Mannose Enters Mammalian Cells Using a Specific Transporter That Is Insensitive to Glucose*

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The concentration of D-mannose in serum is 20–50 μM, but its physiological significance for glycoprotein synthesis is unknown. Here, we show that the uptake of D-mannose by different mammalian cell lines involves a mannose-specific transporter(s) with a K_m, uptake of about 30–70 μM and a V_max which is probably sufficient to account for the bulk of mannose needed for glycoprotein synthesis. Mannose uptake appears to be through a facilitated transport process since it is not inhibited by cyanide. Phloretin completely inhibits mannose uptake, but phloridzin inhibits only 25–30%. Both of these inhibitors can block 2-deoxyglucose uptake in fibroblasts which occurs through the typical glucose transporters. None of 9 other sugars tested inhibited mannose transport. Most importantly, 5 mM D-glucose only inhibits mannose uptake by 50% showing that it is not an efficient competitor. These results suggest that this transporter(s) may use serum mannose for glycoprotein synthesis.

In mammalian cells, mannose for glycoprotein synthesis is assumed to originate mostly, if not entirely, from intracellular synthesis. Man-6-P is probably the main supply of mannose for glycoprotein synthesis. Mannose uptake appears to be through a facilitated transport process since it is not inhibited by cyanide. Phloretin completely inhibits mannose uptake, but phloridzin inhibits only 25–30%. Both of these inhibitors can block 2-deoxyglucose uptake in fibroblasts which occurs through the typical glucose transporters. None of 9 other sugars tested inhibited mannose transport. Most importantly, 5 mM D-glucose only inhibits mannose uptake by 50% showing that it is not an efficient competitor. These results suggest that this transporter(s) may use serum mannose for glycoprotein synthesis.

Experimental Procedures

Materials—D-Glucose, D-mannose, L-mannose, D-galactose, D-fructose, D-ribose, L-fucose, myo-inositol, L-rhamnose, phloretin, and phloridzin were obtained from Sigma. α-Minimal essential medium (α-MEM) and Dulbecco’s modified essential medium (DMEM) were from Life Technologies, Inc. Fetal bovine serum was from Hyclone. Penicillin, streptomycin, and kanamycin were from Life Technologies, Inc. 4-[3H]-Mannose (15 Ci/mmol) and 2-deoxy-[1-3H]-glucose (40 Ci/mmol) were obtained from American Radiolabeled Chemicals, Inc., St. Louis, MO.

Cell Lines and Culture—Normal skin fibroblasts (922sk; CRL 1828), normal rat kidney fibroblasts (NRK-49F; CRL 1570), Madin Darby canine kidney (MDCK NBL-2; CCL 34), and macrophages (RAW 264) were obtained from the ATCC. Rat glial-like cells (B28) were provided by Dr. William Stallcup (13). Mast cells (ABFTL-3) were provided by Dr. Greg Henkel (14).

Fibroblasts (922sk; NRK-49F) were grown in α-MEM supplemented with 10% heat-inactivated fetal bovine serum. 2 mM glutamine, penicillin, streptomycin, and kanamycin were added to the α-MEM containing 10% fetal bovine serum, and antibiotics. Raw (264) and mast cells (ABFTL-3) were grown in RPMI medium supplemented with 10% fetal bovine serum and antibiotics.

Measurement of [3H]-Mannose Uptake—Nearly confluent cells in 35-mm multiwell plates were rinsed with DMEM containing no glucose. Uptake was initiated by the addition of labeling medium containing 20 μCi/ml [3H]-mannose, 2 mM glutamine in DMEM in the absence or presence (0.5 mM) of glucose and incubated at 37 °C for the required time. To study the effect of hexose inhibitors (phloretin and phloridzin), they were included in the labeling medium at the concentrations indicated in Fig. 2. To determine the energy dependence of mannose uptake, cells were preincubated with 1 mM potassium cyanide for 10 min and then labeled with [3H]-mannose (20 μCi/ml) for 10 min at 37 °C. After removal of the radiolabeled medium, cells were washed three times in ice-cold phosphate-buffered saline, harvested by trypsinization, and solubilized in 0.1% SDS. An aliquot of the cell lysate was counted for radioactivity and normalized to protein content.

