STIMULATION OF UDP-N ACETYL GLUCOSAMINE: GLYCOPROTEIN N-ACETYL GLUCOSAMINYL TRANSFERASE ACTIVITY BY CYTIDINE 5'-DIPHOSPHO-CHOLINE[1]

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Summary

Golgi-rich and golgi-depleted heterogenous membrane fractions were prepared from livers of rats fed choline supplemented or deficient diets. Assay of UDP-N-acetyl glucosamine:glycoprotein N-acetyl glucosaminyl transferase activity showed a decrease of the enzyme activity in the golgi-depleted membrane fraction of the choline deficient liver. A marked stimulation of the enzyme activity in the same fraction was observed by in vitro additions of phosphorylcholine plus cytidine 5'-triphosphate or cytidine 5'-diphospho-choline. This stimulation was found to be dose dependent. Two possible mechanisms are suggested for the observed effect.

We have recently reported that phosphorylcholine exerts a stimulatory action on the process of glycoprotein synthesis *in vivo* and in a liver slice system (1). The effect of phosphorylcholine was pH and temperature dependent. This suggested that phosphorylcholine may be involved in some enzymatic reactions responsible for the transfer of amino-sugars into proteins at the level of microsomal membrane. The present report shows a marked stimulation of UDP-N-acetyl glucosamine:

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glycoprotein N-acetyl glucosaminyl (NAcG) transferase activity in the microsomal membranes by phosphorylcholine plus cytidine triphosphate (CTP) or by cytidine diphosphate choline (CDP-Choline) alone.

Materials and Methods

Male Wistar rats (140-180g) were fed with choline supplemented or deficient diet (2). Two sub-fractions of liver were prepared according to the method described by Schachter[2] et al (3), namely, golgi membranes (B) and a heterogenous membrane fraction (C) consisting of nuclei, mitochondria, lysosomes, residual golgi membranes and smooth and rough endoplasmic reticular membranes. The essential features of the method and the distribution of some of the marker enzymes in the liver fractions on a discontinuous sucrose gradient system are illustrated in Fig. 1. The distribution of enzyme activities showed that fraction B was enriched with both sialyl[2] and NAcG-transferase activities, indicating it to be a golgi-rich fraction (3). About 90% of glucose-6-phosphatase[2] activity was present in fraction C indicating that it contained most of the smooth and rough endoplasmic membranes of the cell. UDP-NAcG-^{14}C was purchased from New England Nuclear Corp. and its specific activity was adjusted to 4.1 mc/mmole by adding unlabelled UDP-NAcG (Sigma). Substrate[2] used for the enzyme assay was human plasma α-1-acid glycoprotein pretreated with sialidase, galactosidase and glucosaminidase

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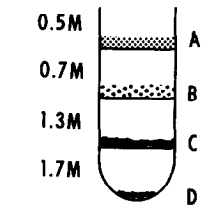

RATIO OF TOTAL ENZYME ACTIVITY (B:C)

NAcG–Transferase	Sialyl–Transferase	Glucose-6-phosphatase
2.4:1	1.6:1	0.1:1

*Average of 3 experiments

Fig. 1. Liver was homogenized in 4 volumes of 0.5 M sucrose buffer (0.1 M Tris-Hcl, pH 7.6, 0.01 M $MgCl_2$, 1% Dextran (mol. wt. 2 x 10^6, from Sigma) with two strokes of a Potter-Elvehjem homogenizer (A.H. Thomas, Type C, was machined for loose fitting, the same pestle and tube were used for all samples) at a speed of 600 rpm. The homogenate was filtered through 4 layers of cheese cloth and diluted with same buffer to a final concentration of 16 ml/ g wet wt. of liver. Four ml of this diluted homogenate (equivalent to 250 mg liver) was layered over above-shown sucrose gradient system and centrifuged at 25,000 rpm in a S.W. 25.1 Spinco rotor for 45 minutes. The volumes of B and C fractions from a single centrifuge tube were usually 2.5 ml and 4 ml and their protein concentrations were 0.4 µg/µl and 2 µg/µl respectively.

(DSGG-protein) (3). We have compared the N-acetylglucosaminyl transferase activity of the golgi fraction (assay system as in Table 1) using endogenous acceptor protein, or the following exogenous acceptors: DSGG-protein, chick albumin, ribonuclease B and a 50% ammonium sulphate precipitatable plasma d>1.21 protein fraction. The enzyme activity with DSGG-protein as acceptor was 10 to 15-fold higher than with any other protein tested.

Results and Discussion

Results in Table 1 show that there is a decrease of N-acetylglucosaminyl transferase activity in the C fraction of choline deficient liver. In vitro addition of phosphorylcholine plus CTP or CDP-Choline caused a remarkable stimulation of the enzyme activity of the C fraction (Table 2). However, the B fraction, although richer in

Table 1. UDP-N-ACETYL GLUCOSAMINE: GLYCOPROTEIN N-ACETYL GLUCOSAMINYL TRANSFERASE ACTIVITY IN MEMBRANE FRACTIONS OF RAT LIVER.

Transfer of N-acetylglucosamine-^{14}C from UDPNAcG-^{14}C (specific activity 4.1 mc/mmole) to acceptor DSGG-protein. Each incubation mixture (total vol. 100 µl) contained: B or C fraction 50 µl, UDPAG-^{14}C 10 µl (48,000 dpm), 2(N-Morpholino)ethane sulfonic acid buffer (pH 5.7) 10 µl, $MnCl_2$ (0.625 µmole) 10 µl, Triton X-100, Sigma (5%) 10 µl, DSGG-protein (1.5 mg) 10 µl. Incubations were routinely done for 30 min. at 37°C and terminated by addition of 2 ml 10% trichloroacetic acid - 2% phosphotungstic acid. Washed precipitates were prepared for the determination of specific activity (dpm/mg protein) as described before (9,10). The enzyme activity was linear with time (tested for 5, 15 and 30 min.). Specific activity on the basis of dpm/mg protein as shown in this table is for convenience of comparison. C fraction contains larger amount of non-enzyme protein. Actual relative yields of the enzyme in B and C fractions are shown in Fig. 1.

