RECONSTITUTION OF A SUCRASE-MEDIATED SUGAR TRANSPORT SYSTEM IN LIPID MEMBRANES

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1. Introduction

Digestive di- and oligosaccharidases are bound to, or are building blocks of, the brush border membrane of the enterocytes. This has been shown consistently by a variety of approaches, including the essentially complete recovery of some disaccharidase activities in isolated brush border membranes [1] and the immunochemical localisation by electron microscopy [2]. (For review, see [3]).

In 1970 Crane et al. [4,5] have investigated the intestinal uptake of glucose from combinations of free glucose and disaccharides. The presence of sucrose, maltose, lactose or trehalose in the medium produces an additional glucose uptake, even if the medium already contains enough free glucose to saturate completely the transport system(s) for the *free* monosaccharide. The extra glucose uptake from the disaccharides must be attributed to additional system(s) not accessible to free glucose. One notable characteristic of this (these) newly detected system(s) is that of not being Na-dependent. (The transport of *free* glucose across the brush border membrane of the small intestine is, of course, highly Na-dependent: for reviews see [6,7]).

We have now made lipid bilayers in the presence of the small intestinal sucrase–isomaltase complex and observed a specific increase of the permeability of the bilayers for the sugars arising from sucrose.

2. Materials and methods

The sucrase–isomaltase complex was obtained in a water-soluble homogeneous form from rabbit small intestine by papain solubilisation, ammonium sulfate fractionation and Sephadex G-200 chromatography [8,9]. It is a glycoprotein with a $s^{o}_{w,20}$ of 9.9 S; a $\bar{v}$ of 0.743; and a pI in the neighbourhood of pH 4 [10]. The carbohydrate moiety (some 15% w/w) consists of D-glucose, D-galactose, mannose, L-fucose, galactosamine and glucosamine. As obtained by this solubilisation procedure, it contains no lipids, no phosphate and no sulfate. Its hydrophobicity factor as deduced from the amino acid composition is < 1.160. The complex consists of two subunits, having each a M.W. of 110–130,000 and endowed with sucrase and isomaltase activity, respectively [9–11].

The complex was mixed with lipids (either total lipids from hamster small intestinal brush borders [12], or from *Micrococcus lysodeikticus* M0128, or a mixture of egg lecithin and cholesterol in n-decan The mixture was placed on the hole in the Teflon septum of the chamber system described by Mueller and Rudin, as modified by Läuger et al. [13]. The area of the hole was 8 mm^2. Once the membrane had blackened, the radioactive substrate ([U-^{14}C] sucrose, [U-^{14}C] glucose, [U-^{14}C] fructose, [U-^{14}C] or [^{3}H] mannitol or [^{3}H] H_2O from Amersham, England or from NEN, Frankfurt, W.Germany) was added to one compartment; after 20 to 60 min samples were withdrawn from each compartment and the radioactivity measured. The gross integrity of the membrane was checked by monitoring the current flux under a potential applied across it (DC up to 70 mV; Ag/AgCl electrodes; Keithley microvolt ammeter 150 B). Inclusion of the sucrase–isomaltase complex into the lipids lead to less stable black membranes.

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Table 1

Permeability coefficients (P) of black lipid membranes containing the sucrase–isomaltase complex or not.

Total lipids from hamster small intestinal brush borders + cholesterol (1:0.75, w/w)	P ± SEM (cm/sec)	Range	n
[^{14}C] Sucrose, no sucrase–isomaltase [a]	$< 10^{-8}$		(7)
[^{14}C] Sucrose, with sucrase–isomaltase [a]	$4.8 \pm 2.2 \times 10^{-5}$	$(0.1 - 20 \times 10^{-5})$	(11)
[^{14}C] Mannitol, with sucrase–isomaltase [b] 10 mM sucrose	$< 10^{-8}$		(6)
Egg lecithin + cholesterol (10:1, w/w)			
[^{14}C] Sucrose, no sucrase–isomaltase [c]	$< 10^{-8}$		(4)
[^{14}C] Sucrose, with sucrase–isomaltase [c]	$7.9 \pm 6.4 \times 10^{-5}$	$(1.34 - 20.7 \times 10^{-5})$	(4)
[^{14}C] Sucrose, with sucrase–isomaltase, 10 mM glucose [c]	$0.32 \pm 0.13 \times 10^{-5}$	$(0.035 - 0.57 \times 10^{-5})$	(4)
[^{14}C] Mannitol, with sucrase–isomaltase [b]	$< 10^{-8}$		(6)
[^{14}C] Glucose, with sucrase–isomaltase [d]	$< 50 \times 10^{-8}$		(6)
[^{14}C] Sucrose, with sucrase–isomaltase (*) [c]	$1.7 \pm 0.57 \times 10^{-5}$	$(0.94 - 2.13 \times 10^{-5})$	(3)
Total lipids from *Micrococcus lysodeikticus* M 0128 (**) + cholesterol (5:1, w/w)			
[^{14}C] Fructose, [^{14}C] glucose, [^{3}H] mannitol, no sucrase–isomaltase [e] glucose + fructose $< 10^{-8}$ mannitol $< 10^{-8}$			(4)
[^{14}C] Fructose, [^{14}C] glucose, [^{3}H] mannitol, with sucrase–isomaltase [e] glucose + fructose $< 10^{-8}$ mannitol $< 10^{-8}$			(6)
[^{14}C] Sucrose, with sucrase–isomaltase [c]	0.375×10^{-5}	$(0.372 - 0.378 \times 10^{-5})$	(2)