1 The abbreviations used are: CDGS, carbohydrate-deficient glycoprotein syndrome; LLO, lipid-linked oligosaccharide; PNGase F, peptide-N′-N-acetyl-[β-glucosaminyl] asparagine amidase; α-MEM, α-minimal essential medium; DMEM, Dulbecco’s modified essential medium; PM1, phosphomannomannosidase.

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Incorporation of \(^{3}H\)mannose into protein was determined by adding 10% trichloroacetic acid to an aliquot of the cell lysate using 100 \(\mu\)g of bovine serum albumin as carrier. After vortexing and standing 10 min on ice, the precipitated protein was collected by centrifugation. The pellet was washed with 10% trichloroacetic acid, neutralized with 0.1 N NaOH, transferred to a scintillation vial, and counted.

Fractionation of \(^{3}H\)Mannose-labeled Products—Fibroblasts grown in glass plates were labeled for 45 min at 37°C with 20 \(\mu\)Ci/ml \(^{3}H\)mannose in DMEM containing 0.5 mM glucose and 2 mM glutamine. After removing the medium, cells were washed a few times with ice-cold phosphate-buffered saline to remove free label, scraped, and extracted three times with 5 ml of CHCl\(_3\)/MeOH (2:1, v/v). This extract contains mostly Dol-P-Man. The residual pellet was dried, sonicated, centrifuged, and washed three times with water to isolate the free oligosaccharides and low molecular weight lipid-linked oligosaccharide (LLO) precursors. The pellet after water wash was extracted three times with 5 ml of CHCl\(_3\)/MeOH/H\(_2\)O (10:10:3, v/v) to isolate LLO. The final pellet was solubilized in 0.1% SDS and digested with peptide-N\(^{4}\)-glucosaminyl)asparagine amidase (PNGase F) to release the protein-bound N-linked oligosaccharides.

RESULTS

\(^{3}H\)Mannose Uptake by Human Fibroblasts—Human skin fibroblasts (922sk) were incubated for short times with serum-free medium and 20 \(\mu\)Ci/ml \(^{3}H\)mannose (1 \(\mu\)M) in the absence or presence of 500 \(\mu\)M glucose. As shown in Fig. 1, glucose made little difference in the rate of \(^{3}H\)mannose uptake, indicating that mannose entry is insensitive to a large excess of glucose. This result is unexpected since both mannose and glucose are assumed to enter the cells by the same transporter and a 500-fold excess of glucose should inhibit \(^{3}H\)mannose uptake. Phloridzin, at all concentrations tested, similarly inhibits the entry of both \(^{3}H\)mannose and 2-\(^{3}H\)deoxyglucose into the cells (Fig. 2A). Phloridzin, a well-known inhibitor of Na\(^{+}\)-dependent active hexose transport (K\(_{\text{m}} < 10 \mu\text{M}\)) (15, 16), showed about a 25% inhibition of \(^{3}H\)mannose and 2-\(^{3}H\)deoxyglucose at a concentration of 50 \(\mu\)M. At higher concentrations, it variably blocked 2-\(^{3}H\)deoxyglucose uptake but had little further effect on \(^{3}H\)mannose uptake (Fig. 2B). Mannose uptake was not significantly inhibited either by including 1 mM potassium cyanide in the labeling medium or by preincubating the cells in 1 mM potassium cyanide for 10 min prior to labeling. So, it is unlikely that mannose enters the cells using Na\(^+\)-dependent active transport. Our results are consistent with mannose entering the cells using facilitative type transporter(s), a portion of which is sensitive to phloridzin.

