GLYCOPROTEIN BIOSYNTHESIS: STIMULATION OF GALACTOSE TRANSFER FROM UDP-[^{14}C] GALACTOSE INTO MICROSOMAL PROTEIN BY CYTIDINE 5'-DIPHOSPHOCHOLINE

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1. Introduction

We have previously reported that CDP-choline exerts a stimulatory effect on the transfer of *N*-acetylglucosamine into exogenous and endogenous proteins in microsomes of rat liver [1–4]. The maximum stimulatory effect of CDP-choline on the membrane-bound *N*-acetylglucosaminyl transferase enzyme was observed in a purified rough microsome fraction and the addition of lecithin or a number of other lipids did not stimulate the enzyme [3, 4]. Furthermore, Triton and CDP-choline appeared to stimulate the enzyme in a synergistic manner. We suggested that lecithin regeneration serves as a novel mechanism for the activation of the membrane-bound enzyme. However, the investigations of two other membrane-bound enzymes, i.e., glucose-6-phosphatase and UDP-glucuronyltransferase, showed no stimulation by CDP-choline although they were stimulated by Triton (Cole and Mookerjea, unpublished observation). This suggested some selectivity with regard to the mechanism of activation by the lecithin regenerating system. The mechanism is probably related to the stimulation of synthesis of macromolecules (glyco- and lipoproteins) which are destined to be secreted into the plasma as soluble products [2, 4, 5]. This concept is supported by the present study showing a marked stimulatory effect of CDP-choline on the UDP-galactose:glycoprotein galactosyl transferase activity in microsomes of rat liver.

2. Materials and methods

Overnight fasted male rats were used for the preparation of microsomes or purified rough microsomes as described previously [3]. The pellets of cell fractions were suspended in 0.25 M sucrose. Unless otherwise specified, each complete enzyme assay mixture (total volume, 200 μl) contained microsome or rough microsome suspension, 100 μl (1.5 to 2.5 mg protein); UDP-[^{14}C] galactose, 5 nmoles (0.02 μCi, 26,000 cpm); MES (2-(*N*-morpholino) ethane sulfonic acid) buffer, pH 6.8, 12.5 μmoles; Mn^{2+}, 1.25 μmoles and 10% Triton X-100, 20 μl. Incubations were done for 60 min at 30° and terminated by the addition of 2 ml 10% trichloroacetic acid (TCA) – 2% phosphotungstic acid (PTA), and the precipitate was filtered under suction through glass fiber filters (Reeve Angel 934-AH). The filters were washed with a large excess of cold 5% TCA–1% PTA containing 0.5% galactose, with ethanol:ether (1:1) and then with ether. The dried filter-discs were counted for radioactivity in a toluene-based scintillation solution. Preliminary trials established that assay by filtration gave the same results as assay by acid-precipitation and by high voltage electrophoresis [3]. Similar conclusions from different assay procedures were also obtained by others [6]. In separate experiments, the standard assay was scaled up 5-fold and incubated for 1 hr in the presence of 8 mM CDP-choline. Acid-precipitable or non-dialysable radioactive products were hydrolyzed and the hydrolysates

Table 1
Properties of UDP-galactose:glycoprotein galactosyltransferase activity in rat liver microsomes and its stimulation by CDP-choline.