* In 5 mM KCl. All other determinations in 5 mM NaCl.

** At 37°. All other determinations at 30°. These lipids were dissolved in n-decane/n-octane (2:1) mixtures. The others were dissolved in n-decane alone.

[a] Sucrose 10 mM; [b] mannitol approx. 0.3 mM; [c] sucrose 0.2–0.6 mM; [d] glucose 10 mM, $P = 50 \times 10^{-8}$ (2); glucose 0.4–0.8 mM, $P < 10^{-8}$ (4); [e] fructose and glucose, 1–3 mM each; mannitol 2–3.7 mM.

3. Results

The main results are condensed in table 1. Black lipid membranes not containing the sucrase–isomaltase complex are essentially impermeable to sucrose, glucose, fructose and mannitol (see also [14]. Membranes containing sucrase–isomaltase have a much larger (by some 3 orders of magnitude) permeability for sucrose, or, more accurately, for the radioactivity stemming from it; (the P-values were calculated on the basis of the radioactivity which had crossed the membrane [14]). The only radioactive compounds which were found in the trans compartment were fructose and glucose as identified by paper chromatography and autoradiography (fig.1). The higher permeability for [^{14}C] sucrose of membranes containing sucrase–isomaltase is independent of Na^+, as shown by the same values being obtained when K^+ was substituted for Na^+.

The sucrase–isomaltase complex seems to specifically increase the permeability for sucrose (and perhaps for other disaecharides, which, however, have not yet been tested). The permeability for mannitol, *free* glucose and *free* fructose in particular is little affected, if at all, by the presence of the sucrase–isomaltase in the lipids used. In addition, double labelling experiments with [^{14}C]-sucrose and [^{3}H] mannitol confirm the specificity for [^{14}C] sucrose of membranes containing sucrase–isomaltase.

