Human Fibroblasts Prefer Mannose over Glucose as a Source of Mannose for N-Glycosylation

EVIDENCE FOR THE FUNCTIONAL IMPORTANCE OF TRANSPORTED MANNOSE

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Mannose in N-linked oligosaccharides is assumed to be derived primarily from glucose through phosphomannose isomerase (PMI). The discovery of mammalian mannospecific transporters that function at physiological concentrations suggested that mannose might directly contribute to oligosaccharide synthesis. To determine the relative contribution of glucose and mannose, human fibroblasts were labeled with either [2-3H]mannose or [1,5,6-3H]glucose at the same specific activity, and the N-linked chains were released by PNGase F digestion. Most of the trichloroacetic acid-precipitable [3H]mannose label was released by this digestion, but only about 10% of the trichloroacetic acid-precipitable material was released from cells labeled with [1,5,6-3H]glucose. Both sugars labeled a similar array of oligosaccharides, and acid hydrolysis of these chains showed that [2-3H]mannose contributed 65–75% of the [3H]mannose in cells labeled for 1 h, despite the 100-fold higher concentration of exogenous glucose.

Mannose consumption and [2-3H]mannose utilization were within the range of rates expected for mannose transport via the mannose-specific transporter. About 7–14% of the [2-3H]mannose is used for glycosylation, while the rest (86–93%) is catabolized to H2O via PMI. Increasing the exogenous mannose concentration beyond mannose transporter saturation results in the conversion of >99% of [2-3H]mannose into H2O. Long term labeling of cells with [2-3H]mannose showed that the specific activity of mannose in glycoproteins reached 77% of the specific activity of [2-3H]mannose added to the medium. These results show that when fibroblasts are provided with physiological concentrations of mannose, they use the mannose-specific transporter to supply the majority of mannose needed for glycoprotein synthesis.

Phosphomannose isomerase (PMI)1 (Fru-6-P ↔ Man-6-P) plays a pivotal role in the second route, and it has long been assumed that most, if not all, of the mannose in macromolecules is derived from glucose. This assumption is based on the universal distribution of PMI (5), and the fact that this enzyme is essential for the survival of yeast grown in the absence of mannose (6).

[2-3H]mannose is practically the only isotope used to specifically label newly synthesized N-linked oligosaccharides (3), since its diversion into any other metabolic pathway begins with PMI, which generates 3H2O. This reaction is irreversible in terms of metabolic labeling, since the product is immediately diverted into 55.5 μm water (3). In contrast, [3H]glucose can be catabolized through glycolysis and the tricarboxylic acid cycle to generate energy or used to synthesize a wide variety of metabolic intermediates including sugars, amino acids, and fatty acids (7, 8). The conspicuous lack of selective incorporation into sugar chains explains why [3H]glucose is rarely used to label them.

Normal blood levels of mannose have been measured at 50–100 μM in a few mammals (9–12), but its potential contribution to glycoprotein synthesis has not been investigated. This is probably due to the assumption that its entry into cells via the common glucose (GLUT) transporters is competed by the 100-fold higher levels of blood glucose (13). However, we recently identified a mannose-specific transporter with a Km,plasto of 35–70 μM that can supply mannose for glycoprotein synthesis under physiological conditions of 5.0 mM glucose and 50 μM mannose (14). This finding prompted us to ask which of these sugars is preferred as a source of mannose for glycoprotein biosynthesis when both are present at their physiological levels.

We find that mannose is highly preferred over the 100-fold greater concentration of glucose for N-linked oligosaccharide synthesis, underscoring the functional significance of the mannose-specific transporter.

EXPERIMENTAL PROCEDURES

Materials—Most of the materials were obtained from Sigma, except for the following: concanavalin A (ConA)-Sepharose (Pharmacia Biotech Inc.), ω-minimum essential medium, Dulbecco's modified Eagle's medium (Life Technologies, Inc.), and fetal bovine serum (Hyclone Laboratories, Logan, Utah).

Radioisotopes—[2-3H]mannose (15 Ci/mmol) and [1,5,6-3H]glucose (20 Ci/mmol) were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Cell Lines—Normal adult fibroblast cultures were obtained from American Type Culture Collection and grown in a medium (CRL 1947

The abbreviations used are: PMI, phosphomannose isomerase; ConA, concanavalin A; PNGase F, peptide-N4-(N-acetyl-d-glucosaminyl) asparagine amidase; CDGS, carbohydrate-deficient glycoprotein syndrome; HPAEC-PAD, high performance anion exchange chromatography with pulsed amperometric detection.

