A Stereospecific myo-Inositol/β-chiro-Inositol Transporter in HepG2 Liver Cells

IDENTIFICATION WITH β-CHIRO-[3-3H]INOSITOL*

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β-chiro-Inositol is an epimer of myo-inositol that is found in certain mammalian glycosylphosphatidylinositol protein anchors and inositol phosphoglycans possessing insulin-like bioactivity. In order to generate a probe for metabolic studies, β-chiro-[3-3H]inositol was synthesized by selective reduction of β-chiro-3-inosose at pH 6.5 with sodium borotritide. β-chiro-[3-3H]inositol was taken up by HepG2 human liver cells through a saturable and stereospecific pathway in which β-chiro-inositol by the myo-inositol competitively but β-chiro-inositol was not recognized. β-Glucose, but not l-glucose, competed for β-chiro-[3-3H]inositol uptake over glucose concentrations of 4–28 mM. Maximum transport capacity was 717 pmol/mg cell protein/3 h with a Kₘ value of 348 μM. Uptake was reduced by 76% when sodium was eliminated from the medium and by 94% when the experiment was performed at 0 °C.

The new myo/β-chiro-inositol transporter is distinct from the sodium-myo-inositol co-transporter found in many tissues and accounts for all of the saturable β-chiro-inositol uptake and for a portion of the saturable low affinity myo-inositol uptake in HepG2 cells. It may allow β-chiro-inositol to be used by cells in the presence of a relatively large amount of competing myo-inositol.

myo-Inositol, one of nine isomers of hexahydroxycyclohexane, comprises almost all of the naturally occurring inositol of mammalian tissues. However, a small amount of chiro-inositol, an optically active epimer of myo-inositol, is also present and may have physiological importance. chiro-inositol was recently found unexpectedly in inositol phosphoglycans thought to be mediators of insulin signaling (1, 2) and in glycosylphosphatidylinositol protein anchors (3, 4). The related compound pinitol, 3-O-methyl-β-chiro-inositol, is a prominent component of dietary legumes such as soybeans (5). Clinical studies have demonstrated that diabetic patients excrete large amounts of β-chiro-inositol in urine related to the degree of diabetic glycemic control (6, 7), although the opposite result has also been reported (8). Treatment of diabetics with insulin resulted in decreased urinary excretion and transient elevation of plasma β-chiro-inositol (6). These experiments show that β-chiro-inositol and related substances are recognized by cell systems, but little is known about the biochemical pathways involved.

To allow further study of this unusual cyclitol with high sensitivity we have prepared radiolabeled β-chiro-inositol by reduction of β-chiro-3-inosose using sodium borotritide. The reaction product, β-chiro-[3-3H]inositol, is taken up avidly by cultured HepG2 liver cells in a pathway that is stereospecific and shared with myo-inositol.

EXPERIMENTAL PROCEDURES

Materials—β-chiro-Inositol (Lot 42136, no longer commercially available) and β-glucose were purchased from Calbiochem. Pinitol was donated by Dr. Andrew Falshaw of Industrial Research Ltd., Lower Hutt, New Zealand, and was also prepared from soybeans by ion exchange chromatography as described previously (5). l-chiro-Inositol was a gift of Dr. Laurens Anderson of the University of Wisconsin. myo-[2-3H]-Inositol, 20 Ci/mmol, was purchased from American Radiolabeled Chemicals (St. Louis, MO). Hexadeuterated racemic chiro-inositol was a gift of Dr. Ken Sasaki (Connaught Centre for Biotechnology Research, Toronto, Canada). Cyanocobalamin B (C-6762) and phlorizin (P-8037) were from Sigma.

