UDP-glucosamine as a substrate for dolichyl monophosphate glucosamine synthesis

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The sugar nucleotide analogue UDP-glucosamine was found to function as a sugar donor in microsomal preparations of both chick-embryo cells and rat liver, yielding dolichyl monophosphate glucosamine (Dol-*P*-GlcN). This was characterized by t.l.c. and retention by DEAE-cellulose. Glucosamine was the only water-soluble product released on mild acid hydrolysis. Dol-*P*-GlcN did not serve as substrate by transferring its glucosamine moiety to dolichol-linked oligosaccharide. Competition experiments between UDP-[³H]glucose and UDP-glucosamine showed Dol-*P*-[³H]glucose synthesis to be depressed by 56 or 73% in microsomes from chick-embryo cells and rat liver respectively. The concentrations of the UDP-sugars in this experiment were comparable with those occurring in galactosamine-metabolizing liver. These findings suggest that Dol-*P*-GlcN, formed as a metabolite of D-galactosamine, may interfere with Dol-*P*-dependent reactions.

INTRODUCTION

The biosynthesis of complex carbohydrates proceeds via pathways involving lipid intermediates, sugar nucleotide substrates, glycosyltransferases, processing by glycosidases and finally packaging of end products (Schachter & Roseman, 1980; Hubbard & Ivatt, 1981; Kornfeld, 1982). Some sugar analogues have been shown to interfere with protein glycosylation (Schwarz & Datema, 1982*a,b*). Analogues such as 2-deoxyglucose, 2-deoxy-2-fluoroglucose and 2-deoxy-2-fluoromannose have to be metabolized to their respective UDP- and GDP-derivatives to exert their effects (Schwarz *et al.*, 1979; Schwarz & Datema, 1982*a,b*). Another glucose analogue modified at C-2 is D-glucosamine, the UDP-derivative of which is formed in D-galactosamine-metabolizing cells (Maley *et al.*, 1966, 1968; Keppler *et al.*, 1970*a*). UDP-glucosamine can serve as substrate for enzymes acting on UDP-glucose (Tarentino & Maley, 1976; Weckbecker & Keppler, 1982). Utilization of UDP-glucosamine may lead to aberrant glucosamine-containing oligosaccharides and the question is raised as to whether the dolichol pathway is affected and if so, which step in the sequence of reactions is most susceptible. Moreover, we have reinvestigated whether the inhibitory effect of D-glucosamine on glycosylation reactions in virus-infected cells (Scholtissek *et al.*, 1975; Schwarz & Datema, 1982*a,b*) could be related to the formation of UDP-glucosamine.

MATERIALS AND METHODS

Chemicals, enzymes and isotopes

T.l.c. sheets coated with silica-gel G-60 or with cellulose were from E. Merck (Darmstadt, Germany). DEAE-cellulose DE52 was from Whatman (Maidstone, Kent, U.K.). UDP-glucose pyrophosphorylase (100 units/mg), alkaline phosphatase (1900 units/mg), snake-

venom phosphodiesterase (52 units/mg), UDP-glucose and GDP-mannose were from Boehringer Mannheim (Mannheim, Germany); specific activities were defined by the supplier. Dol-*P* was from Sigma (St. Louis, MO, U.S.A.) and Dol-*PP*-(GlcNAc)₂ was prepared as described previously (Schwarz & Datema, 1982*c*). L-Mannono-1,5-lactone was obtained from K & K Laboratories (Plainview, NY, U.S.A.). UDP-*N*-acetyl-D-[U-¹⁴C]-glucosamine (235 Ci/mol), GDP-D-[1-³H]mannose (12.6 Ci/mmol), D-[1-³H]galactosamine (12.4 Ci/mmol), D-[1-¹⁴C]galactosamine (51 Ci/mol), D-[1-³H]glucosamine (24.8 Ci/mmol), Dol-*P*-[³H]Man (0.9 Ci/mmol) and Dol-*P*-[³H]Glc (6.5 Ci/mmol) were obtained from New England Nuclear (Dreieich, Germany). UDP-D-[U-¹⁴C]-glucose (292 Ci/mol), UDP-D-[6-³H]glucose (93.1 Ci/mmol) and GDP-D-[U-¹⁴C]mannose (307 Ci/mol) were from Amersham Buchler (Braunschweig, Germany); [6-³H]glucose 1-phosphate was produced from UDP-[6-³H]glucose by treatment with snake-venom phosphodiesterase.

