PRELIMINARY NOTES

BBA 71 022

Incorporation of [1-^{14}C]glucosamine by rat intestinal microvillus membrane

Synthetic pathways involved in the production of glycoproteins of the cell surface have not been studied extensively. COOK, LAICO AND EYLAR[1] showed that [^{14}C]glucosamine was incorporated into glycoprotein of a smooth endoplasmic reticulum fraction which contained plasma membranes, and suggested that their data described the biosynthesis of surface membrane glycoprotein. Incorporation of label into an isolated plasma membrane fraction was, however, not established. The small intestine presents a unique opportunity for the study of surface glycoproteins, since methods exist for the isolation of highly pure plasma membranes[2]. Moreover the intestinal luminal surface possesses intense staining properties which are characteristic of glycoproteins[3]. ITO[4] has shown by electron microscopy that this staining is confined predominantly to a filamentous coat attached to the luminal surface of the microvillus plasma membrane. This surface coat appears to be part of the surface membrane rather than a layer of mucus, since it respects cell boundaries[4,5] and resists removal by mucolytic agents[4].

Since [1-^{14}C]glucosamine is an excellent labelled precursor of plasma and tissue glycoproteins[6], studies were undertaken to determine whether it might be incorporated into acid-precipitable glycoproteins of intestinal plasma membranes as a preliminary step in the study of their synthesis.

The purpose of this communication is to indicate that [1-^{14}C]glucosamine is incorporated into, and can be isolated with, microvillus plasma membranes, and to present preliminary evidence which suggests that glycoprotein may be transported to the membrane from a microsomal site.

Table I shows the acid-precipitable radioactivity in intestinal subcellular fractions and intestinal contents 6 h following the intraperitoneal injection of [1-^{14}C]-glucosamine. At this time interval 31 % of the total radioactivity found in the intestinal mucosa was present in the membrane-rich brush border fraction. The specific activity of the brush border fraction was higher than that of any of the remaining fractions whether expressed per mg of protein or per μg of hexosamine. Labelling of the brush border fractions cannot, therefore, be due to contamination by other subcellular components. Although the mucus-rich intestinal content contained approximately one third as much radioactivity as the intestinal mucosa, the low specific radioactivity of hexosamine in this fraction indicates that it too cannot be responsible for the brush border radioactivity.

In order to show that [1-^{14}C]glucosamine was incorporated into the microvillus plasma membrane, and not into a contaminant of the brush border fraction, purified microvillus membranes were isolated under similar experimental conditions and incorporated radioactivity compared with that of the membrane marker invertase

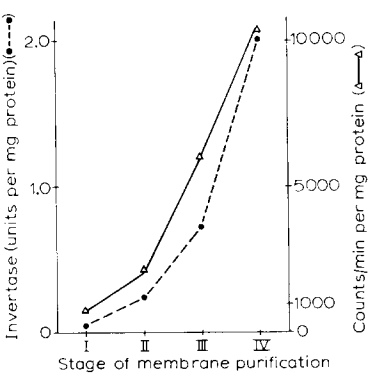
TABLE I

RADIOACTIVITY INCORPORATED INTO ACID-PRECIPITABLE PROTEIN AND HEXOSAMINE OF INTESTINAL SUBCELLULAR FRACTIONS

Male Wistar rats, unfasted, 150–200 g, received 10 μC [1-^{14}C]glucosamine (New England Nuclear, 50 μC/mg) in 0.2 ml isotonic saline intraperitoneally. 6 h later the small intestine was removed. Intestinal content was obtained by washing the intestinal lumen with 20.0 ml isotonic saline. Brush borders were isolated without purification. Other subcellular fractions were prepared from the brush border supernatant by differential centrifugation following the addition of sufficient sucrose to bring the concentration to 0.3 M. Acid-precipitable fractions were prepared by precipitation with 10% trichloroacetic acid and 1% phosphotungstic acid, followed by lipid extraction with chloroform–methanol (1:1, v/v). Protein and hexosamine were determined by the methods of LOWRY *et al.*[8] and ALLISON AND SMITH[9]. Proteins and hexosamines were dissolved in 0.4 M NaOH and distilled water, respectively, added to BRAY'S[10] solution and radioactivity was determined in a liquid scintillation counter.

Fraction	*% of total radioactivity in mucosa*	*Counts/min per mg protein*	*Counts/min per µg hexosamine*
Homogenate		650	34.0
Brush borders	31	2230	72.0
Mitochondria	12	444	39.0
Microsomes	17	650	24.0
Supernatant	39	513	17.0
Intestinal content		1000	14.3

(EC 3.2.1.26)[2] at several stages of purification. As shown in Fig. 1, each stage of membrane purification, indicated by an increment in the specific activity of invertase, is associated with a corresponding increase in specific radioactivity. It is apparent, therefore, that [1-^{14}C]glucosamine is incorporated by and remains tightly bound to the plasma membrane during its isolation.



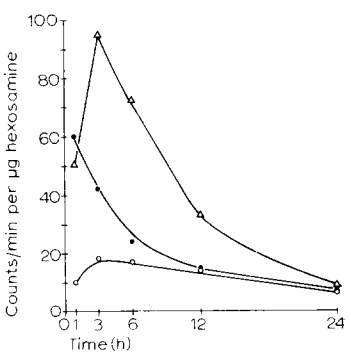


Fig. 1. Comparison of specific activities of invertase and acid-precipitable radioactivity at successive stages of membrane purification. I, homogenate; II, brush borders as isolated in Table I; III, brush borders purified as previously described[2]; IV, microvillus membrane[2]. Experimental procedure was the same as described in Table I. Invertase was assayed as described by DAHLQVIST[11].

Fig. 2. Change in specific activity of acid-precipitable hexosamine of intestinal subcellular fractions with time. [1-^{14}C]Glucosamine, 10 μC, was injected intraperitoneally and data obtained as described in Table I. Rats were sacrificed following injection at the time intervals shown. Results shown are for the brush border fraction (△—△), the microsomal fraction (●—●), and the supernatant fraction (○—○).

The specific activity of acid-precipitable hexosamine in the microsomal, supernatant and brush border fractions of the intestinal mucosa from 1 to 24 h following [1-^{14}C]glucosamine injection is shown in Fig. 2. At 1 h the microsomal specific activity was greater than that of the brush border but diminished rapidly thereafter. The specific activity of the brush border fraction was greatest at 3 h, falling to negligible levels at 24 h. The specific activity of the supernatant hexosamine was also greatest at 3 h, but remained low at all time periods. Early labelling of glycoprotein by the intestinal mucosa, therefore, occurs at an intracellular microsomal site while labelling of the plasma membrane is delayed. The microsomal and brush border curves do not conform strictly to that of a precursor product relationship since their periods of maximal specific activity do not coincide[7]. It is possible that the delayed rise of the brush border specific activity curve reflects the transport of glycoprotein from a microsomal to a brush border site. If this is the case, the transport process must require several hours for completion. It seems most likely that glycoprotein is transported in particulate form since the low specific activity of the supernatant hexosamine apparently excludes transport in soluble form. As yet, however, attempts to identify a precursor of membrane glycoprotein in the particulate fractions of the mucosa have not been successful.

These results are consistent with the concept that surface glycoproteins of the intestine are an integral part of the plasma membrane, and indicate that information regarding their synthesis may be obtained by labelling them *in vivo* with [1-^{14}C]-glucosamine.

This work was supported by Grant No. MA2340 of The Medical Research Council of Canada. The author wishes to express his gratitude to Miss A. PEDNEAU, and Mrs. G. SAMANEK for their excellent technical assistance.

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Received February 19th, 1968