Analytical Procedures—HPLC was performed with an amino-propyl-silica column (Supelcosil-LC-NH2, Supelco, Bellefonte, PA) using acetone/trililic acid mixtures as mobile phase. Although good HPLC separation of most inositols has been reported with a calcium-form cation exchange resin designed for carbohydrates (9, 10), the separation between chiro-inositol and myo-inositol was incomplete. A previous HPLC separation of inositols reported by Ghias-Ud-Din et al. (11) showed excellent resolution of chiro-inositol and myo-inositol, but the column chemistry was proprietary and uncharacterized. At the suggestion of Dr. Daniel V. Phillips of the University of Georgia we tested the reported separations using an amino-propyl-silica column and have obtained very similar results, suggesting that the original column chemistry may have been aminopropyl-silica. Chiro-inositol and myo-inositol were separated at base line (retention volumes 10.8 and 12.8 min, respectively), using a mobile phase of 76:24 acetone/trililic acid with detection at OD 206. This HPLC system is very useful for the separation of inositols, inososes, and inositol methyl ethers.

For GC/MS analysis inositol samples were derivatized by incubating with 10% pentfluoropropionic anhydride in acetonitrile at 65 °C for 30 min. The derivatizing reagent was evaporated almost to dryness, and the samples were reconstructed in acetonitrile and injected onto a 25 × 0.23-mm inner diameter Chirasil-Val fused silica capillary column with 0.2-μm film thickness (Alltech Associates, Deerfield, IL) and analyzed on an HP 5970 electron impact ionization positive ion mass spectrometer. A temperature program of 100 °C for 1 min followed by a pro-

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Results of labeled D-chiro-inositol. 

Preparation of D-chiro-[3-3H]inositol—D-chiro-3-inosose was prepared from 1-bromo-2,3-dihydroxy cyclohexa-4,6-diene as described previously (12). Because this material tends to form decomposition products on storage it was purified by HPLC on an amnropropyl-silica column in 78:22 acetonitrile:water mobile phase immediately before labeling. To 250 µl of 20 mg/ml inosose in water we added 28 µl of 1 M sodium phosphate buffer, pH 6.5 (final concentration 0.1 M), and then 1 Ci of sodium borotritide (15 Ci/mmol), and the reaction was allowed to stand at room temperature overnight. The reaction products were separated by preparative high voltage paper electrophoresis and deionized with a small amount of mixed bed ion exchange resin. The final yield was 100 mCi D-chiro-[3-3H]inositol (24% of the inose precursor). The specific activity was 15 Ci/mmol.

Cell Culture—HepG2 human liver cells and IMR-90 human diploid fibroblasts were obtained from the American Type Culture Collection (Rockville, MD) and grown in Eagle's minimal essential medium containing 0.1% nonessential amino acids, 1.0 mM sodium pyruvate, and 10% fetal bovine serum. The medium was replaced twice weekly, and cells were divided 1:3 at weekly intervals. Experimental dishes were plated into 12-well cluster dishes (wells 2 cm in diameter) and grown for 4–8 days before use. Experimental wells were analyzed in triplicate. D-chiro-[3-3H]inositol Binding—Assays and cell washing were carried out with PBS that contained 137 mM NaCl, 8.1 mM Na₂HPO₄, 2.7 mM KCl, 1.5 mM KH₂PO₄, 0.9 mM CaCl₂, 0.5 mM MgCl₂, pH 7.4. In certain experiments sodium salts were replaced with lithium salts. Cells were washed twice with PBS at 37°C and then incubated for 3 h with 0.4 ml of PBS containing 1.0–1.5 µCi/ml D-chiro-[3-3H]inositol with or without competitors. At the end of the experiment the cells were placed on ice, washed 4 times with cold PBS, and dissolved in 0.5 ml of 0.1 N NaOH. Aliquots were taken for Bradford protein analysis (13) and liquid scintillation counting, both after neutralization with HCl.

Data Analysis—Experiments were routinely run in triplicate and analyzed with the general linear model of the Statistical Analysis System (SAS) software. Results are presented as mean ± S.E. Kinetic analyses were performed with SAAM-II (Simulation, Analysis and Modeling) obtained from the Resource Facility for Kinetic Analysis at the University of Washington (Seattle, WA).

