ENZYMATIC TRANSFER OF ^{14}C-GLUCOSAMINE FROM UDP-N-ACETYL-^{14}C-GLUCOSAMINE TO ENDOGENOUS ACCEPTORS IN A GOLGI APPARATUS-RICH FRACTION FROM LIVER[1]

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Summary

A Golgi apparatus-rich fraction isolated from rat liver catalyzes the transfer of glucosamine from UDP-N-acetylglucosamine to endogenous protein acceptors. The Golgi-associated transfer accounts for almost half of the total activity of the original homogenate. Plasma membrane and endoplasmic reticulum fractions are practically devoid of activity. GDP-mannose and GTP stimulate this transfer, possibly by sparing UDP-N-acetylglucosamine from enzymatic hydrolysis. UDP-glucose, UDP-galactose, UTP, UDP, and UMP are inhibitory. Digestion with pronase converts the radioactive product of this transfer into a trichloroacetic acid-soluble form. All of the radioactive material cochromatographs with glucosamine, following acid hydrolysis of pronase glycopeptides.

In a recent report (1), we have described the incorporation of glucosamine into endogenous glycoprotein acceptors in smooth microsomes isolated from rat liver. Since this cellular subfraction contains Golgi bodies, which are claimed (2,3) to be implicated in the biosynthesis of the carbohydrate moiety of glycoproteins, it seemed reasonable to examine an isolated Golgi fraction for the incorporation of glucosamine into glycoproteins. This communication describes the enzymatic transfer of glucosamine from UDP-N-acetylglucosamine to endogenous protein acceptors within a Golgi apparatus-rich fraction obtained from rat liver.

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Methods and Materials

Golgi membranes were prepared from liver homogenates of fasted male Wistar rats by sedimentation in a sucrose-dextran density gradient. Details of isolation, and the chemical and enzymatic characterization of this fraction will be published elsewhere (4,5,6)*. UDP-N-acetylglucosamine-1-C^{14} (specific activity 12.3 μC/μmole) was synthesized as previously described (7). UDP-glucose, UDP-galactose, and GDP-mannose were purchased from Sigma. Pronase was from Calbiochem.

Transfer of glucosamine-^{14}C to protein was measured as radioactivity precipitated with 3 ml of 5% trichloroacetic acid. Precipitates were washed twice with 3 ml of trichloroacetic acid, once with 2 ml of ethanol, 2 ml of ethanol/ether (1:1, v/v), and 2 ml of ether. Dried precipitates were suspended in 1 ml of hydroxide of hyamine, and heated at 65° until dissolved. The digested samples were then added to 10 ml of Liquifluor, and radioactivity was measured by liquid scintillation spectrometry.

Protein was estimated by the method of Lowry (8). Sugars were detected on papers following chromatography with a triphenyl tetrazolium chloride reagent (9).

Results

Fig. 1 shows the incorporation of glucosamine into trichloroacetic acid-insoluble material as a function of time. Addition of unlabeled GDP-mannose (40 mμmoles) to the standard incubation mixture stimulated both the rate and extent of transfer. It is possible that this stimulation results primarily from decreased hydrolysis of UDP-N-acetylglucosamine, since the addition of GTP has a similar effect. However, this fraction does catalyze the transfer of mannose from GDP-mannose to trichloroacetic acid-precipitable protein, but not to an extent which would account for the observed stimulation on the

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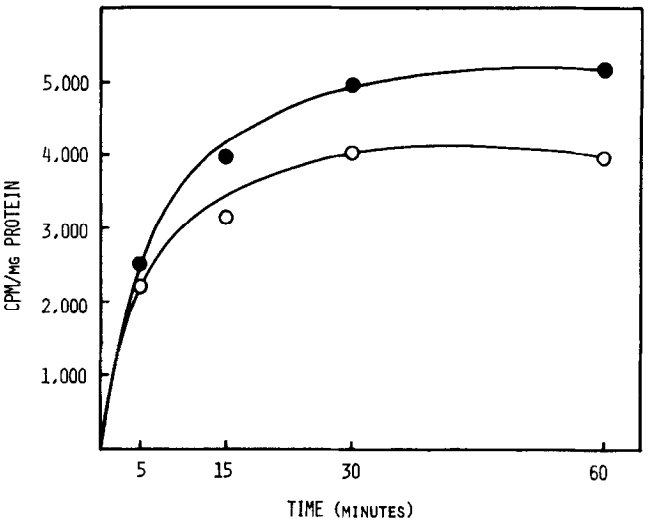