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Eukaryotic cells contain mannose primarily in N-linked oligosaccharides and glycopospholipid anchors (1, 2). The only known pathway providing mannose for these molecules requires the conversion of Man-6-P ↔ Man-1-P → GDP-Man and dolichyl-P-Man (3, 4). Man-6-P can be formed in two ways; either directly from mannose using hexokinase or derived from the pathway of GIC → Glc-6-P ↔ Fru-6-P ↔ Man-6-P (Fig. 1).
in Dulbecco's modified Eagle's medium; CRL 1828 in α-minimum essential medium) containing 10% heat-inactivated fetal bovine serum and 2 mM glutamine.

**Labeling of Cells with [2-3H]Mannose or [1,5,6-3H]Glucose—** Fibroblasts were labeled at 37 °C with either [2-3H]mannose (10 μCi/ml) or [1,5,6-3H]glucose (1 mM) for required time periods in Dulbecco's modified Eagle's medium containing 5 mM glucose, 50 mM mannose, and 2 mM glutamine. After removal of the radioactive medium, cells were quickly washed three times with ice-cold phosphate-buffered saline and harvested by trypsinization, sonicated, and solubilized in 0.1% SDS.

In the initial experiments, aliquots of the cell lysates were digested with and without PNGase F, precipitated with trichloroacetic acid, solubilized in 0.1% SDS, and counted after solubilization in 0.1% SDS as described before (15).

**Analysis of Labeled Sugars in N-Linked Oligosaccharides—** To analyze the released labeled sugar chains, the cell lysates were precipitated with trichloroacetic acid, solubilized in 0.1% SDS, and chromatographed on a Sephadex G-50 column. Labeled proteins were collected from the void volume, acetone-precipitated, solubilized in 0.1% SDS, and digested with PNGase F to release the oligosaccharides (15). The digests were again run on a G-50 column in 50 mM Tris-HCl buffer, pH 7.5, containing 0.1% SDS to collect the released oligosaccharides. These samples were passed through a C18 cartridge to remove the detergents; or SDS was precipitated with KCl, and the sample was desalted on Biogel P2 column.

To account for the possible loss of radioactivity during sample preparation, all of the steps were also carried out in a single tube. The percentage of radioactivity released from the cell lysate by PNGase F digestion was the same using both methods.

**ConA-Sepharose Chromatography—** Oligosaccharides in 0.5 ml were applied to 1 ml of ConA-Sepharose column in phosphate-buffered saline, washed with 5 x 1 ml of the same buffer, and eluted with 300 mM NaCl (5 x 1 ml) (fraction I) (16).

**Monosaccharide Analysis—** Oligosaccharides were hydrolyzed in 4 x trifluoroacetic acid at 100 °C for 4 h, and the acid was removed by evaporation. Labeled amino sugars (glucosamine plus N-acetylgalactosamine) were measured by binding to a Dowex 50 cation exchange column. The amount of each hexose was determined by treating the sample with specific enzymes followed by QAE-Sephadex anion exchange chromatography to determine the amount of neutral and anionic material. All enzymatic treatments were carried out in a 100-μl volume of 100 mM Tris-HCl at 37 °C for 2 h. Kinase reactions were done in the presence of 5 mM MgCl₂ and 2 mM ATP. Total hexose content was estimated by incubating the sample with 5 μU of hexokinase (EC 2.7.1.2) at pH 8.0. The amount of glucose was measured by incubation with 1 IU of glucokinase (EC 2.7.1.2) from Bacillus stearothermophilus) at pH 8.5. The amount of galactose was determined as a label that remained neutral after sequential treatments with galactose oxidase (EC 1.1.3.9 from Dactylium dendroides) at pH 7.2 and hexokinase. Standard sugar mixtures were analyzed simultaneously.