RESULTS

Synthesis of D-chiro-[3-3H]inositol—Reduction of D-chiro-3-inosose with sodium borotritide yields both D-chiro-inositol and allo-inositol (Fig. 1). Our initial experiments with unlabeled sodium borohydride showed that when unbuffered water was used as the solvent only 15.6% of the product was chiro-inositol, confirming a report that approximately 90% of the borohydride reaction product was the unwanted epimer allo-inositol (12). However, when the reduction was performed in the presence of 0.1 M sodium phosphate buffer, pH 6.5, approximately equal amounts of chiro- and allo-inositol were obtained. Fig. 2A shows a GC/MS-selected ion tracing at m/z 565 and 569, a characteristic ion of pentafluoropropionyl-derivatized inositols, for a nonradioactive pH 6.5 reaction product using a Chiral-Sep Val GC column. In two such experiments D-chiro-inositol was 47.5 and 52.5% of the recovered inositols, a substantial improvement over unbuffered water. Fig. 2B displays the reaction product of Fig. 2A using an expanded time scale, and Fig. 2C shows on the same expanded scale a racemic hexadeuterated chiro-inositol internal standard. The product of the pH 6.5 borohydride reduction comigrated with D-chiro-inositol and contained none of the L enantiomer. Myo-inositol was also absent from the reaction product as expected.

HPLC-purified D-chiro-3-inosose (5 mg) was then reduced with sodium borotritide as described under “Experimental Procedures” and purified by high voltage preparative paper electrophoresis. The product migrating in the position of chiro-inositol contained 100 mCi of tritium and was homogeneous, comigrating with authentic chiro-inositol on silica gel G TLC in 10:3 acetone:water (data not shown) and on HPLC with 76:24 acetonitrile:water as mobile phase (Fig. 3). By HPLC the preparation was free of radio labeled myo-inositol and allo-inositol (elution positions marked with arrows in Fig. 3). This material was used for further studies.

Uptake of D-chiro-[3-3H]inositol by Cultured Cells—When HepG2 liver cells were incubated at 37°C with 1.5 µCi/ml D-chiro-[3-3H]inositol in PBS as described under “Experimental Procedures” time-dependent uptake was observed for at least 3 h (Fig. 4). When the incubation was done at 0°C instead of 37°C, uptake was reduced by 94% (Fig. 4, open symbol), demonstrating that nonspecific adsorption to the cells and plastic dishes was very small.

Very little of the cell-associated tracer was found in phospholipids or proteins after 3 h of incubation. Cells from an experiment similar to that shown in Fig. 4 were extracted with 1.5 ml of 1:2 chloroform:methanol containing 0.05 n HCl for 30 min at room temperature, and the insoluble protein pellet was removed by centrifugation. Then 0.5 ml of chloroform and 0.9 ml of water were added, and the organic and aqueous phases were separated, dried, and counted. The aqueous phase contained 95.6 ± 3.5% of the recovered DPM, the organic phase contained 0.35 ± 0.06%, and the protein pellet contained 4.0 ± 0.7%.

The specificity of D-chiro-[3-3H]inositol transport is shown in Fig. 5. For Fig. 5A HepG2 cells were incubated with D-chiro-
[3-H]inositol with and without 1 mg/ml of competing unlabeled inositol. d-chiro-Inositol displaced 43% of the radioactive tracer ($p < 0.0001$), and pinitol (the structurally related 3-O-methyl-d-chiro-inositol found in soybeans) displaced 27% of the tracer ($p < 0.0001$). However, L-chiro-inositol was ineffective, the tracer uptake remaining 99.2 ± 1.4% of the control ($p = 0.67$).