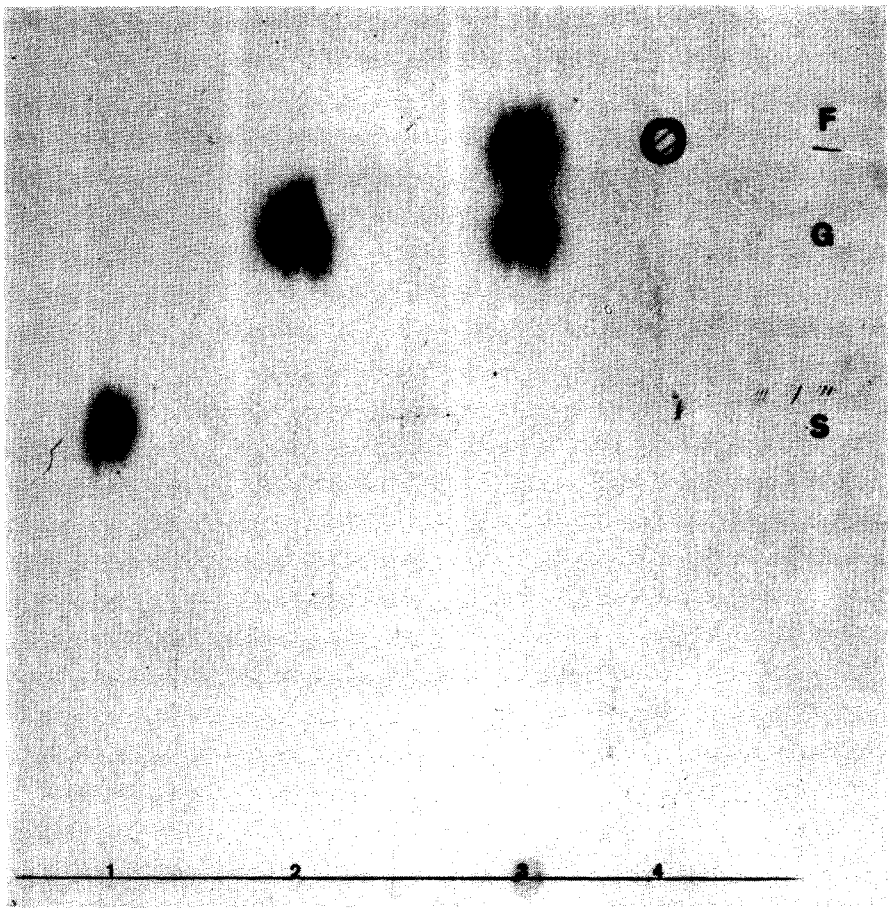


Fig. 1a. Identification of the radioactive compounds crossing black lipid membranes containing sucrase–isomaltase complex and stemming from [^{14}C] sucrose. Autoradiography of a paper chromatogram (Whatman Nr.1; n-butanol, pyridine, water, 30:10:15; developed three times in the descending direction):
1) [^{14}C] sucrose standard; 2) [^{14}C] glucose standard; 3) sample from the trans compartment; 4) cold fructose standard.

produced by other water soluble proteins in lipid bilayers (see e.g. [15]), and possibly due to the formation of non specific "pores" or "channels". (Occasionally, however, long lasting membranes were found to be less discriminating).

The water ([^{3}H] H_2O) permeability of lecithin/cholesterol membranes containing the sucrase–isomaltase complex was $2 \pm 0.86 \times 10^{-3}$ (3) ($\bar{x} \pm$ S.E.M. (n)) and was identical with that of membranes of lecithin alone (see, for example, [16]). The effect of the sucrase–isomaltase does not depend critically on the composition of the lipids used.

4. Discussion

As mentioned at the beginning, in intact small intestine the glucose arising from sucrose, in addition to being transported by the system(s) for free glucose, can be transported by another system, whose diagnostic characteristics are:
i) that of not being available to *free* glucose,