Determination of K\(_{\text{uptake}}\)—Similar short-term labeling with increasing amounts of mannose was done in the presence of 0.5 mM glucose to determine the K\(_{\text{uptake}}\) of mannose. The amount of cell-associated mannose was calculated based on the known specific activity of mannose in the medium. A biphasic saturation curve was obtained using a wide range of mannose concentrations, suggesting that mannose enters the cell using two different transporters. In three different labelings, K\(_{\text{uptake}}\) of mannose was calculated at 30–70 \(\mu\)M and a V\(_{\text{max}}\) of 3.0–9.0 nmol/mg/h. A representative experiment is shown in Fig. 3A (K\(_{\text{uptake}}\) 35 \(\mu\)M; V\(_{\text{max}}\) of 3.2 nmol/mg/h). This is significant because the plasma mannose concentration has been measured at 20–50 \(\mu\)M (5, 6), suggesting that such a transporter could be effective at physiological levels of mannose. Our calculated K\(_{\text{uptake}}\) is considerably lower than those usually seen for facilitative type or Na\(^{+}\)-dependent type glucose transporters which are normally in the millimolar range (8, 11). At higher concentrations of mannose, a second saturation curve can be plotted which gives a K\(_{\text{uptake}}\) of 850 \(\mu\)M (Fig. 3B) and a V\(_{\text{max}}\) of 78 nmol/mg/h. This K\(_{\text{uptake}}\) is comparable to the range (0.8–8.6 \(\mu\)M) reported for 2-deoxyglucose entry using the GLUT1 transporter that is normally found in all cultured cells (17, 18). Since GLUT 1 can also transport mannose (2), it is likely to account for the uptake of mannose at high concentrations as seen in Fig. 3B. When the results obtained using all mannose concentrations are plotted as V\(_{\text{d}}\) versus V\(_{\text{max}}\) (Fig. 3C), two kinetically distinct transporters are evident.

Incorporation of \(^{3}H\)Mannose into Glycoproteins—To determine whether the high affinity transporter provides mannose for glycoprotein synthesis, fibroblasts were labeled for 45 min with a constant amount of \(^{3}H\)mannose and variable amounts of unlabeled mannose. We calculated the amount of mannose in the various fractions based on the specific activity of \(^{3}H\)mannose in the medium, assuming that the endogenous pools rapidly equilibrate with the label in the medium. This is probably valid since the GDP-Man pool in mammalian cells is quite small (2–5 pmol/10\(^6\) cells) and would completely turn over within a few minutes.\(^2\) As shown in Fig. 4, the curves for total cell-associated mannose and that in glycoproteins are similar, with about 25–30% incorporated into glycoproteins. About 85% of the trichloroacetic acid-precipitable \(^{3}H\) label in protein was released with PNGase F and consisted of mostly high mannose oligosaccharides (data not shown). The PNGase F-insensitive

\(^2\) Several laboratories have estimated the GDP-Man level as 2–25 pmol/10\(^6\) cells (Refs. 20, 38, and 39). Since different cell lines have been found to have \(\sim 300 \mu\)g of protein in 10\(^6\) cells, we normalized the GDP-Man content to 6–80 pmol/mg. We have calculated (see “Discussion”) that cells require approximately 1.5 nmol of mannose per h for glycoprotein synthesis. That means the GDP-Man pool should turn over 20–250 times per h.
label is probably in glycolipid anchors. The remaining cell-associated label is found in lipid-linked (20–25%) and free oligosaccharides (15%) and in low molecular weight precursors (15%). Although [2-3H]mannose can be metabolized by the glycolytic pathway and lost in the medium as 3H2O, this accounts for only about 15–20% of the total, which is comparable to our previous results (19).

The calculated Vmax of 5.1 nmol/mg/h and $K_{accu}$mulation of 80 μM are comparable with those obtained in the short term labeling experiments. These results suggest that much of the mannose predicted to be taken up by the high affinity transporter is used for oligosaccharide synthesis. Similar kinetic values were also obtained for macrophages, NRK, MDCK, glial-like, and mast cells (see Table I). The occurrence of similar saturation curves and kinetic values in several different cell types means that mannose transport for glycoprotein synthesis may be a common feature of mammalian cells.