Fig. 6 gives kinetics of d-chiro-inositol transport by HepG2 cells. Tracer and unlabeled d-chiro-inositol were mixed in PBS and incubated with HepG2 cells for 3 h. Fig. 6A shows that d-glucose reduced tracer uptake progressively over the concentration range of 4–28 mM ($p = 0.0087$ for an effect of glucose by analysis of variance). However, L-glucose showed no tracer displacement even at 28 mM, and tracer uptake remained 96.4 ± 5.5% of the control ($p = 0.67$).

d-chiro-[3-H]inositol transport was dependent upon extracellular sodium ion. After replacing the sodium chloride and sodium phosphate in PBS by lithium salts the uptake of 1

![Graph showing the purity of d-chiro-[3H]inositol.](image)

**Fig. 3. Purity of d-chiro-[3H]inositol.** A sample consisting of 20 μl of a 20 mg/ml solution of authentic chiro-inositol containing 2000 dpm of d-chiro-[3-H]inositol was chromatographed on aminopropyl silica in 76:24 acetonitrile:water with monitoring of the optical density at 206 nm and radioactivity present in 0.5–1-ml fractions. The optical density peak at 4 ml is an injection artifact.

![Graph showing uptake of d-chiro-[3H]inositol by HepG2 cells.](image)

**Fig. 4. Uptake of d-chiro-[3H]inositol by HepG2 cells.** Cells were washed twice with warm PBS and incubated at 37°C for the indicated times with PBS containing 1.5 μCi/ml d-chiro-[3-H]inositol. At the end of the experiment cells were washed 4 times with cold PBS and solubilized in 0.1 N NaOH. The open circle depicts an identical experiment in which the cells were incubated with d-chiro-[3-H]inositol at 0°C rather than 37°C. Error bars represent standard deviation of triplicate wells and where missing are within the symbol.

![Graph showing specificity of d-chiro-[3H]inositol transport in HepG2 cells.](image)

**Fig. 5. Specificity of d-chiro-[3H]inositol transport in HepG2 cells.** 1.5 μCi/ml tracer in PBS either without addition (control) or with competitor was incubated with cells for 3 h at 37°C. The cells were then washed and dissolved in sodium hydroxide. A, competition by 5.6 mM chiro-inositol enantiomers or 5.2 mM pinitol. B, competition by glucose enantiomers. Error bars depict the S.E. of triplicate wells.

The critical question of the relation between d-chiro-inositol uptake and myo-inositol uptake was studied in the following experiments. First, as seen in Fig. 7, unlabeled myo-inositol and d-chiro-inositol appeared to compete equally for uptake of d-chiro-[3-H]inositol tracer in HepG2 cells ($p = 0.18$ for a difference between the two inositols by 2-way analysis of variance). However, when myo-[2-3H]inositol was used as a tracer instead a much different picture emerged, as seen in Fig. 8. Here myo-inositol was a much more potent competitor, reducing tracer binding by 78% at a concentration of 0.17 mM where no corresponding effect of d-chiro-[3-H]inositol was observed. However, at higher concentrations of competitors there was clearly some incomplete displacement of the myo-[3H]inositol tracer. Thus, the two inositol tracers appeared to differ kinetically. Fig. 8B gives an Eadie-Hofstee semireciprocal plot of the dis-
A curve-linear relation in myo-transporters was examined by performing D-glucose transporters and sodium-dependent sugar placements of the plot became linear with reduction in tracer uptake, respectively. (Table I). Both D-glucose and L-glucose were recognized by the myo-[3H]inositol uptake pathway and produced 55 and 45% in the presence of 5.6 mM D-glucose of the data of Fig. 6

Fig. 6. Kinetics of D-chiro-[3H]inositol. HepG2 cells were incubated for 3 h in PBS containing 1.0 μCi/ml tracer and various concentrations of unlabeled D-chiro-inositol. A, displacement of tracer by unlabeled D-chiro-inositol. Points represent triplicate wells ± S.E., and error bars where missing are within the symbol. B, Eadie-Hofstee plot of the data of Fig. 6A after subtracting the nonspecific uptake observed in the presence of 5.6 mM D-chiro-inositol.