**Paper Chromatography—** The neutral monosaccharides were also analyzed by paper chromatography using Whatman 1 MP paper and developed for 18 h with ethyl acetate/pyridine/butanol/butyric acid/water (10:10:5:1:5, v/v). Standards were visualized by silver nitrate analysis by paper chromatography using Whatman 1 MM paper and UV light (10:10:5:1:5, v/v). Standards were visualized by silver nitrate

**H₂O Determination—** An aliquot of the medium used to radio label the cells was evaporated to dryness, suspended in water, and counted to determine radioactivity remaining in the medium. The amount of H₂O formed was taken as the difference between the initial amount of radioactivity in the medium and that remaining after evaporation plus cell lysate radioactivity.

**Mannose in the labeling medium was determined by the method of Etchison and Freeze (18). Glucose in the medium was assayed using a modification of the glucokinase procedure described in Ref. 18.

**Determination of Specific Activity of [2-3H]Mannose Incorporated into Glycoconjugates—** Radiolabeled cells were harvested by scraping and centrifugation at 1000 x g for 5 min. The cells were washed three times with isotonic saline and then lysed in deionized water at a concentration of 1–5 mg/ml. Radiolabeled glycoconjugates were precipitated with 4 volumes of 2-propanol at ~20 °C overnight. The precipitate was washed twice with 2-propanol and resuspended in 70% ethanol by sonication. A portion containing 0.1–0.5 mg of protein was dried in vacuo and hydrolyzed with 100 μl of 2.5 N trifluoroacetic acid at 100 °C for 4 h under nitrogen. The hydrolysate was dried in vacuo, resuspended in 200 μl of 0.1 M HCl, and deionized by passing through a 0.5-ml bed of Dowex 50 mixed bed resin. The effluent containing neutral sugars was dried in vacuo, and a portion was analyzed by HPAC-PAD on a CarboPac PA1 column by the Glycobiology Core Facility at the University of California, San Diego, Cancer Center. The column effluent was collected, and fractions were assayed by liquid scintillation counting. The specific activity of [3H]mannose from hydrolysates was determined using a standard curve generated by analyzing [3H]mannose standards (10–250 μCi/mmol) under identical conditions.

**RESULTS**

**Incorporation of [3H]Mannose and [3H]Glucose into Glycoproteins—** Mannose and glucose can both be converted into Man-6-P and then incorporated into sugar chains of glycoproteins (Fig. 1). Assuming that both labeled sugars enter a common pool of Man-6-P that rapidly equilibrates with the exogenous label, the radioactivity in mannose from each source should be proportional to the contribution from the pathways. To determine which sugar is preferred for N-glycosylation, we incubated identical cultures of human fibroblasts with 5 mM glucose and 50 μM mannose containing either 1 μCi/ml of [3H]glucose or 10 μCi/ml of [3H]mannose for 1 h. The labeled cells were washed, lysed, and precipitated with trichloroacetic acid either before or after digestion with PNGase F to release the N-linked chains (15). Results from multiple labelings (Table I) show that about 40% of the total cell-associated [3H]mannose is trichloroacetic acid-precipitable and that PNGase F digestion releases 80–90% of it, in agreement with the results of previous labeling studies using 1 μM mannose and 0.5 mM glucose (15). In contrast, only 10% of the label from cells incubated with [3H]glucose is trichloroacetic acid-precipitable, and of this, only 1–2% is released by PNGase F digestion. These results suggested that little of the [3H]glucose was incorporated into any of the sugars found in N-linked chains and that mannose was highly preferred over glucose as a precursor.

**Isolation and Analysis of N-Linked Chains Labeled with [3H]Mannose and [3H]Glucose—** Accurate determination of the relative contributions by each labeled precursor required the isolation and analysis of radiolabeled oligosaccharides. A critical assumption for quantitative measurements using these radiolabels is that the intracellular precursor pools have the same specific activity as the exogenous label. To insure equilibration, labeling was extended to 6 and 16 h. The labeled cell lysates were sequentially precipitated with trichloroacetic acid and acetone and then digested with PNGase F to release the N-linked oligosaccharides as described under “Experimental Procedures.” Fig. 2 shows representative elution profiles of samples with and without digestion. About 75% of the radioactivity in [3H]mannose-labeled protein was released, but only about 10% of the label was released from [3H]glucose-labeled protein.