Synthesis of UDP-[1-³H]glucosamine and UDP-[1-³H]-galactosamine

Enzymes of the galactose pathway were employed for the synthesis of labelled UDP-hexosamines in the following incubation mixture (250 µl): 10 mM-ATP, 15 mM-UDP-glucose, 0.18 mM-D-[1-³H]galactosamine (5.5 Ci/mmol), 70 mM-Tris, pH 8.7, 14 mM-magnesium acetate, 1 mM-EDTA, 10 mM-cysteine, 5 µM-glucose 1,6-bisphosphate in the presence of galactokinase (16 kunits/litre), hexose-1-phosphate uridylyltransferase (9 kunits/litre), UDP-glucose 4-epimerase (10 kunits/litre), and phosphoglucomutase (24 kunits/litre). UDP-hexosamines reached a maximum after an incubation for 7 h at 37 °C as checked by h.p.l.c. (Weckbecker & Keppler, 1983). For isolation of the UDP-hexosamines, the deproteinized mixture was separated by preparative h.p.l.c. on a Partisil-10 SAX column using the volatile triethylammonium acetate buffer (Weckbecker & Kep-

Abbreviations used: Dol-*P*, dolichyl monophosphate; Dol-*PP*, dolichyl diphosphate.

pler, 1984). Fractions containing UDP-hexosamines were freeze-dried and chromatographed by borate h.p.l.c. (Weckbecker & Keppler, 1983) for the separation of UDP-[1-³H]GlcN and UDP-[1-³H]GalN. They were subsequently subjected to DEAE-Sephadex A-25 chromatography in triethylammonium acetate buffer in order to eliminate borate salts. The reaction products were co-eluted in three different h.p.l.c. systems with unlabelled standard UDP-hexosamines (Weckbecker & Keppler, 1983). The same synthetic procedure was employed for synthesis of UDP-[1-¹⁴C]glucosamine (51 Ci/mol). Unlabelled UDP-glucosamine was synthesized enzymically by using UDP-glucose pyrophosphorylase (Weckbecker & Keppler, 1982).

Preparation of a particulate enzyme fraction from chick-embryo cells and rat liver

A crude membrane preparation was isolated from primary chick-embryo cells (prepared from 11-day-old chick embryos) by using a modified procedure of Krag & Robbins (1977) (see Schwarz & Datema, 1982c). Rat liver microsomes (microsomal fractions) were prepared as described by Shidoji & DeLuca (1981). The final membraneous pellets were suspended in a buffer comprising 20 mM-Tris/HCl, pH 7.5, 150 mM-NaCl, 0.4 mM-MgCl₂ and 0.4 mM-MnCl₂ at a protein concentration of 20 g/litre. Protein was measured by using the Bio-Rad dye-binding assay, with bovine serum albumin as standard (Bradford, 1976).

Synthesis *in vitro* of lipid-linked saccharides

Standard assay mixtures contained 13 mM-Tris/HCl, pH 7.5, 100 mM-NaCl, 0.3 mM-MgCl₂, 0.3 mM-MnCl₂ and particulate enzyme (0.8 mg of protein) in a final volume of 60 µl. Incubations were performed at 37 °C. Syntheses of Dol-PP-(GlcNAc)₂, Dol-P-Man and Dol-P-Glc were measured by incubating 0.05 µCi of the appropriate ¹⁴C-labelled sugar nucleotide donor with standard assay mixture for 20 min, 10 min and 15 min respectively (Schwarz & Datema, 1982c).

Glycosyl transfer to preformed Dol-PP-(GlcNAc)₂Man₉ from UDP-Glc or UDP-GlcN was carried out as described previously (Schwarz & Datema, 1982c): standard assay mixture was first incubated with UDP-GlcNAc (22 µM) to form a pool of Dol-PP-(GlcNAc)₂, followed by incubation with GDP-Man (5 µM) to form Dol-PP-(GlcNAc)₂Man₉. At the end of the incubation period, 10 vol. of ice-cold Tris-buffered saline [20 mM-Tris/HCl (pH 7.5)/0.15 M-NaCl] containing 0.1 M-L-mannono-1,5-lactone to inhibit mannosidase activity were added and the membranes isolated by centrifugation at 100 000 g for 60 min at 4 °C. The resulting pellet was resuspended in the buffer used in the preparation of the original microsomal membrane fraction. The reactions were initiated by addition of UDP-[³H]Glc (1 µM) or UDP-[³H]GlcN (1 µM). After 15 min incubation at 37 °C the reactions were terminated by the addition of ice-cold chloroform/methanol (2:1, v/v) (2 ml).