The uptake of myo-[3H]inositol tracer by unlabeled myo-inositol after subtraction of nonsaturable uptake. A curve-linear relation is seen. When the uptake of Fig. 8B was corrected by subtracting myo-inositol uptake attributable to the myo-inositol/ D-chiro-inositol transport pathway of Fig. 7 (see Fig. 8 legend), the plot became linear with $K_m = 37.5 \, \mu M$ and maximum uptake capacity of 1240 pmol/mg.

The uptake of myo-[3H]inositol differed from that of D-chiro-[3H]inositol in the stereospecific discrimination of glucose (Table I). Both D-glucose and L-glucose were recognized by the myo-[3H]inositol uptake pathway and produced 55 and 45% reduction in tracer uptake, respectively.

The relation of the myo/D-chiro-inositol transporter to facilitated glucose transporters and to sodium-dependent sugar co-transporters was examined by performing D-chiro-inositol uptake studies in the presence and absence of cytochalasin B, which binds to and inhibits facilitated glucose transporters, and phlorizin, which does not affect facilitated glucose transporters but inhibits sodium-dependent transporters (Fig. 9). Saturable D-chiro-[3H]inositol uptake was not affected significantly by cytochalasin B but was reduced 93% by phlorizin ($p < 0.0001$).

Uptake of D-chiro-[3H]inositol was also studied in the presence of 5.6 mM unlabeled D-chiro-inositol as a competitive inhibitor. The competitive inhibitor was corrected for nonspecific uptake, and the curves were extrapolated to infinite concentration (Fig. 6B). The inhibition constant ($K_i$) was determined to be 348 μM. The maximum uptake capacity ($B_{max}$) was calculated to be 717 pmol/mg.

Fig. 7. Competition of myo-inositol for D-chiro-[3H]inositol uptake. HepG2 cells were incubated for 3 h at 37°C with 1 μCi/ml D-chiro-[3H]inositol tracer plus the indicated concentrations of unlabeled inositols. Triplicate wells were analyzed.

IMR-90 diploid human fibroblast strain. The amount of uptake observed during a 3-h incubation at 37°C using 1.0 μCi/ml tracer was 1897 ± 125 dpm/mg cell protein, 25% of the value for HepG2 cells. Uptake was not affected by 1 mg/ml L-chiro-inositol but was significantly reduced by 24% with 1 mg/ml D-chiro-inositol ($p = 0.04$) and by 49% with 1 mg/ml myo-inositol ($p = 0.003$). Therefore, a pathway for D-chiro-inositol uptake appears to be present in these cells but is expressed at a lower level than in HepG2 cells.

TABLE I. Rate Constants for D-Chiro-[3H]inositol Uptake in HepG2 Cells

| Inositol Competitor, mM | Uptake / Free |
|------------------------|--------------|
| 0                      | 2000         |
| 2                      | 1500         |
| 4                      | 1000         |
| 6                      | 500          |

DISCUSSION

The synthesis of a radioactive D-chiro-inositol tracer should facilitate studies of D-chiro-inositol-containing structural and regulatory molecules in mammalian cells. Tritium labeling of D-chiro-inositol was achieved by boro-tritide reduction of D-chiro-3-inosose (Fig. 1). The yield of D-chiro-inositol as a percentage of the inositol reaction product was increased to 50% from about 10% as previously reported (12) by performing the reaction at pH 6.5 in phosphate buffer, as determined in nonradioactive preparations. In a radioactive preparation after purification by paper electrophoresis, the final tracer was analyzed by HPLC on aminopropyl-silica in an acetonitrile:water solvent system, which gave good resolution of various inositols and inososes and showed that the final product was pure (Fig. 3). GC/MS analysis of a similar unlabeled reaction product showed that only the D-enantiomer of chiro-inositol was produced (Fig. 2).