The released chains from samples at each time point were analyzed by anion exchange chromatography on QAE-Sephadex (Table II). Essentially all of the N-linked chains labeled for 1 h were neutral. This was consistent with previous results (15, 19) showing that they are nearly all high mannose, not complex-type oligosaccharides. At longer labeling times, a higher proportion of the oligosaccharides labeled with each sugar were anionic, and all of the charge was due to sialic acids, since they were neutralized by digestion with sialidase (data not shown).
FIG. 1. Metabolic pathway for incorporation of exogenous mannose and glucose into glycoproteins. The box defines the cell membrane that contains both a mannose-specific transporter (Man Transporter) and common GLUT transporters (Glc Transporter), which primarily transport glucose. The relative concentration of exogenous Man (1) and Glc (100) is shown above the respective transporter. \(^{3}H\)-Labeled mannose \((\text{Man}^*)\) can only be incorporated into glycosylation precursors or glycoproteins in the form of N-linked oligosaccharides or glycolipid anchors. Metabolism to any other pathway begins with loss of \(^{3}H_2O\). \(^{3}H\)-Labeled glucose can be converted into Man-6-P and then into glycoproteins. Alternatively, it can be converted into Gal or HexNAc or remain as Glc. The majority of glucose is carried to glycolysis and the tricarboxylic acid cycle for energy or for synthesis of other molecules. Degradation results in conversion into \(^{3}H_2O\). Dol-P-Man, dolichyl P-Man.

TABLE I
PNGase F digestion of proteins labeled with \(^{3}H\)monosaccharides

Results are shown from three different labelings of fibroblasts (CRL 1828) with either 10 \(\mu\)Ci/ml \(^{3}H\)mannose or 1 mCi/ml \(^{3}H\)glucose in the presence of 50 \(\mu\)M d-mannose and 5 mM d-glucose. Cell lysates were incubated with or without PNGase F and trichloroacetic acid (TCA) precipitated to determine the amount of labeled oligosaccharides released. Radioactivity released from \(^{3}H\)mannose- and \(^{3}H\)glucose-labeled samples were defined as 100, and the relative contribution by each sugar was calculated as a percentage of the sum.

| Labeling sugar | Cell lysate | TCA-precipitable | PNGase F-released | Relative contribution |
|----------------|-------------|------------------|-------------------|----------------------|
| \(^{3}H\)Mannose | 544–615 | 223–240 | 201–240 | 86–93 |
| \(^{3}H\)Glucose | 30,180–38,188 | 3169–3434 | 19–34 | 7–14 |

Source of Mannose for Glycoproteins

\[^m\]D-Mannose and 5 \[^m\]D-glucose. Cell lysates were incubated with or without PNGase F and trichloroacetic acid (TCA) precipitated to determine the amount of labeled oligosaccharides released. Radioactivity released from \(^{3}H\)mannose- and \(^{3}H\)glucose-labeled samples were defined as 100, and the relative contribution by each sugar was calculated as a percentage of the sum.

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Analysis of Monosaccharides Labeled with \(^{3}H\)Mannose and \(^{3}H\)Glucose—The released oligosaccharides were hydrolyzed, and the labeled monosaccharides were quantitatively analyzed by both specific enzymatic treatments and by paper chromatography. Both methods gave similar results. None of the \(^{3}H\) label derived from either sugar bound to QAE-Sephadex; however, after incubation with hexokinase and ATP, nearly all (95%) bound, showing that the labels were present entirely in hexoses or hexosamines that had been converted into their 6-phosphate derivatives by hexokinase. Glucokinase analysis (20) showed that only a small amount (3–8%) of \(^{3}H\)glucose remained as glucose. The amount of label in galactose was determined by first treating the mixture with galactose oxidase and then hexokinase followed by QAE-Sephadex analysis. This treatment specifically oxidizes galactose at the C-6-position, forming an aldehyde that cannot be converted into galactose-6-P. If \(^{3}H\)galactose is present, this treatment is expected to increase the amount of residual neutral material when the hydrolysate is incubated with hexokinase and ATP. An example of the results of these treatments is shown in Fig. 3. Galactose represented 6–12% of the radioactivity from chains labeled with \(^{3}H\)mannose, but none was present in those labeled with \(^{3}H\)glucose. The amount of label in amino sugars that bound to Dowex-50 cation exchange column was 10–17%.

After removing the amino sugars, the neutral sugars were separated by paper chromatography. Peaks corresponding to mannose and fucose were present in the hydrolysate labeled with \(^{3}H\)mannose, and peaks corresponding to the positions of galactose, glucose, mannose, and fucose were evident in the \(^{3}H\)glucose-labeled material (not shown). The relative distribution of each sugar is shown in Table III.