Dol-P (0.3 mg/ml final concn.) was added as a suspension in 0.2% (v/v) Triton X-100. Control incubations (without Dol-P) were performed at the same final concentration of Triton X-100 (0.017%). Reactions were terminated by the addition of ice-cold chloroform/methanol (2:1, v/v) (2 ml). Washing of the chloroform/methanol (2:1, v/v) extract and further extraction with

chloroform/methanol/water (10:10:3, by vol.) have been described (Sharma *et al.*, 1974; Schwarz & Datema, 1982c).

Separation techniques

The following t.l.c. systems were used: (1) silica-gel G-60 with chloroform/methanol/NH₃/water (65:35:4:4, by vol.) and (2) cellulose with ethyl acetate/pyridine/acetic acid/water (5:5:1:3, by vol.). Radioactive material was located with a Berthold LB 2821 automatic scanner. Dol-P-sugars were isolated by ion-exchange chromatography in columns (1 cm × 2 cm) of DEAE-cellulose (acetate form) equilibrated in 99% (v/v) methanol. Samples were applied to the column in butanol (1 ml), and Dol-P-linked compounds were eluted with 0.2 M-ammonium acetate in 99% methanol after first washing the column with 99% methanol to remove neutral glycolipid.

The h.p.l.c. apparatus has been described in detail elsewhere (Weckbecker & Keppler, 1983).

Cell-incubation experiments

Cultures of primary chick-embryo cells (16 cm-diameter plates) were seeded at such a concentration (2.5 × 10⁶ cells/ml of medium) to give confluent monolayers after 48 h. Cells were maintained in Earle's medium supplemented with 10 mM-glucose and were infected with an influenza A virus (fowl-plague virus, strain Rostock, H7N1) at an input multiplicity of 20–50 plaque-forming units/cell. After adsorption (30 min), the cells were washed twice with warm phosphate-buffered saline (170 mM-NaCl/3.4 mM-KCl/10.1 mM-Na₂HPO₄/1.8 mM-KH₂PO₄/0.5 mM-MgCl₂/0.7 mM-CaCl₂, pH 7.6), flooded with 10 ml of medium and incubated at 37 °C. At 1 h after infection, [³H]GlcN (4 Ci/mol) was added to give a final concentration of 10 mM and the cells were labelled for 4 h. Plates were washed three times with ice-cold phosphate-buffered saline and immediately frozen in liquid N₂ before extraction with HClO₄.

HClO₄ extraction of chick-embryo cells and h.p.l.c.

Chick-embryo cell monolayers (6 × 10⁷ cells/16 cm dish) were frozen and extracted by addition of 1.5 ml of cold 0.9 M-HClO₄. The acidic cell extract was centrifuged for 5 min at 12000 g and 4 °C. The volume of the supernatant was measured by weighing. For neutralization, solid KHCO₃ was added and the precipitating KClO₄ was removed by centrifugation in the cold. The neutral cell extracts (50 µl) were separated by anion-exchange h.p.l.c. using phosphate buffer and flow gradients on a Partisil-10 SAX column (Holstege *et al.*, 1982). Absorbance was recorded at 262 nm; nucleotides were quantified by referring to standard solutions analysed enzymically. The radioactivity from [³H]GlcN in the effluent was determined in 6 or 15 s fractions in a liquid-scintillation counter. The ion-pair reverse-phase h.p.l.c. on a RP-18 column (Krug *et al.*, 1984) and the anion-exchange h.p.l.c. in borate buffers (Weckbecker & Keppler, 1983) have been described previously.