D-chiro-3-[3H]inositol tracer appears to be taken up by HepG2 liver cells through a novel pathway for which we suggest the name myo/D-chiro transporter. myo-Inositol and D-chiro-inositol are recognized equally (Fig. 7), but uptake is highly stereospecific in that L-chiro-inositol is not recognized at all (Fig. 5A). This enantiospecificity implies that the transporter is also asymmetric with a defined spatial orientation, as would be expected in a transport protein. The myo/D-chiro transporter shares some features of the well-studied transporter for myo-inositol, which is found in many cells and tissues (14–18). For example, it requires the extracellular sodium ion and has appreciable cross-competition with D-glucose (Fig. 5B).

Glucose over a range of 4 mM (similar to fasting plasma concentrations) to 28 mM (found in very poorly controlled diabetic patients) reduces D-chiro-inositol uptake in cultured liver cells. This provides a presumptive mechanism for the observation that diabetic subjects excrete D-chiro-inositol in the urine in proportion the degree of hyperglycemia present (6). In the
Kidney excessive glucose might interfere competitively with D-chiro-inositol reabsorption and increase the amount excreted. It also suggests the possibility that the presence of hyperglycemia might interfere with tissue uptake of D-chiro-inositol elsewhere in the body and reduce intracellular D-chiro-inositol stores.

However, the myo/D-chiro transporter differs from the myo-inositol transporter in several respects. First, the two inositols compete equally in the former but D-chiro-inositol competes poorly if at all in the latter (Fig. 8A versus Fig. 7). Second, L-glucose is not recognized by the myo/D-chiro transporter (Fig. 5B), but it competes almost as well as D-glucose in the myo-inositol pathway (Table I). The lack of stereospecificity for glucose in the myo-inositol transporter that we observed confirms a previous report in which the two glucose enantiomers were equally competitive in reducing 3H-myosinositol uptake in endothelial cells (17). Finally, the Km values of the two transporters for myo-inositol are markedly different. In our hands the Km for myo-inositol of the myo-inositol transporter is 43.4 μM if uncorrected data are used and 37.5 μM if the corrections detailed in the legend to Fig. 8 are performed. Both of these values are close to values of 12–41 μM reported previously for high affinity uptake in cultured cells (16, 17) and are also close to the reported human plasma free myo-inositol concentration of 24.5 μM (6). In contrast, the Km of the myo/D-chiro transporter for both inositols is 348 μM. Thus, this pathway would not be saturated at normal plasma concentrations of myo-

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**TABLE I**

| Competition of glucose enantiomers for myo-[3H]inositol uptake |
|---------------------------------------------------------------|
| HepG2 cells were incubated for 3 h with 1.9 μCi/ml myo-[3H]inositol and the indicated competitors. Results are presented for triplicate wells. All competitors were effective, p < 0.0005. |
| **Enantiomer** | **Uptake** |
|----------------|------------|
| Tracer alone | 147,257 ± 5470 |
| Tracer + 28 mM D-glucose | 70,127 ± 2295 |
| Tracer + 28 mM L-glucose | 85,007 ± 2333 |
| Tracer + 5.6 mM myo-inositol | 7829 ± 146 |

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**Fig. 8. Kinetics of myo-[3H]inositol uptake.** A, HepG2 cells were incubated with 1 μCi/ml myo-[2-3H]inositol in PBS in the presence of unlabeled inositols. Values are for triplicate wells. B, Edie-Hofstee plot of the unlabeled myo-inositol displacement curve of panel A after subtraction of the nonspecific uptake observed in the presence of 5.6 mM myo-inositol. C, same as part B after further subtraction of the uptake attributable to the myo-inositol/D-chiro-inositol uptake pathway. The latter was computed as the displacement of tracer by D-chiro-inositol divided by the displacement of tracer by myo-inositol multiplied by the total myo-inositol mass uptake.

