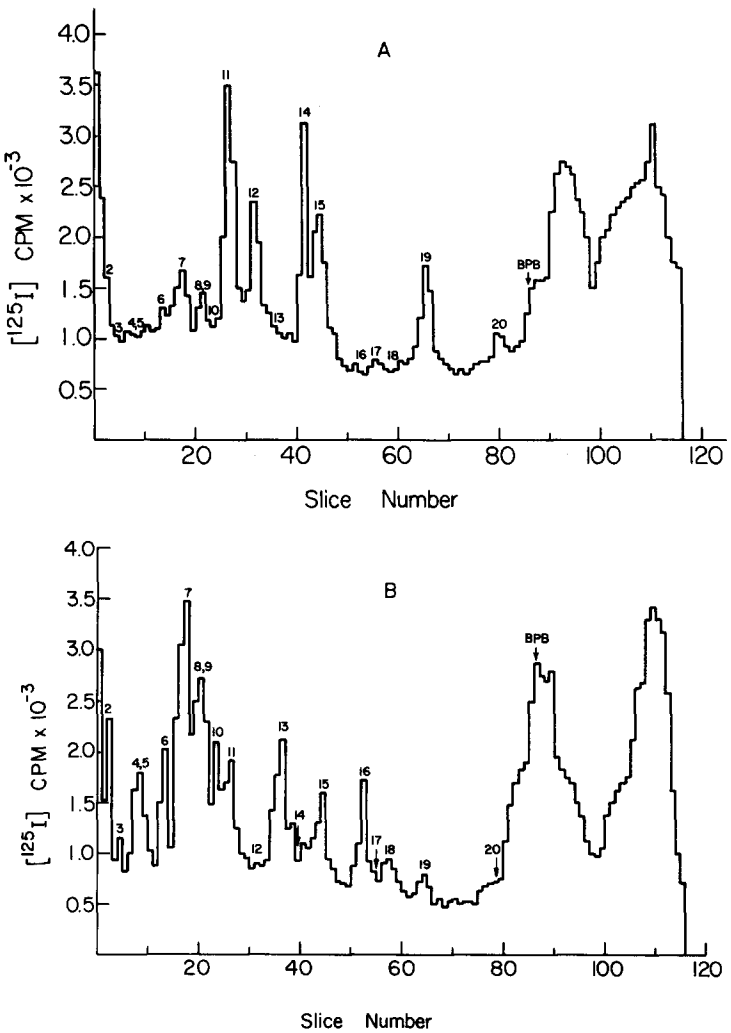

Fig. 2. (A) [^{125}I]iodonaphthylazide incorporation into brush border membrane. Labeling was carried out as described in Methods. 45 μg of [^{125}I]iodonaphthylazide-labeled membranes were treated and electrophoresed under conditions identical to those described in Fig. 1. The numbers included in the graph refer to the numbered protein bands displayed in Fig. 1. BPB indicates the mobility of bromophenol blue in the gel. (B). Chloramine T catalysed iodination of microvillus membranes. The iodination was performed as detailed in Methods. Treatment and electrophoresis of 40 μg of labeled membrane protein is identical to Fig. 2A. The position of protein bands corresponding to Fig. 1 is indicated by the numbers above the graph. BPB denotes the position of bromophenol blue.

bound iodonaphthylazide as previously reported [7]. 70% of the total non-bound iodonaphthylazide was removed in step 2, while 20%, 8% and 2% were removed in steps 4, 5 and 6 respectively. The complete pelleting of brush border membranes was judged by the absence of sucrase activity, an enzymatic microvillus membrane marker, from the supernatant. That all membrane protein was effectively recovered during the washing procedure was additionally demonstrated by the unchanging specific activity of several membrane-bound enzymes, sucrase, alkaline phosphatase, and aminopeptidase (see Table II). Complete removal of bovine serum albumin was ascertained by protein measurements and by the observation that albumin-washed and non-albumin-washed microvillus membranes gave identical electrophoretic protein patterns.