RESULTS

Formation of Dol-P-GlcN from UDP-GlcN *in vitro* using a microsomal preparation from chick-embryo cells

UDP-[³H]GlcN was synthesized and incubated with standard assay mixture. Label was incorporated into

Fig. 1. Analysis of the chloroform/methanol extract from the incubation of UDP-[^3H]GlcN with chick-embryo cell microsomes *in vitro*

UDP-[^3H]GlcN (0.05 μCi) was incubated with chick-embryo cell microsomes for 20 min at 37 $^{\circ}\text{C}$ and a chloroform/methanol (2:1, v/v) extract prepared. This chloroform/methanol (2:1, v/v) extract was subjected to t.l.c. using system 1. Inset: mild-acid hydrolysate of the chloroform/methanol (2:1, v/v) extract analysed by t.l.c. system 2. The numbered arrows indicate the positions of the following reference compounds: 1, Dol-*P*-Glc; 2, Dol-*PP*-(GlcNAc) $_2$; 3, GlcN; 4, GalN.

chloroform/methanol (2:1, v/v)-soluble material linearly over a 30 min incubation. Addition of Dol-*P* stimulated incorporation 2-fold. This material was analysed by t.l.c. and DEAE-cellulose ion-exchange chromatography. On t.l.c. (system 1) the label migrated as a single peak like a Dol-*P*-linked sugar rather than as a Dol-*PP*-linked saccharide (Fig. 1). The label also bound to DEAE-cellulose equilibrated in 99% methanol and was eluted with 0.2 M-ammonium acetate, as did Dol-*P*-Glc in a parallel run. Under these conditions Dol-*PP* derivatives remain bound to the column (Datema *et al.*, 1980). These results show that a monophospho-linked dolichol derivative has been formed. Glucosamine was confirmed as being the glycosyl moiety by the following criteria: (1) only 33% of the label was made water-soluble under mild-acid-hydrolysis conditions [1 M-HCl in 50% (v/v) propanol for 15 min at 50 $^{\circ}\text{C}$] sufficient to hydrolyse Dol-*P*-Glc completely. Full hydrolysis was accomplished by treatment with 1 M-HCl in 50% propanol for 30 min at 100 $^{\circ}\text{C}$. This acid-stability is characteristic of hexosamine-containing phosphate esters (MacDonald, 1972); (2) after release of the glycosyl moiety from the Dol-*P*-saccharide by acid hydrolysis as described above, the water-soluble product was analysed by t.l.c. (system 2), which showed the label to co-migrate with authentic D-glucosamine (Fig. 1, inset). Thus UDP-GlcN is a substrate for Dol-*P*-GlcN synthesis *in vitro*, indicating that it is behaving as an analogue of UDP-Glc in the dolichol pathway.

When UDP-[^3H]GalN was incubated with standard assay mixture, no label was incorporated into a chloroform/methanol (2:1, v/v)-soluble product. Thus

UDP-GalN is not a substrate for Dol-*P*-saccharide formation.

Comparison of the apparent K_m and V_{max} values for UDP-GlcN (8.4 μM and 0.4 pmol of GlcN transferred $\cdot\text{min}^{-1}\cdot\text{mg}$ of protein $^{-1}$ respectively) with those for UDP-Glc (1.0 μM and 1.0 pmol of Glc transferred $\cdot\text{min}^{-1}\cdot\text{mg}$ of protein $^{-1}$ respectively) indicate that UDP-GlcN is not as effective a substrate as UDP-Glc for UDP-Glc:Dol-*P* glucosyltransferase.

The question was now raised as to whether Dol-*P*-GlcN could 'glucosaminylate' Dol-*PP*-(GlcNAc) $_2$ Man $_6$ in a reaction analogous to that of glucose transfer from Dol-*P*-Glc in the formation of Dol-*PP*-(GlcNAc) $_2$ Man $_6$ Glc $_3$. Under conditions in which substantial glucose transfer to Dol-*PP*-(GlcNAc) $_2$ Man $_6$ from UDP-[^3H]Glc via Dol-*P*-Glc (1.1 pmol transferred/15 min) was measured, no transfer of GlcN to lipid-linked oligosaccharide was observed. Only Dol-*P*-GlcN synthesis from UDP-GlcN was detected.

Dol-*P*-GlcN synthesis in rat liver microsomes

To ensure that the results obtained with chick-embryo cell microsomes are equally valid for liver, microsomes were prepared from rat liver and incubated with UDP-[^3H]GlcN in a manner identical with that used for chick-embryo cell microsomes. Within a 30 min incubation period, 0.5 and 2.1 pmol of [^3H]GlcN were transferred/mg of protein by rat liver microsomes in the absence and in the presence of exogenous Dol-*P* (0.3 mg/ml) respectively. The product of this transfer reaction was extracted with chloroform/methanol (2:1, v/v) and shown to possess properties identical with those of Dol-*P*-GlcN synthesized by the chick-embryo cell membranes (results not shown). Therefore rat liver microsomes are also capable of synthesizing Dol-*P*-GlcN from UDP-GlcN.