**Fig. 9. Effect of inhibitors on saturable D-chiro-[3H]inositol uptake.** Left, triplicate wells of HepG2 cells were incubated as described under “Experimental Procedures” with PBS containing either 0.1% ethanol (control) or 0.1% ethanol plus 80 μM cytochalasin B. Right, cells were incubated with PBS containing no addition (control) or 1 mM phlorizin. Nonsaturable uptake observed in the presence of 5.6 mM unlabeled D-chiro-inositol under the same conditions was subtracted from the total uptake to give saturable uptake. Error bars depict S.D.
inositol and would allow uptake of both inositols under physiological conditions. The myo-choro transporter appears to be unrelated to facilitated glucose transporters because it is sodium-requiring, phlorizin-inhibitable, and unaffected by cytochalasin B (19).

Our data show that HepG2 cells, which are transformed cells derived from liver, have a high affinity saturable transporter for myo-inositol (Fig. 8). Previous studies of dissociated normal liver cells have described only pathways for myo-inositol uptake that are poorly saturable or nonsaturable at millimolar levels of myo-inositol (20–22). However, in that work the ability of unlabeled myo-inositol to displace trace amounts of $^3$H-myoinositol was not reported explicitly, and it is possible that a component of high affinity uptake was also present. myo-Inositol transport pathways in liver require further study. Figs. 6A and 7 show that nonsaturable pathways (or pathways with $K_m$ values much higher than 0.348 mM) account for a portion of the uptake of a-choro-inositol, and it is possible that this uptake might be similar to the nonsaturable myo-inositol pathway of liver.

Our findings may have implications with respect to the transport of myo-inositol as well as a-choro-inositol. As discussed above, previous reports have shown that tissues and cells have high affinity saturable myo-inositol uptake ($K_m$ of about 30 µM) and nonsaturable uptake that is apparently carrier-mediated. But in addition, studies in which radioactive tracer was displaced by unlabeled competitor uniformly have shown a third pathway consisting of saturable sites of apparently lower affinity, which are frequently quite prominent and are recognized by nonlinear Eadie-Hofstee plots (15, 16). These sites are absent in oocytes injected with myo-inositol transporter transcripts (18). Our data suggest that at least part of this low affinity saturable pathway is the myo-choro transporter (Fig. 8). The Eadie-Hofstee plot of the displacement of $^3$H-myoinositol tracer by unlabeled myo-inositol is curved even though nonsaturable binding has been subtracted, suggesting that two saturable uptake sites are present (Fig. 8B). Although it is theoretically possible to deconvolute this curve into two sites mathematically, this is not practical because of an inherent lack of precision in estimating the parameters of the two sites, as noted previously with kidney cells (13). However, by considering the additional information contained in the experiment shown in Fig. 8 in which displacement of $^3$H-myoinositol tracer by both myo-inositol and a-choro-inositol was done, it is possible to improve estimation of the high affinity myo-inositol uptake pathway. In the resulting analysis we made two simplifying assumptions: 1) both inositols compete equally for the myo-choro transporter (see Fig. 7); 2) only myo-inositol is recognized by the high affinity myo-inositol transporter (see Fig. 8A). When the cell myo-inositol uptake was corrected for the amount passing through the lower affinity myo-choro transporter the Eadie-Hofstee plot became linear (Fig. 8B and C). This analysis suggests that at least some of the saturable but low affinity myo-inositol uptake observed in HepG2 cells can be accounted for by the myo-choro transporter.

These results show that an alternative pathway exists for inositol transport in cultured cells, which can be revealed by use of radiolabeled a-choro-[3-$^3$H]inositol. Further studies are needed to evaluate the physiological significance, tissue specificity, and potential importance of this pathway in the regulation of cell signaling and protein structure.

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Richard E. Jr. Ostlund, Robert Seemayer, Surendra Gupta, Robert Kimmel, Ellen L. Ostlund and William R. Sherman

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