The final recovered membranes of step 8 were solubilized with 1% sodium dodecyl sulfate under reducing conditions before being subjected to electrophoresis in a 7.5% polyacrylamide/sodium dodecyl sulfate gel system. The pattern of [^{125}I]iodonaphthylazide incorporation is given in Fig. 2A. Additionally, isolated brush border membranes were labeled with ^{125}I, utilizing chloramine T, as described in Methods. The resulting pattern of insertion of ^{125}I into the microvillus membrane is depicted in Fig. 2B. Variation in the pattern of radioisotope incorporation between the two labeling procedures is strikingly evident. Membranes labeled with iodonaphthylazide showed predominant incorporation into bands 11, 12, 14, 15 and 19. Higher molecular weight bands 2, 7, 8/9 and 20 were minimally labeled. Two peaks of radioactivity could be noted below the bromophenol blue marker, presumably due to incorporation of the azide into lipids. Contrastingly, chloramine T iodinated membranes demonstrated predominant incorporation into bands 2, 4/5, 6, 7, 8/9, 10, 11, 13, 15 and 16.

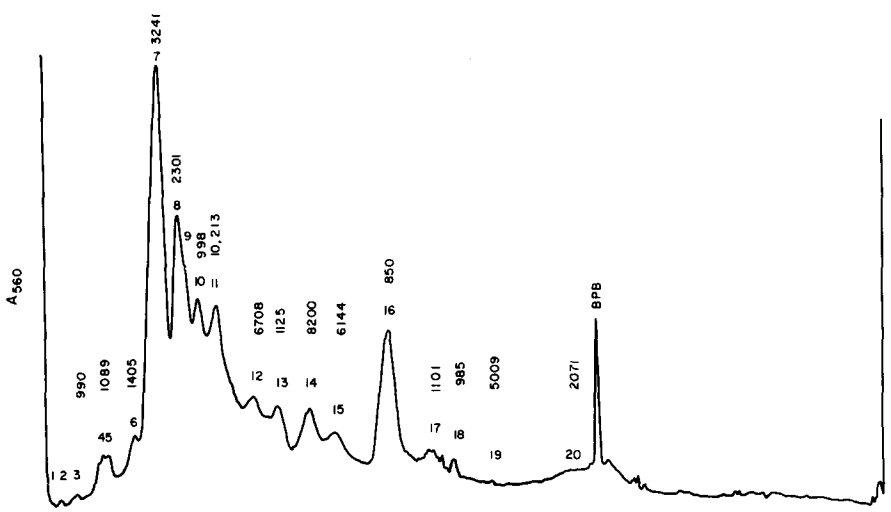

Fig. 3. [^{125}I]iodonaphthylazide incorporation into brush border membrane containing elevated amounts of actin. Gels were first stained with Coomassie Blue. Complete protein bands were then separately cut from the gel and counted. The densitometric trace of the electrophoretic protein pattern is numbered to indicate the identity of the protein bands. The position of the marker bromophenol blue is indicated by BPB.

Comparatively lower labeling of bands 3, 12, 14 and 17—20 occurred while strong insertion of ^{125}I into lipids was observed.

Brush border membranes isolated according to Hopfer et al. [1] can be prepared to contain relatively large amounts of actin, a protein (M_r = 45 000) which is thought to originate from the core fraction [19—21]. When incomplete homogenization of isolated brush borders (step 3 of ref. 1) is carried out core material can be observed to be partially attached to or included in the membrane vesicle in electron micrographs of negatively stained preparations (Sigrist-Nelson, K., unpublished observations). Fig. 3 presents a densitometric trace from a stained polyacrylamide gel of such a preparation. Electrophoretically, this membrane preparation appears to deviate from the usual preparation only in that it contains comparatively large amounts of actin, band 16. The