Interference of UDP-glucosamine in the synthesis of Dol-*P*-saccharides

When UDP-GlcN was included in assay mixtures containing microsomal preparations from chick-embryo cells and synthesizing Dol-*P*-Glc, Dol-*P*-Man and Dol-*PP*-(GlcNAc) $_2$ from their respective sugar nucleotides, all three reactions were suppressed to different extents. In the presence of 10 μM -UDP-GlcN, Dol-*PP*-(GlcNAc) $_2$ synthesis was suppressed by 61%, whereas 200 μM -UDP-GlcN was required to suppress Dol-*P*-Glc and Dol-*P*-Man synthesis to the same extent. Addition of exogenous Dol-*P* relieved the suppression in all three cases, suggesting that UDP-GlcN is competing with the physiological sugar nucleotide for Dol-*P*.

Whether formation of Dol-*P*-GlcN could effectively compete with that of Dol-*P*-Glc should depend upon the ratio of UDP-GlcN to UDP-Glc. In liver metabolizing either GalN or GalN plus uridine, this ratio of the sugar nucleotides is approx. 500 μM : 10 μM and 1000 μM : 200 μM respectively (Keppler *et al.*, 1970a; Reynolds & Reutter, 1973; Decker & Keppler, 1974). Mimicking these conditions *in vitro*, Dol-*P*-[^{14}C]Glc synthesis from 10 μM -UDP-[^{14}C]Glc in the presence of 500 μM -UDP-GlcN was inhibited by 73% and 56% when microsomes from rat liver and chick-embryo cells respectively were used. At 200 μM -UDP-[^3H]Glc and 1000 μM -UDP-GlcN, Dol-*P*-[^3H]Glc synthesis was inhibited by 34% when liver microsomes, and 6% when microsomes from chick-embryo cells, were used.

Fig. 2. Radio-h.p.l.c. of a phosphatase-treated HClO_4 extract of influenza-virus-infected chick-embryo cells treated with 10 mM- $[\text{^3H}]\text{GlcN}$

The arrow indicates the retention time of standard UDP-GlcN. This fraction was collected and rechromatographed on an ion-pair reverse-phase h.p.l.c. system (inset) to demonstrate that detectable amounts of UDP-GlcN are not formed from GlcN. The shaded peak areas represent radioactivity derived from $[\text{^3H}]\text{GlcN}$.

UDP-glucosamine is a metabolite of D-galactosamine and not of D-glucosamine

GlcN inhibits the multiplication of enveloped viruses by interfering with viral glycoprotein synthesis (Scholtissek *et al.*, 1975; Schwarz & Datema, 1982*a, b*), and it has been shown to act at an early stage of lipid-linked oligosaccharide synthesis (Datema & Schwarz, 1979). In order to test whether these effects are mediated by metabolism of GlcN to UDP-GlcN that may lead to a trapping of Dol-P due to Dol-P-GlcN formation, cultures of primary chick-embryo cells infected with influenza virus were treated with 10 mM- $[\text{^3H}]\text{GlcN}$ for 4 h. Anion-exchange h.p.l.c. analysis of the alkaline phosphatase-treated nucleotide extract showed that some radioactivity is eluted with the retention time of a UDP-GlcN reference (Fig. 2). However, when this fraction was collected and rechromatographed by using an ion-pair reverse-phase system, none of the label was eluted with the retention time of UDP-GlcN (Fig. 2, inset). For comparison, an anion-exchange h.p.l.c. profile of a HClO_4 extract of liver from rats treated with GalN (Fig. 3) shows the expected formation of the UDP-GlcN.

DISCUSSION

The metabolic conversions of the cytotoxic agent GalN (Maley *et al.*, 1966, 1968; Keppler *et al.*, 1968; Decker & Keppler, 1972, 1974) results in the formation of unphysiological UDP-GlcN and UDP-GalN (Fig. 3) (Keppler *et al.*, 1970*a*). The effects of GalN may include an interference at the sugar-nucleotide level leading to impaired glycoconjugate synthesis in GalN-metabolizing cells (Keppler *et al.*, 1970*b*; Bauer *et al.*, 1974; Tarentino & Maley, 1976). We therefore determined the substrate properties of UDP-GlcN in reactions of the dolichol pathway. Additionally, we tested the possibility of UDP-GlcN formation in cells treated with GlcN, since this amino sugar is also known to alter cellular nucleotide contents and to interfere with glycoprotein synthesis (Scholtissek *et al.*, 1975; Schwarz & Datema, 1982*b*).