Utilization of the \(^{3}H\)-Labeled Sugars—When the exogenous concentration of mannose is 50 \(\mu\)M, most of it enters the cell through the high affinity mannose transporter (14). This man-
nose can be used for glycoprotein synthesis or catabolized, and we have previously estimated the rate of mannose uptake at 8–16 nmol/mg/h in several cell lines (14). To determine the fate of [3H]mannose taken up by the cells under physiological conditions, we measured the cell-associated radioactivity, trichloroacetic acid-precipitable material in the cells and medium, and the amount converted into 3H2O during its entry into glycolysis (Table IV). Direct enzymatic assay of mannose in the medium and utilization of radiolabeled [3H]mannose agree with each other very well. These two independent measurements indicate that the initial uptake rate appears fast during the first hour (29.2 nmol/mg/h) and then decreases to an average of about 7–8 nmol/mg/h protein at longer times. These average rates of mannose uptake are comparable with our previous estimates expected for uptake by the mannose transporter (14). Our results showed that there is less mannose available during the longer labelings. Most of the transported [3H]mannose (85–93%) is converted into 3H2O under physiological conditions. In other experiments using higher nonphysiological concentrations of mannose (0.2–1.0 mM), the proportion of transported [3H]mannose converted into 3H2O increased to 99% (data not shown). Approximately 7–15% of the transported [3H]mannose is nonvolatile and incorporated into cellular or secreted glycoproteins and various glycoprotein precursors such as lipid linked oligosaccharides and sugar phosphates (3).

Average rate of [3H]glucose uptake was 40–150 times higher than mannose, and 1–2% of the label was converted into cellular material, of which 20% was trichloroacetic acid-precipitable.

**Relative Contributions of [3H]Mannose and [3H]Glucose to N-Linked Chains**—The major purpose of this study was to determine the relative contributions of [3H]mannose and [3H]glucose to mannose in N-linked oligosaccharide chains. Knowing the amount of PNGase F-releasable 3H label (Table II), the distribution of labeled monosaccharides was determined (Table III).
Equivalent numbers of CRL 1947 cells in multiwell plates were labeled with either 10 μCi/ml \(^{3}H\)mannose or 1 mCi/ml \(^{3}H\)glucose in the presence of 50 nmol of D-mannose and 5000 nmol of D-glucose in a volume of 1 ml in Dulbecco’s modified Eagle’s medium as described under “Experimental Procedures.” Utilization of mannose and glucose was measured either by enzymatic assay or by calculation based on the radiolabel left in the medium after evaporation and correction for the amount of secreted glycoproteins.

| Labeling sugar | Time   | Enzymatic assay | \(^{3}H\) label | Uptake \(^a\) | Catabolized \(^b\) | Cell lysate | TCA pptable \(^c\) | PNGase F-released | Mannose \(^d\) | Contribution to mannose \(^e\) |
|---------------|--------|-----------------|----------------|-------------|----------------|-------------|----------------|----------------|----------------|----------------|
| \(^{3}H\)mannose | 1      | 3.0             | 5.0            | 29.2        | 27.3           | 1.86        | 0.87           | 0.70           | 0.63           | 58%            |
|               | 6      | 7.5             | 7.5            | 8.1         | 6.94           | 1.16        | 0.26           | 0.21           | 0.19           | 41%            |
|               | 16     | 16.0            | 18.5           | 7.8         | 7.17           | 0.63        | 0.23           | 0.18           | 0.16           | 47%            |
| \(^{3}H\)glucose | 1     | 60              | 255            | 1146        | 1129           | 17          | 4.59           | 0.46           | 0.34           | 35%            |
|               | 6      | 700             | 920            | 1007        | 989            | 18          | 3.31           | 0.39           | 0.27           | 59%            |
|               | 16     | 2400            | 2355           | 1168        | 1155           | 13          | 2.65           | 0.29           | 0.18           | 53%            |

\(^a\) Amount of sugar consumed (average of enzymatic assay and \(^{3}H\) label columns) was used to calculate the uptake rate.

\(^b\) Calculated from the amount of radiolabel converted into \(^{3}H_2O\) after correcting for secreted glycoproteins.

\(^c\) Trichloroacetic acid-precipitable.