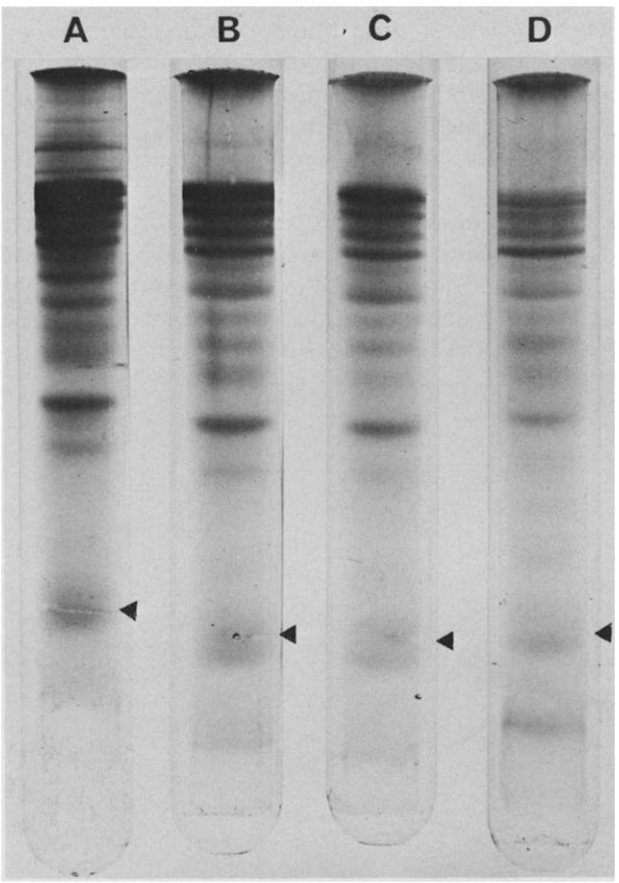

Fig. 4. Papain digestion of microvillus membranes. Membranes were digested with papain as described in Methods. At the given time points equal aliquots (containing 100 μg membrane protein) of membrane-papain incubation medium were removed, added to iodoacetate and centrifuged at 31 000 × *g* for 30 min. The pellets, suspended in identical amounts of buffer, were reduced with mercaptoethanol and treated with 1% sodium dodecyl sulfate before electrophoresis. The migration of bromophenol blue is indicated by an arrow. A. Untreated: membranes added directly to iodoacetate after addition of papain; B, 5 min; C, 10 min; D, 30 min.

membrane preparation was consequently labeled with iodonaphthylazide. The radioisotope incorporation into individual protein bands is given on the scan. The labeling pattern observed in Fig. 3 is similar to that present in Fig. 2A. In both cases band 16 shows almost no incorporation of the iodinated naphthylazide, though band 16 comprises a relatively large amount of the total protein in Fig. 3.

Papain digestion of microvillus membranes

Microvillus membranes, prepared containing elevated amounts of actin, were digested by papain for various lengths of time. Fig. 4 depicts the changing polypeptide pattern when samples of the recovered membrane pellet were electrophoresed. Papain effectively removed bands 1—6 and 17. Bands 7—10, 13, 16 and 18 were greatly reduced. Bands 11, 12, 14 and 15 appeared to be little affected or unchanged by papain. Though band 19 is poorly visible in the photograph, it did not appear to be extensively digested by papain.

Fig. 5 shows the ensuing pattern of liberated protein and radioactivity when microvillus membranes, labeled with [^{125}I]iodonaphthylazide were subjected to papain digestion. Both the supernatant and pellet radioactivity remained constant and unchanging throughout the digestion period (Fig. 5A), while the pellet protein decreased 36% and the supernatant protein increased a corresponding amount (Fig. 5B).

Similarily to the above experiment, chloramine T iodinated brush border membranes were also digested with papain. However, unlike Fig. 5, a distinct decrease in pellet radioactivity (33%) and an increase in supernatant ^{125}I could be detected (Fig. 6A). As in Fig. 5B both decrease in pellet protein and increase in protein appearing in the supernatant occurred (Fig. 6B).

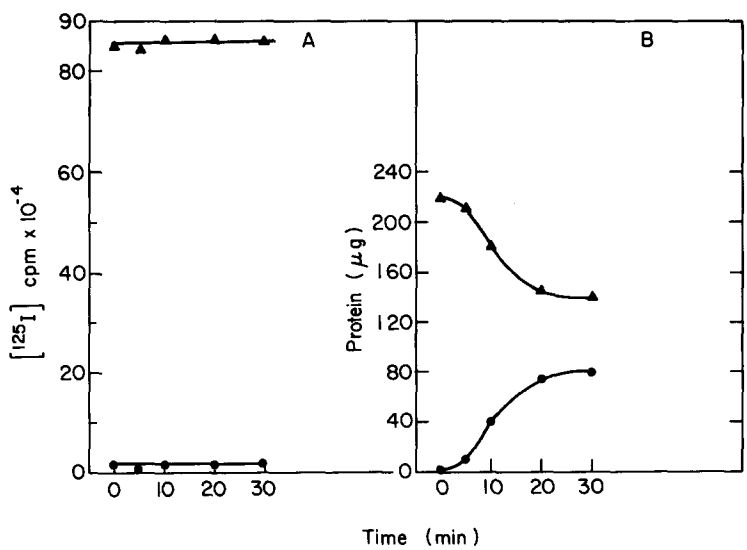

Fig. 5. Papain digestion of [^{125}I]iodonaphthylazide labeled brush border membranes. Release of radioactivity (A) and protein (B). •, Supernatant; ▲, pellet.