UDP-GlcN, a metabolite of GalN and not GlcN (Figs 2 and 3) (Maley *et al.*, 1968; Krug *et al.*, 1984) can replace UDP-Glc in reactions catalysed by glycogen synthetase (Tarentino & Maley, 1976), galactose-1-phosphate uridylyltransferase (Weckbecker & Keppler, 1982) and UDP-Glc dehydrogenase (Weckbecker & Keppler, 1984), and has now been shown to serve as substrate for

Fig. 3. Nucleotide profile of a HClO_4 extract of rat liver

High tissue contents of UDP-GlcN and UDP-GalN were produced by treatment of female Wistar rats with D-galactosamine (3 mmol/kg intravenously) and uridine (4 mmol/kg intraperitoneally) for 5 and 3 h respectively. Livers were freeze-clamped, homogenized in five parts of 0.6 M- HClO_4 and neutralized as described in the Materials and methods section. The hepatic content of UDP-hexosamines amounted to 3.2 mmol/kg as analysed by h.p.l.c. (Weckbecker & Keppler, 1983). Inset: rechromatography of the UDP-hexosamine-containing fraction using borate buffers yielded contents of UDP-GlcN and UDP-GalN of 2.1 and 1.1 mmol/kg of liver respectively.

microsomal enzyme preparations forming Dol-*P*-GlcN. Subsequent glucosaminyl transfer to Dol-*PP*-(GlcNAc)₂Man₉ could not be detected, indicating that Dol-*P*-GlcN cannot substitute for Dol-*P*-Glc, the immediate precursor of glucose residues in Dol-*PP*-Glc₃(GlcNAc)₂Man₉. UDP-GlcN was also shown to suppress the formation of Dol-*P*-Man and Dol-*PP*-(GlcNAc)₂ as well as that of Dol-*P*-Glc. This suppression is due to Dol-*P*-GlcN synthesis competing with the synthesis of the physiological Dol-linked saccharides. In the case of Dol-*P*-Man and Dol-*PP*-(GlcNAc)₂ synthesis there is competition between two different reactions, with a common requirement for Dol-*P* as co-substrate. The ratio of UDP-GlcN to UDP-Glc was shown to influence markedly the extent of Dol-*P*-Glc formation *in vitro* in that a ratio of 50 (500 μM : 10 μM) inhibited the reaction by 73%, whereas a ratio of 5 (1000 μM : 200 μM) allowed almost uninhibited utilization of UDP-Glc. Since a ratio of 50 is characteristic of cells metabolizing GalN alone,

an interference with Dol-*P*-Glc synthesis *in vivo* might occur under these conditions. Moreover, the intracellular synthesis of Dol-*P*-GlcN as the mechanism of the diminished Dol-*P*-Glc formation seems to be possible. In preliminary experiments with hepatocytes metabolizing [¹⁴C]GalN, a ¹⁴C-labelled compound with the properties of a Dol-*P*-sugar, presumably Dol-*P*-[¹⁴C]GlcN, has been isolated (W. McDowell, G. Weckbecker, D. O. R. Keppler & R. T. Schwarz, unpublished work). Dol-*P*-GlcN formation may lead to a trapping of Dol-*P*, since we have shown *in vitro* that the GlcN moiety is not transferred to Dol-*PP*-(GlcNAc)₂Man₉. Dol-*P* is known to be limiting *in vivo* (Hemming, 1977). An interference with the dolichol pathway by the formation of Dol-*P*-GlcN would be consistent with a perturbation of glycoprotein synthesis in GalN-metabolizing cells (Bauer *et al.*, 1974).

The lack of UDP-GlcN in GlcN-treated chick-embryo cells confirms the earlier results of Koch *et al.* (1979), who

studied this question with less refined separation methods (paper chromatography) and concluded that GlcN itself mediates the inhibition of viral glycoprotein synthesis (Koch *et al.*, 1979). Despite the fact that UDP-GlcN is only formed in cells metabolizing GalN (Figs 2 and 3), it is interesting that GlcN can act as an inhibitor of lipid-dependent glycosyl transfer reactions both in its free state and as a sugar-nucleotide derivative.

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