Specificity of Mannose Transporter—To determine whether the mannose uptake was specific and that the transported mannose contributed to glycoprotein synthesis, we incubated cells with 1 μM [3H]mannose and increasing amounts of nonlabeled mannose or with other sugars for 45 min. The longer incubation times were needed to allow significant incorporation into glycoprotein. Irrespective of the sugar present, 25% of the radiolabel present in the cell lysate was trichloroacetic acid-precipitable (data not shown). Table II shows the cell-associated radiolabel. Mannose itself was the only effective competitor. Glucose had a much less pronounced effect; even at a 5000-fold excess over mannose, glucose inhibited [3H]mannose uptake by only 50%. 2-Deoxyglucose which is a known competitor of glucose uptake, had minimal effects on [3H]mannose transport and suggests that it is not recognized by the putative mannose transporter. Thus, efficient mannose uptake and incorporation into glycoproteins is quite tolerant of physiological concentrations of glucose. Galactose and other sugars such as xylose, rhamnose, fucose, fructose, N-acetylgalactosamine, L-mannose, and myo-inositol produced negligible inhibition at 5000-fold excess (Table II). Only talose which is the C-4 epimer of mannose inhibited the mannose uptake by 50%. Conversely, 1 mM mannose produces significant inhibition of [3H]deoxyglucose uptake (not shown), as expected, since at high concentrations, mannose can be carried by the more common hexose transporters (2).

**DISCUSSION**

We present evidence that human fibroblasts efficiently transport mannose and use it for glycoprotein synthesis. The three essential findings are: 1) transport is mannose-specific, 2) it is only slightly inhibited by glucose at normal plasma concentrations, and 3) the calculated $K_{uptake}$ for mannose in the medium, $V_{max}$, and $V_{min}$ were calculated using Lineweaver-Burk plots.

**Table I**

| Cell type          | $K_{accumulation}$ | $V_{max}$ |
|--------------------|--------------------|-----------|
| Fibroblast (922sk) | 84                 | 15.9      |
| NRK (CRL 1570)     | 88                 | 7.6       |
| Macrophages (RAW264)| 64                 | 5.4       |
| Glial-like (B28)   | 83                 | 11.0      |
| Mast (ABFTL-3)     | 97                 | 9.2       |
| MDCK (CCL 34)      | 119                | 17.5      |
Normal plasma glucose (5.0 mM) is about 1.75 nmol/h which is uptake by the high affinity transporter in the presence of experimentally determined in different cell lines (20). Mannose uptake by the high affinity transporter in the presence of various additional sugars for 45 min at 37°C and measured the cell-associated radiolabel as described under “Experimental Procedures.”

| Sugar                  | [3H]Mannose/mg (cpm × 10⁻³) |
|------------------------|------------------------------|
| Control (0.5 mM d-glucose) | 1266 (100)*                   |
| d-Glucose (mM)          |                               |
| 1                      | 936 (74)                      |
| 5                      | 620 (49)                      |
| 10                     | 125 (9.9)                     |
| 2-Deoxy-d-glucose (mM)  |                               |
| 1                      | 1126 (89)                     |
| 10                     | 772 (61)                      |
| d-Mannose (mM)          |                               |
| 0.06                   | 870 (64)                      |
| 0.25                   | 455 (36)                      |
| 0.5                    | 227 (18)                      |
| 1.0                    | 87 (6.9)                      |
| Other sugars (5 mM)     |                               |
| d-Galactose             | 1093 (86.4)                   |
| N-Acetyl-d-glucosamine  | 1157 (91.4)                   |
| myo-Inositol            | 1136 (89.8)                   |
| d-Xylose                | 1045 (82.6)                   |
| L-Rhamnose              | 1282 (101.3)                  |
| d-Talose                | 639 (50.5)                    |
| L-Fucose                | 1007 (79.6)                   |
| d-Fructose              | 1225 (96.8)                   |
| L-Mannose               | 1007 (79.6)                   |

* Numbers in the parentheses indicate percent of control which is defined as 100%, and that in the presence of additional sugar is presented as a percentage of this value.