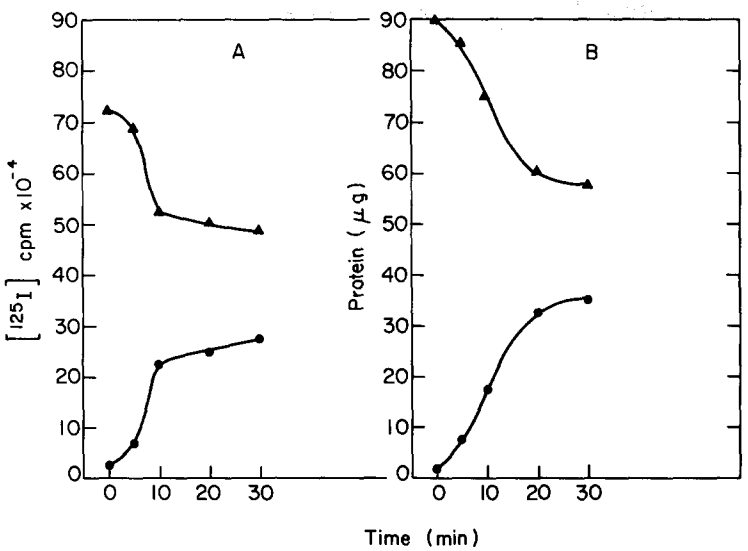

Fig. 6. Papain digestion of chloramine T iodinated microvillus membranes. Release of radioactivity (A) and protein (B). ●, Supernatant; ▲, pellet.

Supernatants from papain digested iodonaphthylazide- and chloramine T iodinated membranes were analyzed by sodium dodecyl sulfate gel electrophoresis. Protein bands, corresponding to those bands reduced in the membrane pellet by papain in Fig. 4, were in evidence for both supernatants. While the liberated proteins were labeled radioactively in the case of the chloramine T iodinated membranes, no radioactivity was present in the iodonaphthylazide-labeled proteins liberated by papain (data not shown).

Effect of iodonaphthylazide labeling on the functional properties of the brush border membrane

Membrane bound enzymatic activity was monitored before labeling with iodonaphthylazide, directly after irradiation and at step 8, after extensive washing. Additionally, enzymatic activities of the hydrolases were assayed after irradiation in the absence of iodonaphthylazide. Table II lists enzymatic activities for three varied brush border membrane-associated enzymes: sucrase, alkaline phosphatase and aminopeptidase. Neither irradiation nor the presence of the iodonaphthylazide served to decrease the hydrolases' specific activity.

Transport of tritiated D- and L-glucose into the brush border vesicles is illustrated in Fig. 7. The uptake experiment was initiated by the addition of the membrane vesicles to the incubation medium, a NaSCN gradient thus initially existing. At early time points D-glucose transport demonstrated concentrative uptake, D-glucose being accumulated above equilibrium levels. The kinetics of D-glucose transport are faster than those of L-glucose. Both stereoisomers reached identical equilibrium values, indicating entry into the same intact intravesicular space. Identical curves for glucose transport were obtained both before the membranes were subjected to irradiation and after irradiation in the presence and absence of nonradioactive iodonaphthylazide.

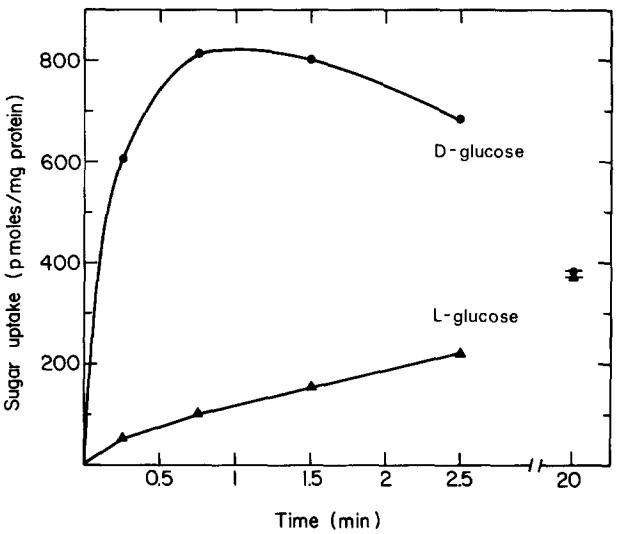

Fig. 7. Transport of D- and L-glucose into iodonaphthylazide-labeled microvillus membranes. Membranes were labeled with nonradioactive iodonaphthylazide (40 μg/mg membrane protein). Content of the incubation medium is reported in Methods. The uptake was initiated by addition of the membranes vesicles to the incubation medium.