		cpm (pmoles)/mg protein*	
A. Requirements		*–CDP-choline*	*+CDP-choline (2 mM)*
Complete		182 (34.9)	725 (130)
–DTT		138 (26.6)	579 (111)
$-Mn^{2+}$		5 (0.9)	24 (4.6)
$-Mn^{2+}$, $+Mg^{2+}$		6 (1.1)	32 (6.1)
$-Mn^{2+}$, $+Ca^{2+}$		7 (1.4)	56 (10.8)
–Triton		21 (4.0)	48 (9.1)
B. pH optima			
pH			
MES 5.5		13 (2.5)	40 (7.7)
MES 6.0		187 (35.9)	647 (124)
MES 6.8		182 (34.9)	725 (139)
TRIS 6.0		145 (27.9)	582 (111.7)
TRIS 7.0		95 (18.1)	604 (115.9)
TRIS 8.0		17 (3.3)	211 (40.5)
TRIS 9.0		12 (2.2)	90 (17.3)
C. Acceptor proteins			
None		99 (19.0)	496 (95.2)
Fetuin	0.5 mg	180 (34.5)	811 (155.7)
	1 mg	260 (50.0)	995 (191.0)
Ribonuclease B	0.5 mg	269 (51.7)	696 (133.6)
	1 mg	159 (30.5)	596 (114.4)
Ovalbumin	0.5 mg	259 (49.8)	1413 (271.3)
	1 mg	432 (82.8)	1843 (354.0)
Bovine serum albumin	1 mg	59 (11.4)	430 (82.6)

* For experiments A and B, 2.4 mg and for experiment C, 2 mg of microsomal proteins were incubated for 1 hr. Conditions of complete assay mixture are described in the text.

were found to contain galactose as the only radioactive product after ion-exchange and paper chromatography following a method essentially identical to that described recently [6]. Uniformly labelled UDP-[^{14}C] galactose was purchased from New England Nuclear, Dorval, Quebec; Triton X-100, ovalbumin, ribonuclease-B, CDP-choline and other nucleotides were from Sigma; fetuin and bovine serum albumin were from Calbiochem and Armour, respectively.

3. Results and discussion

Table 1 shows the results of various properties of the galactosyltransferase reaction. Experiment A established the requirements of dithiothreitol, Triton and Mn^{2+}. Mg^{2+} and Ca^{2+} could not replace Mn^{2+}. The optimum pH was between 6 and 7 using MES or Tris buffers (experiment B). Experiment C showed that fetuin and ovalbumin, in contrast to ribonuclease B, responded to dose when used as exogenous acceptors. The characteristic stimulatory effect of CDP-choline on the transferase reaction was evident in the optimized and in some sub-optimum conditions of the reaction. Fig. 1 shows the time (A) and protein (B) dependence of the galactosyltransferase reaction and its stimulation by CDP-choline. The dose-dependent effect of CDP-choline is shown in fig. 1 (C).

It is noteworthy that almost 10% (0.48 nmoles) of the exogenous galactose presented to the system (2 mg microsome protein and 8 mM CDP-choline)