\(^d\) Measured from PNGase F-released oligosaccharides and corrected for radioactivity in mannose.

\(^e\) Mannose derived from both glucose and mannose together has been taken as 100%. The relative contribution of each sugar is calculated.

Quantitative Analysis Shows That Mannose Is Highly Preferred over Conversion from Glucose—Radioactivity from \(^{3}H\)mannose is clearly incorporated into N-linked chains more efficiently than radioactivity from \(^{3}H\)glucose, suggesting that mannose is preferred over glucose for glycoprotein synthesis. However, glucose may substitute for mannose in fibroblasts if the concentration of the latter is limited.

**FIG. 4.** Specific activity of \(^{3}H\)mannose incorporated into cellular macromolecules versus labeling time. Cells were radiolabeled with [2-\(^{3}H\)mannose (10 μCi/ml) or [1,5,\(^{3}H\)]glucose (250 μCi/ml) in Dulbecco’s modified Eagle’s medium containing 5 mM glucose and 50 μM mannose and 10% fetal bovine serum. Fresh labeling medium was added every 24 h. At the indicated times of labeling, radiolabeled cellular macromolecules were precipitated and hydrolyzed with trifluoroacetic acid, and released monosaccharides were quantified by HPAEC-PAD analysis and liquid scintillation counting of the column effluent as described under “Experimental Procedures.” In a second experiment (B) the medium was also supplemented with 1 mM sodium pyruvate, and an excess of radiolabeling medium was added daily (5 ml/25 cm\(^2\) for days 1–3 and 10 ml/25 cm\(^2\) for day 4). These data are shown as the double reciprocal plot for determination of maximal specific activity of incorporated \(^{3}H\)mannose attainable by extrapolation to infinite labeling time. The results of two experiments are shown in Fig. 4. As the cells grew, the specific activity of mannose in those labeled with \(^{3}H\)mannose increased. Extrapolation to infinite labeling time showed that the specific activity (155 mCi/mmol) reached 77% of the specific activity of \(^{3}H\)mannose in the labeling medium (200 mCi/mmol) (Fig. 4A). This was confirmed in a second experiment in which the cells were incubated along with so-
Source of Mannose for Glycoproteins

Mannose is a hexose sugar that is an important component of glycans, which are carbohydrate moieties found on glycoproteins and glycolipids. Mannose is a key sugar in the biosynthesis of complex carbohydrates, providing structural diversity to glycoproteins. The source of mannose for glycoprotein synthesis is a topic of interest because it affects the properties and functions of these macromolecules.

In mammalian cells, mannose is absorbed via the mannose transporter. However, glucose uptake is more efficient, and glucose can be oxidized directly for energy production. This competition for uptake results in a significant inhibition of mannose uptake by glucose at physiological concentrations. This phenomenon is known as the glucose starvation effect.

Mannose can be catabolized through the pentose phosphate pathway (PPP) to generate 3H2O or be converted to [2-3H]Man-6-P and then to other glycosylation intermediates and finally to macromolecular products. These decisions are made based on the concentration of mannose and the physiological conditions at which glycoproteins are synthesized.

Since mannose is a preferred substrate for the formation of fucose and N-linked oligosaccharides, its concentration is critical for proper glycoprotein synthesis. Experiments using [1-3H]mannose and [3H]glucose have shown that mannose is the preferred substrate under most conditions, although this preference can be reversed by increasing the glucose concentration.

Discussing the implications of these findings, it is clear that mannose is a key substrate for glycoprotein synthesis. However, the specific utilization of mannose versus glucose depends on the cellular context and the physiological conditions. The results also highlight the importance of elucidating the mechanisms that govern the competition between mannose and glucose for uptake and utilization.

**Discussion**

Identifying the source of mannose for oligosaccharide synthesis became a critical question when we found that mannose uptake near the concentrations of glucose. Experiments with [1-3H]mannose and [3H]glucose showed that the specific activity of mannose in glycoproteins was significantly higher than that of glucose. This finding led us to conclude that mannose was the preferred substrate for glycoprotein synthesis.

Cells were also incubated over several days with medium containing 50 μCi [3H]mannose and 25 mM glucose rather than the physiological concentration of 5 mM. Extrapolation to infinite labeling time shows that the specific activity of mannose in glycoproteins increased in the high glucose medium, reaching only 25% of that seen in 5 mM glucose medium. The decrease probably results from the 3-4-fold greater inhibition of mannose entry via the mannose transporter.