Discussion

Electrophoretic fractionation of sodium dodecyl sulfate/mercaptoethanol treated microvillus membranes yielded 20 clearly discernable Coomassie Blue staining bands with apparent molecular weights ranging from 380 000 to 17 000. The protein subunit pattern observed is similar to that reported by Maestracci et al. [20] for human intestinal brush border membrane. The determined apparent molecular weight for sucrase agree well with previously published values [11,22,23]. Chloramine T iodination of the electrophoretically characterized membrane labeled predominantly higher molecular weight polypeptides. Conversely [^{125}I]iodonaphthylazide was attached primarily to lower molecular weight bands. Strong labeling by iodonaphthylazide was noted for protein bands of 99 000, 86 000, 65 000, 54 000 and 30 000 daltons. Minimal labeling occurred in higher molecular weight bands of 300 000, 135 000, 130 000, 125 000 and 17 000.

In further experiments brush border membranes were isolated with elevated levels of actin. The iodonaphthylazide failed to label the actin band indicating the specificity of the photoactive label despite the fact that the nonmembraneous core protein represented a large amount of the total protein present. Interestingly, Mooseker and Tilney [21] have reported that the core of actin filaments are attached to the inner surface of the microvillus membrane by densely staining material identified as the Z-band or α-actinin, a 95 000 dalton protein. In our system iodonaphthylazide extensively labeled a papain nondigested protein of 99 000 daltons. Final confirmation of the identity of this protein awaits isolation and chemical characterization procedures.

Papain has long been reported to be the protease of choice for digesting

membrane proteins from microvillus membrane [24—31]. The disappearance of knobbed structures from the external surface of the membrane have attested to the effectiveness of this enzyme in liberating membrane proteins exposed to the aqueous environment. The papain digestion experiments presented here are in agreement with previously published work and support the concept that the iodonaphthylazide labels only those portions of membrane proteins which are embedded in the lipid bilayer. Upon digestion of iodonaphthylazide-labeled membrane with papain no radioactivity was released although proteins were liberated from the membrane. Chloramine T iodinated membranes, on the other hand, released radioactivity when incubated with papain. Furthermore protein bands digested by papain were either not labeled or labeled to a limited extent by the iodonaphthylazide. That certain bands, such as band 7, sucrase which could be digested by papain, were labeled with the iodonaphthylazide is not surprising. Sigrist et al. [23] have solubilized microvillus membrane sucrase-isomaltase complex both by papain treatment and Triton-X-100. Limited papain digestion of the Triton-solubilized sucrase-isomaltase produced an enzyme electrophoretically identical to the papain-solubilized sucrase-isomaltase and low molecular weight fragments of low polarity [32]. These data and others (refs. 29—32, and Sigrist, H., Mueller, M. and Semenza, G., in preparation) suggest that the outward directed bulk of the glycoprotein, carrying the hydrolase active site is integrated in the membrane by a hydrophobic anchor. Similar observations have been made by Maroux and Louvard [33] on the intestinal brush border aminopeptidase and maltase. The limited labeling of sucrase and other high molecular weight bands by iodonaphthylazide may be accounted for by such a model where in the iodonaphthylazide may insert into the hydrophobic segment within the membrane. That protein band overlaying may occur where more than one protein occupies the same position in the gel cannot, however, be ruled out.

Finally it is an important finding that neither enzymatic nor transport activities of the membrane are affected by the label or the irradiation process. Data presented on both aspects of membrane function are in agreement with previously reported findings [1,14,34]. That functional properties of the membrane remain intact is an essential requirement for possible reconstitution studies and is certainly desirable for protein chemistry investigations.

In conclusion, we believe photoactive hydrophobic labels will prove to be powerful tools in membrane biochemistry, therewith giving insight as to which protein molecules are deeply embedded in the lipid bilayer and adding a new dimension to membrane biochemical investigations.

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