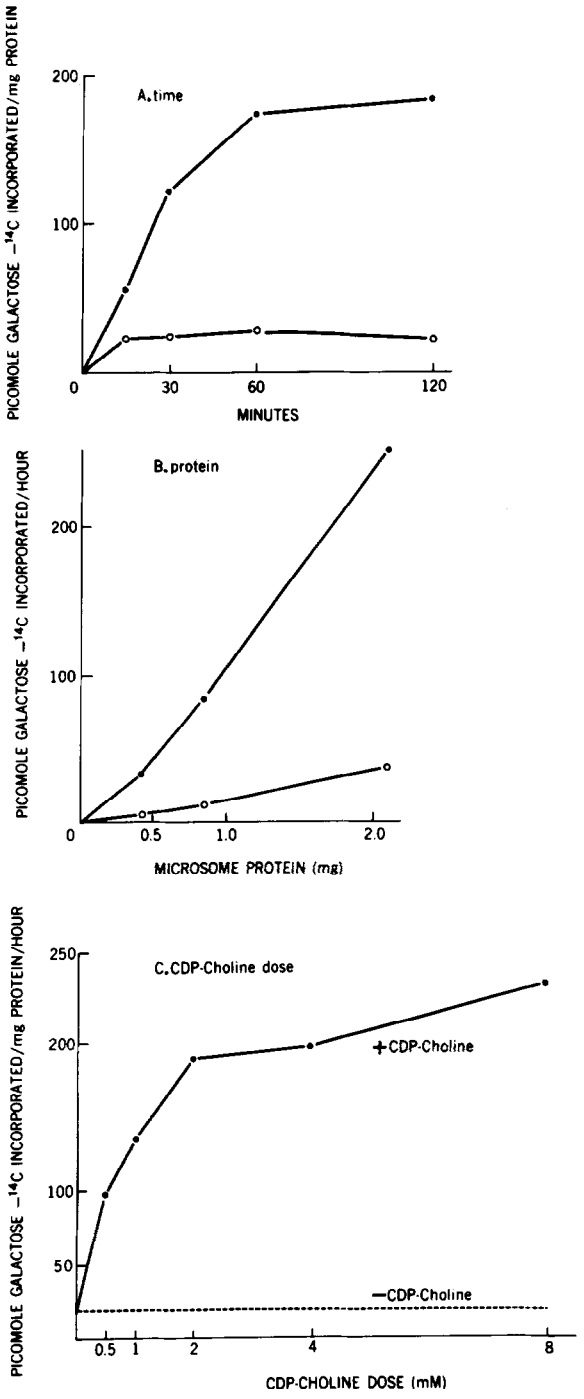

Fig. 1. Time (A), protein (B) and CDP-choline dose (C) dependence of UDP-galactosyl glycoprotein:galactosyltransferase reaction. For A and B, 2 mM CDP-choline (●—●—●) was used. (○—○—○): Incubations without CDP-choline.

was incorporated compared to the corresponding control (without CDP-choline) value of only 0.8% (0.04 nmoles). Table 2 shows a comparison of effects of CDP-choline and other nucleotides on the galactosyltransferase reaction. Setting the stimulatory effect of CDP-choline as 100%, the effects of other nucleotides in equimolar concentrations were in the range of 20–50%. This activation could be the result of sparing effects of the nucleotides on the small amount of labelled nucleotide sugar used for the assay. But CDP-choline appears to cause a greater stimulation than could be accounted for by the nucleotide-sparing effect. This was also true for the CDP-choline effect on *N*-acetylglucosaminyltransferase reaction [3]. Also phosphorylcholine itself has been shown to increase glucosamine incorporation into glycoprotein in a liver slice system [2]. The results in fig. 2 show that the stimulatory effect of CDP-choline was present even when the nucleotide sugar concentration in the assay system was increased 10-fold to near saturation, thus providing further evidence that CDP-choline effect could not be due to only a sparing action on the labelled nucleotide sugar.

The synergistic effect of CDP-choline and Triton on the transferase activity is illustrated in fig. 3. The enzyme activity is almost negligible in absence of both Triton and CDP-choline. An increase of Triton concentration to 0.5% has a small stimulatory effect on the enzyme. But with the addition of CDP-choline there is a remarkable stimulation of the enzyme activity in the presence of optimal amounts of Triton. Further addition of Triton has an inhibitory effect, although increased amounts of CDP-choline (up to 8 mM, fig. 1C) in presence of optimum amounts of Triton seem to continuously increase the enzyme activity. A similar cooperative effect of Triton and CDP-choline has been observed for the *N*-acetylglucosaminyltransferase enzyme [3]. These results are highly suggestive of a membrane involvement of the observed effect of CDP-choline. In experiments with excitable membranes, it has been shown that Triton X-100 increases the motion of membrane proteins, and thereby exposes an important fraction of proteins to the solvent environment [7]. The phosphorylcholine moiety of CDP-choline is incorporated extremely rapidly into the membrane lecithins of the incubation system used for glycosyltransferase

Table 2
Effect of CDP-choline and other nucleotides on UDP-[^{14}C]galactose incorporation into endogenous microsomal protein.

Concentration (mM)	(pmoles incorporated/mg protein)*					
	CDP-choline	CMP	CDP	CTP	ATP	GTP
0.5	79	24	32	25	30	28
1.0	117	23	41	27	40	58
2.0	170	42	47	37	77	85

* Control (–nucleotide) incorporation of 20 pmoles has been subtracted from each value.