Mannose uptake by the high affinity transporter in the presence of normal plasma glucose (5.0 mM) is about 1.75 nmol/h which would be roughly sufficient to supply all of the mannose required for glycoprotein synthesis. Since we see similar results in macrophages, NRK, MDCK, glial-like, and mast cells, it suggests that mannose-specific transport may be a common feature of mammalian cells.

At the cellular level, mannose is used for biosynthesis of oligosaccharides and glycosphospholipid anchors (21), and, under normal circumstances, it does not appear to make a large contribution to general energy metabolism (22, 23). Several studies have shown that mannose can be converted into glycogen (22, 24), and, in general, at high concentrations (>5 mM) it is metabolized like glucose at both the cellular and organismic levels (25–30). We could find no studies that used the normal physiological concentration of mannose for any experiments. This lack of attention probably results from the assumption that glucose normally provides sufficient mannose for glycoprotein synthesis. Also, it has been assumed that plasma mannose entry should not be significant because the overwhelming excess of glucose would compete out mannose transport through the typical hexose transporter. Our results bring these assumptions into question. However, at this point we do not know the relative contributions of glucose and mannose to glycoprotein synthesis in any system. In some cases, exogenous mannose may be a significant or preferential precursor for glycoprotein synthesis.

The plasma mannose presumably comes from a combination of dietary sources (23, 24), normal oligosaccharide processing (31), and from turnover of endogenous glycoproteins and free oligosaccharides (32–34). Since mammalian cells generally contain a higher proportion of complex oligosaccharides compared to unprocessed high mannose-type chains, it is clear that the majority of nine mannose residues initially incorporated into the lipid-linked oligosaccharide (LLO) precursor will be lost as free mannose. When this amount is added to that generated by catabolism of LLO in cultured cells (40), perhaps as much as 75–80% of the mannose initially incorporated into LLO does not become incorporated into stable protein products. It would be reasonable to have a salvage system that recycles the large fraction of rapidly turning over mannose. Mammalian cells are equipped with a diverse collection of broad specificity and highly specific α-mannosidases (35) that degrade oligosaccharides within the cell, but the fate of the released mannose has not been studied. Some of it would probably reach the plasma where it could be reabsorbed or excreted. Studies in rabbit kidney shows that mannose reabsorption is blocked by phloretin but not by phlorizin whereas glucose reabsorption is blocked by both the inhibitors (29). These results were interpreted to mean that there were different binding sites on the same transporter, but they could also be interpreted as mannose and glucose using different transporters.

Clearly, mannose can be derived from glucose. The assumption that glucose is the primary source of mannose seems to be based on the ubiquitous distribution of phosphomannomutase isomerase (PMI) and the fact that PMI deficiency is lethal in yeast (36). This comparison may not be appropriate since the demand for extended high mannose-type oligosaccharide synthesis in yeast is much greater and the specific activity of their PMI is 20–100 times higher than in mammalian cells (37). There are no comparable mutations in mammalian cells or specific inhibitors of PMI to directly determine its contribution to glycoprotein synthesis.

Our previous findings that CDGS cells are deficient in mannose uptake and underglycosylate their proteins may be partially explained by an altered mannose transporter system, but this will require identification of the mannose transporter(s) itself.

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REFERENCES

1. Schwartz, N. B. (1992) in Textbook of Biochemistry with Clinical Correlations: Carbohydrate Metabolism II: Special Pathways (Delvin, T. M., ed) pp. 359–386, John Wiley & Sons, Inc., New York.

2. Gould, G. W., Thomas, H. M., Jones, T. J., and Bell, G. I. (1991) Biochemistry 30, 5139–5145.

3. Miyamoto, K., Hase, T., Takagi, T., Fujii, T., Taketani, Y., Minamai, H., Oka, T., and Nakabou, Y. (1993) Biochem. J. 295, 211–215.