Fig. 1. Effect of GDP-mannose on glucosamine transfer. The standard incubation mixture contained in a total volume of 100 µliters: 30-80 µliters Golgi suspension (0.3 - .5 mg protein), 0.8 mµmoles (15,000 cpm) UDP-N-acetylglucosamine-^{14}C, 0.5 µmoles $MgCl_2$, 0.1 µmoles EDTA, and 4.0 µmoles Tris-maleate buffer, pH 7.1, with the addition of none (○), 40 mµmoles GDP-mannose (●). Incubations were carried out at 37° for the length of time indicated. Reactions were terminated by the addition of 3 ml of 5% trichloroacetic acid, as described in the text.

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basis of newly synthesized acceptor sites alone. The following substances are inhibitory at 4 x 10^{-4}M: UDP-galactose, 22%; UDP-glucose, 26%; UTP, 22%; UDP, 31%; UMP, 39%.

The initial rate of transfer is linear with respect to protein concentration (Fig. 2), and exhibits optima at pH 6.1 and pH 7.1, indicating the presence of at least two distinct glucosamine transfer activities.

The Golgi-associated transfer of glucosamine to endogenous acceptor accounts for 48% of the total activity of the crude homogenate (at approx. 150 times the specific activity). Plasma membrane and endoplasmic reticulum fractions were practically devoid of activity (0 and 5% respectively, of the activity of the total homogenate).

Incubation with pronase resulted in the conversion of all of the radioactive

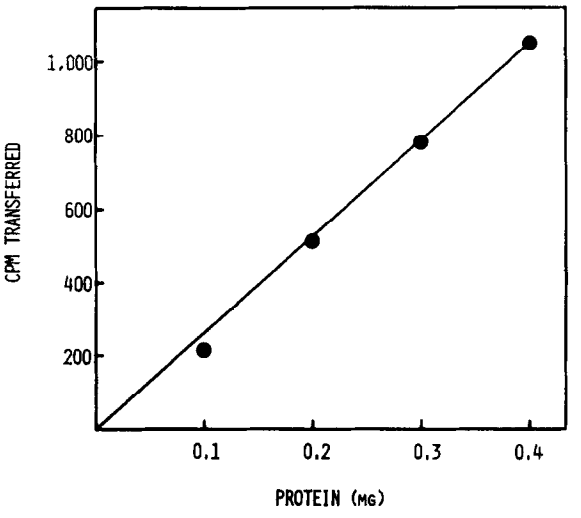

Fig. 2. Effect of enzyme concentration on initial rate of transfer. The conditions of incubation are as described in the legend to Fig. 1. Incubation time was 4 min.

product into a form soluble in 5% trichloroacetic acid. This pronase-digested product was eluted near the void volume of a Sephadex G-50 column, which indicates that the radioactive label is still bound to materials of relatively high molecular weight.

Acid hydrolysis (3N HCl, 100°, 6 hr) of the pronase-digested material yielded glucosamine as the sole radioactive product, as identified by cochromatography with authentic glucosamine on paper, in a solvent system consisting of ethyl acetate-pyridine-H_2O (12:5:4).

Discussion

The results of several *in vivo* studies (10, 11, 12) indicate that most glucosamine residues are transferred to glycoproteins subsequent to the ribosomal stage of protein synthesis, as the completed polypeptide is transported through the channels of the endoplasmic reticulum, prior to secretion by the cell. The finding (1) that the transfer of glucosamine to endogenous acceptor is much higher in microsomes derived from smooth endoplasmic reticulum

than in those derived from rough endoplasmic reticulum, is consistent with this view. The smooth microsomal fractions which were employed in these earlier studies most probably contain plasma membrane fragments and Golgi bodies in addition to smooth membranes. Since the radioautographic techniques which are used to support the claim (2,3) that glyco proteins are synthesized within Golgi bodies are not definitive (i.e. a reaction in the Golgi region after administration of isotopic glucose may reflect transport and packaging, rather than actual synthesis), we decided to examine a Golgi fraction for the presence of both glucosaminyl transferase enzymes and glycoprotein acceptors in vitro. The above results indicate that such a fraction does indeed catalyze the transfer of glucosamine to endogenous protein acceptors, which are presumed to be intermediates in the biosynthesis of glycoproteins.

The products of this reaction(s), and the properties of the transferases involved are being investigated in greater detail. In addition, the isolated Golgi fraction is being examined for the presence of other glycosyl transferases and macromolecular acceptors.

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