Together these labeled sugars account for 99-100% of the mannose in glycoproteins and indicate that only a very small portion of mannose in glycoproteins could be derived from unlabeled sources such as amino acids or pyruvate via gluconeogenesis.

The interpretation of our results on the relative utilization of [3H]mannose versus [3H]glucose in the analysis of N-linked chains relies on several assumptions. The first is that there is a single pool of Man-6-P that is freely accessible to both Man and Fru-6-P and that subsequent conversions of Man-6-P to Man-1-P and GDP-Man cannot discriminate the origin of Man-6-P. As far as we are aware, there is no evidence for separate pools of these intermediates. Second, the specific activity of the Fru-6-P and Man-6-P pools must quickly attain that of exogenous [3H]mannose and [3H]glucose labels. The Man-1-P, Man-6-P, and GDP-Man pools are very small in cultured cells.

Given our measured rate of utilization of both labeled mannose and glucose by the cells, it is likely that the internal pools equilibrate with the exogenous radiolabeled medium within seconds or, at most, a few minutes. This is especially important for the 1-h incubations. Continuous labeling of cells with mannose and glucose for 6 and 16 h further ensured equilibration of the label.

The data in Table IV show that mannose consumption measured by direct enzymatic assay and that estimated by accounting for 3H2O and [3H]mannose in the glycosylation pathway closely agree with each other, and mannose uptake rates calculated are comparable with the predicted estimates for mannose transporter. As mannose is consumed from the medium, its relative contribution to glycosylation could decrease, but some of this loss is undoubtedly due to N-linked oligosaccharide processing. As long as exogenous mannose remains available, mannose uptake rates can be used to infer the physiological levels of mannose uptake.

The direct demonstration that the specific activity of mannose incorporated into glycoproteins can reach 77% of the specific activity of mannose added to the medium shows that under these physiological conditions, fibroblasts rely primarily on mannose for glycosylation rather than converting glucose into mannose via the well known phosphomannose isomerase-based pathway. The relatively long time needed to achieve maximal labeling with [3H]mannose could reflect a preferential reutilization of mannose salvaged from glycoprotein catabolism. Alternatively, the radiolabel might be self-diluting. A single conversion of [3H]mannose-6-P → fructose-6-P + 3H2O by PMI could only be catabolized through PMI to generate 3H2O or be converted to [2-3H]Man-6-P and then to other glycosylation intermediates and finally to macromolecular products. These decisive advantages make it practically the universal choice for specific labeling of N-linked chains.
is sufficient to produce a nonlabeled molecule, but the energetically neutral reaction ($K_{eq} = 1.03$) could sometimes be reversed to generate an unlabeled mannose-6-P that could participate in glycosylation reactions. The 23% contributed by other sources appears to be primarily from glucose, but a small amount could arise from gluconogenesis. Including pyruvate in the medium did not affect the ultimate specific activity of $[^3H]$mannose in glycoconjugates, but the calculated time required to reach this level was longer than in its absence. At present we cannot tell whether this is due to culture conditions or other factors. It is important to point out that the relative contribution of $[^3H]$mannose to glycoprotein synthesis is nearly the same whether measured by isotope dilution analysis or by counting samples labeled with each precursor in short term labelings. The origin of mannose in the blood is unknown. Some is probably derived from the diet; however, neither the content nor the bioavailability of mannose in foods has been investigated. Mannose may also be derived from normal oligosaccharide processing or from glycoprotein-bound or free oligosaccharide degradation (1, 41). Clearly, glucose can be converted into mannose, but the amount may be cell type- or tissue-dependent. If the mannose transporters are major suppliers of mannose for glycoprotein synthesis in mammalian systems, we would expect to find mannose in the blood of all species. Regardless of their specific diets, mammals should also have an intestinal transport system that delivers mannose to the blood. A key factor for supplying mannose is likely to be the efficiency of mannose-specific transporters. The identification of mannose as a major source for glycosylation in fibroblasts has physiological, nutritional, and medical implications beyond a potential therapy for CDGS type 1 (15, 4440–4455). Several reports have underscored the problem of underglycosylation of important glycoproteins (49–51). Based on the results presented here, mannose may promote more efficient glycosylation of proteins than glucose.

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