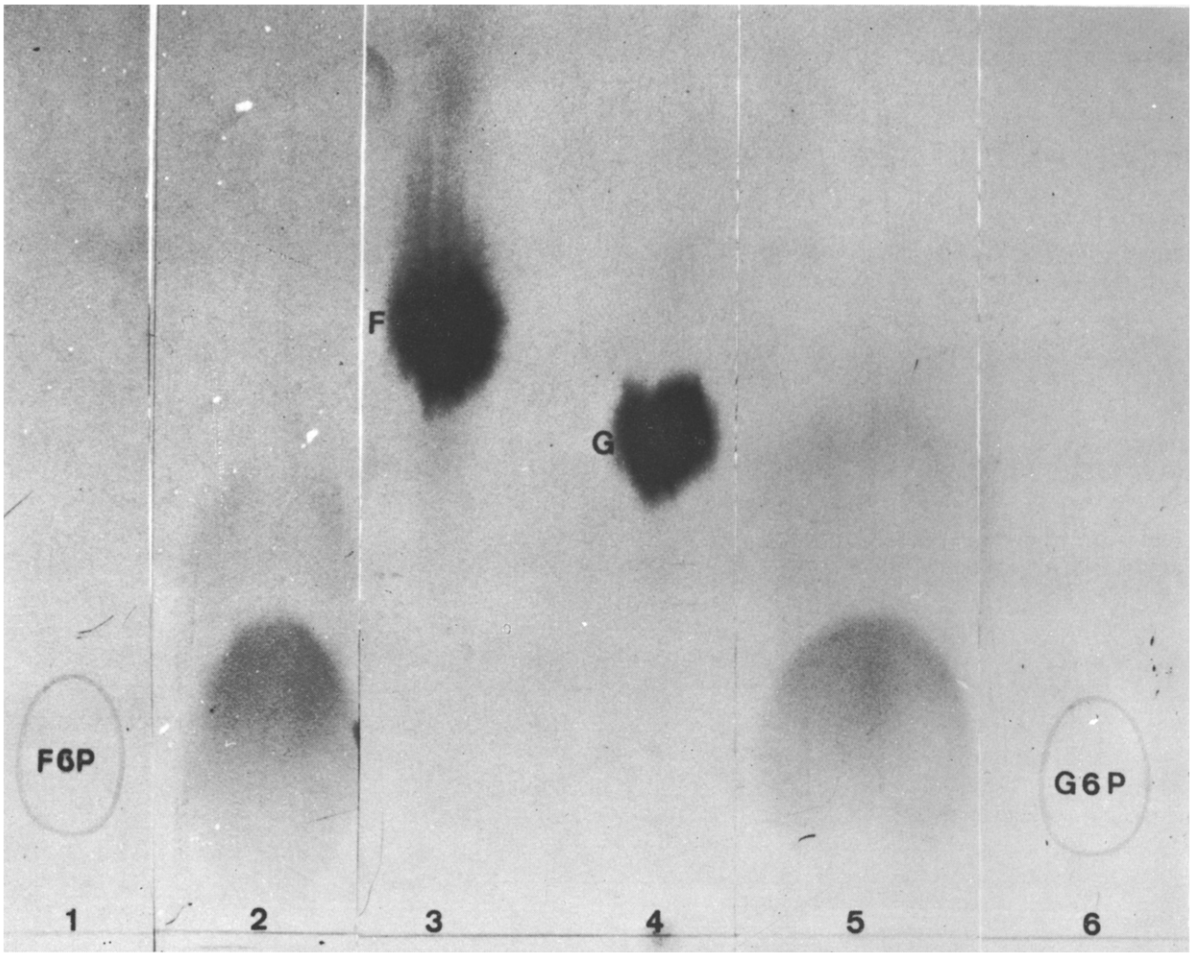


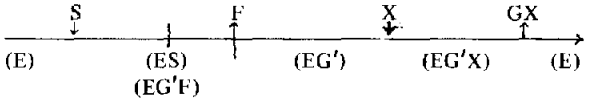
Fig.1b. Identification of the radioactive compounds crossing black lipid membranes containing sucrase–isomaltase complex and stemming from [^{14}C] sucrose. The individual radioactive spots from the chromatogram of fig.1a, row Nr.3, were eluted with water and incubated with hexokinase and ATP-Mg. After heat inactivation of the hexokinase and addition of cold fructose and fructose-6-phosphate (row Nr.2), or of cold glucose and glucose-6-phosphate, respectively (row Nr.5), the samples were rechromatographed in the same system as in fig.1a (developed twice): 1) cold fructose-6-phosphate standard; 3) [^{14}C] fructose standard; 4) [^{14}C]-glucose standard; 6) cold glucose-6-phosphate standard. The change in chromatographic behaviour following hexokinase treatment is apparent.

ii) that of not being Na-dependent. Clearly, the synthetic sucrase–isomaltase/black lipid membrane transport system does indeed have these two characteristics. Therefore, a transport system similar to, and perhaps identical with, one of those which are operative in intact small intestinal cells has been reconstituted *in vitro*. To the best of our knowledge, this is the first report in which a homogeneous protein, solubilized from a biological membrane, has apparently produced a reconstitution of the original transport system in artificial lipid membranes, thereby providing the functional demonstration of acting as a membrane carrier.

The available recent data of Crane's group, which are still in a preliminary form [4,5], do not indicate whether fructose, in addition to glucose, is trans-

The data in table 1 are not compatible, therefore, with a generalized increase in permeability of the kind ported by the disaccharidase-associated system(s). Our observation (fig.1) that fructose is transported by the synthetic system in amounts approximately equivalent to those of glucose, agrees with an older observation of Crane's group [17] that the fructose stemming from sucrose has a kinetic advantage for transport, over free fructose, in much the same way as it occurs for glucose.

The well-known difficulties inherent in the black membrane technique have prevented any accurate kinetic analysis of the reconstituted system. However, if one assumes that the reaction mechanism of sucrase is unchanged by its being included in the lipid membrane, one may venture to discuss our data as follows. By steady-state analysis the sucrase activity in the solubilized sucrase–isomaltase complex was shown to follow a Ping Pong Bi Bi mechanism [18]:



where S is sucrose, F fructose, X water or its equivalent (in the hydrolytic activity) or an acceptor (in the transglucosidase activity), GX either glucose or a transglucosidation product.

Although we do not know whether fructose can be liberated into the cis compartment also (too much sucrase activity was present in the compartment), it does certainly appear in the trans compartment (fig.1a). Since *free* monosaccharides are very little transported, if at all, (table 1), the monosaccharides appearing in the trans compartment must have crossed the membrane either i) as sucrose, which was hydrolyzed subsequently, or ii) as ES or EG'F. Alternative (ii) implies coupling between transport and hydrolysis. If true, transport should occur at either of the levels indicated by $\}$, depending on whether fructose can be liberated into the cis compartment also, or into the trans compartment alone.

Experiments are presently in progress to elucidate these points, as well as the following: a) The sucrase–isomaltase complex used was solubilised from the membrane by papain treatment [8,9]. How does it compare with the enzyme complex originally present in the membrane, particularly as far as its lipophilicity is concerned? b) Does the sucrase–isomaltase complex form "specific pores" or "channels" in the torus, or perhaps in the double layer? Or does it rather span across the membrane reaching both water compartments with its hydrophilic areas and adjoining the hydrophobic lipid chains with its lipophilic areas? At present we favour the latter model, because it has been impossible to see any effects when the sucrase–isomaltase complex was added to already formed black membranes, and because the tendency to aggregate at high ionic strength [10] is compatible with the presence of hydrophobic areas at the surface of the complex. Finally, proteins even smaller than the sucrase–isomaltase complex are known to span across biological membranes [19], and electron microscope studies also indicate that proteins and lipids probably aggregate sidewise in a two-dimensional array to form biological membranes [20,21].

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