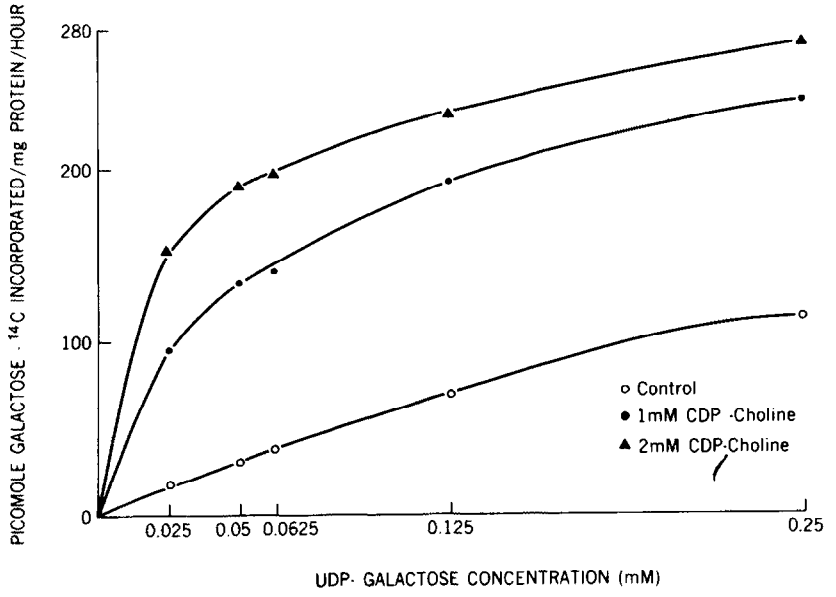

Fig. 2. The effect of the concentration of UDP-galactose on [^{14}C]galactose incorporation into endogenous acceptors.

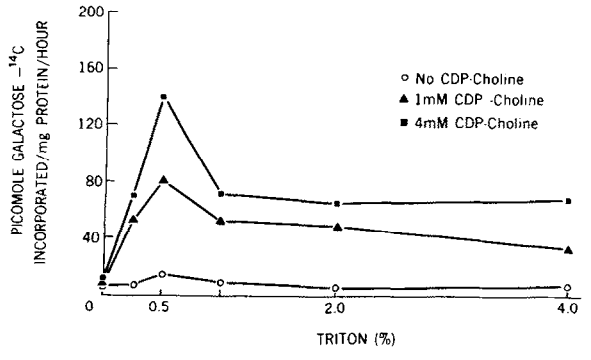

Fig. 3. Interrelationship between CDP-choline and Triton doses on the galactosyltransferase activity.

assay [3, 5]. A combination of rapid regeneration of membrane lecithin and Triton stimulation of membrane proteins may be an unique mechanism for the activation of these membrane-bound enzymes responsible for synthesizing secretory glycoproteins.

Acknowledgements

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References

[1] S. Mookerjea and A. Chow, Biochem. Biophys. Res. Commun. 399 (1970) 486.
[2] S. Mookerjea, Federation Proc. 30 (1971) 143.
[3] S. Mookerjea, Can. J. Biochem., in press.
[4] S. Mookerjea, in: Protides of Biological Fluids, 19th Colloquium, ed. H. Peeters (Pergamon Press, 1972) p. 135.
[5] T.J. Delahunty and S. Mookerjea, Biochem. J. 125 (1971) 96P.
[6] S. Roth, E.J. McGuire and S. Roseman, J. Cell Biol. 51 (1971) 536.
[7] J.P. Changeux, R. Blumenthal, M. Kasai and T. Podleski, in: Molecular Properties of Drug Receptors, eds. R. Porter and M. O'Connor (J. and A. Churchill, 1970) p. 197.