4. Mayer, P., Maianu, L., and Garvey, W. T. (1992) Diabetes 41, 274–285.

5. Soyama, K. (1984) Clin. Chem. 30, 293–294.

6. Akazawa, S., Metzger, B. E., and Freinkel, N. (1986) J. Clin. Endocrinol. Metab. 62, 984–989.

7. Yurchenco, P. D., Caccaniga, C., and Atkinson, P. H. (1978) Methods Enzymol. 50, 175–204.

8. Gould, G. W., and Holman, G. D. (1993) Biochem. J. 295, 329–341.

9. Asano, T., Katagiri, H., Takata, K., Lin, J. L., Ishihara, H., Inukai, K., Tsukuda, K., Kikuchi, M., Hirano, T., Noguchi, K., and Oka, Y. (1991) Biochem. J. 266, 2463–2466.

10. Lefevre, P. G., and Marshall, J. K. (1999) J. Biol. Chem. 274, 3022–3026.

11. Hedges, M. A., and Rabin, D. G. (1999) J. Biol. Chem. 274, 3022–3026.

12. Henkel, G., and Brown, M. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7737–7741.

13. Chardon, P., Pécocq, J., Mehta, A., and Kemp, P. J. (1994) Am. J. Physiol. 267, L390–L397.

14. Lee, W. S., Kanai, Y., Wells, R. G., and Hediger, M. A. (1994) J. Biol. Chem. 269, 12032–12039.

15. Germinario, R. J., Oliveria, M., and Taylor, M. (1987) Clin. Invest. Med. 10, 295–302.

16. Germinario, R. J., and Walken, B. M. (1992) J. Cell. Biol. 116, 156–163.

17. Pannenkoven, K., and Freeze, H. H. (1995) Biochem. Biophys. Res. Commun. 205, 517–522.

18. Rush, J. S., and Waechter, C. J. (1995) Anal. Biochem. 244, 494–501.
21. Varki, A. (1994) Methods Enzymol. 230, 16–32
22. Bailey, W. H., and Roe, J. H. (1943) J. Biol. Chem. 152, 135–146
23. Herman, R. H. (1971) Am. J. Clin. Nutr. 24, 488–498
24. Davis, S. E., and Lewis, B. A. (1975) in Carbohydrate Symposium; Physiological Effects of Food Carbohydrates, pp. 296–311
25. Harding, V. J., Nicholson, T. F., and Armstrong, (1934) J. Biol. Chem. 128, 2036–2042
26. Wood, F. C., Jr., and Cahill, G. F. J. (1963) J. Clin. Invest. 42, 1300–1312
27. Sloviter, H. A., and Kamimoto, T. (1970) J. Neurochem. 17, 1109–1111
28. Dringen, R., Bergbauer, K., Wiesinger, H., and Hamprecht, B. (1994) Neurochem. Res. 19, 23–30
29. Silverman, M., Aganon, M. A., and Chinard, F. P. (1970) Am. J. Physiol. 218, 743–750
30. McNamara, P. D., Rea, C., Ozegovic, B., and Segal, S. (1976) Am. J. Physiol. 231, 9–13
31. Kornfeld R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664
32. Villers, C., Cacan, R., Mir, A. M., Labiau, O., and Verbert, A. (1994) Biochem. J. 298, 135–142
33. Cacan, R., Villers, C., Melard, M., Kaiden, A., Krag, S. S., and Verbert, A. (1992) Glycobiology 2, 127–136
34. Spiro, M. J., and Spiro, R. G. (1991) J. Biol. Chem. 266, 5311–5317
35. Daniel, P. F., Winchester, B., and Warren, C. D. (1994) Glycobiology 4, 551–566
36. Payton, M. A., Rheinnecker, M., Klig, L. S., De Tiani, M., and Bowden, E. (1991) J. Bacteriol. 173, 2006–2010
37. Gracy, R. W., and Noltmann, E. A. (1968) J. Biol. Chem. 243, 3161–3168
38. Rimoldi, D., Creek, K. D., and De Luca, L. M. (1990) Mol. Cell. Biochem. 93, 129–140
39. Pels Rijcken, W. P., Overdijk, B., Van den Eijnden, D. H., and Ferwerda, W. (1995) Biochem. J. 305, 865–870
40. Panneerselvam, K., and Freeze, H. H. (1996) J. Clin. Invest. 97, 1478–1487
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