Rats	Specific Activity*	
	Fraction B (dpm/mg protein/hrx10^5)	Fraction C (dpm/mg protein/hrx10^4)
Choline supplemented (average)	2,85,2.50,2.55,1.87 (2.44)	1.77,1.70,1.83,1.06 (1.59)
2-days choline deficient (average)	2.46,2.99,1.92,1.68 (2.26)	1.40,0.88,1.22,0.82 (1.08)
Decrease, %	7	32

* Values are from four independent experiments. Liver homogenate samples from paired groups were always centrifuged in the same rotor.

enzyme activity, did not respond to phosphorylcholine plus CTP or CDP-Choline (data not shown). We have also observed (unpublished) in a slice incubation system (1) that glucosamine-1-^{14}C incorporation into the proteins of the C fraction, but not of B, was stimulated by phosphorylcholine (B and C

Table 2. ACTIVATION AND DOSE RESPONSE OF CDP-CHOLINE ON NAcG TRANSFERASE ACTIVITY IN C MEMBRANE FRACTION.

Additions	Dose (µg)	Specific Activity * (dpm/mg protein/hr x 10^4) Fraction C from choline supplemented rats.	Increase %	Fraction C from 2-days choline deficient rats.	Increase %
None**	-	1.06	-	0.82	-
PC	50	1.14	7.5	0.84	2.4
	100	1.12	5.6	-	-
PC plus CTP†	5	2.32	118	1.09	33
	10	3.06	188	1.50	83
	20	3.09	184	2.14	161
CDP-Choline	5	2.06	94	1.51	84
	10	3.14	196	1.86	127
	20	3.84	262	2.60	217
	40	3.20	202	2.81	243

* Determined as in Table 1. ** Complete incubation mixture and conditions were same as in Table 1. Additions were done in a volume of 10 µl. † PC dose was 100 µg, only CTP dose was varied. PC, CTP and CDP-choline were purchased from Sigma.

fractions were separated by the procedure described in Fig. 1 at the end of 2 hrs of incubation). Structural studies on plasma α_1-acid glycoprotein have established (4,5,6) that the carbohydrate moieties are linked to the protein through the reducing group of N-acetyl glucosamine and the amide nitrogen of aspargine (β-aspartyl N-acetyl glucosaminylamine). It has also been suggested that during biosynthesis, the N-

acetyl glucosamine and mannose residues of the inner core of the mature plasma glycoproteins are attached in the microsomal membranes soon after polypeptide synthesis (3,6,7). The non-reducing terminus of the oligosaccharide prosthetic group, on the other hand, is probably incorporated into glycoprotein in the Golgi apparatus (3). The stimulation of both in vitro and in a liver slice system of N-acetyl glucosamine incorporation into glycoprotein occurs in fraction C but not in the Golgi-rich fraction B. This finding suggests that the action of phosphorylcholine plus CTP or of CDP-choline is at the level of biosynthesis of the inner core of the polysaccharide prosthetic groups. Studies with low density lipoproteins of hen's egg yolk has shown a glucosamine aspargine linkage for the glycopeptide (8). If this is also true for rat plasma low density lipoproteins, a decrease of N-acetylglucosaminyl transferase activity in choline deficient rats (Table 1) and its activation by in vitro addition of phosphorylcholine plus CTP and by CDP-choline (Table 2), might establish a regulatory step for the low density lipoprotein synthesis which has been inferred from our previous work in vivo and in vitro (9-13). Role of CDP-Choline in lecithin synthesis has been established by the work of Kennedy and co-workers (14). Concentration of this co-factor is not decreased in choline deficiency (15). Extremely rapid turnover of CDP-choline has been observed for the exchange of phosphorylcholine moiety into lecithin in vivo (16), which indicates that the measurement of the steady-state level of CDP-Choline may not be very meaningful to explain its role, in either rapid exchange with lecithin or activation of NAcG-transferase activity. On the other hand, the decrease of phosphorylcholine level in choline de-

ficiency (17), effect of phosphorylcholine on glycoprotein synthesis in liver slices (1), and the results of the present work strongly favor a new role of phosphorylcholine and CDP-Choline in glycoprotein synthesis. Two possible mechanisms may be suggested from these studies. CDP-Choline may act as a co-factor for the enzyme activity and/or its role in the rapid exchange of phosphorylcholine moiety into lecithin may catalytically activate the microsomal membrane function. Stimulation of glycoprotein synthesis by the NAcG transferase enzyme, which is tightly bound on the membrane, may be just one manifestation of the activation of other functions of the membrane. Work is in progress to test these possibilities.

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References

1. Mookerjea, S., Park, C.E. and Chow, A. Biochem. Biophys. Res. Commun. 36, 851, 1969.
2. Mookerjea, S. Can. J. Biochem. 43, 1733, 1965.
3. Schachter, H., Jabbal, I., Hudgin, R.L., Pinteric, L., McGuire, E.J. and Roseman, S. J. Biol. Chem., March 10 issue, 1970 (In Press).
4. Satake, M., Okuyama, T., Ishihara, K. and Schmid, K. Biochem. J. 95, 749, 1965.
5. Wagh, P.V., Bornstein, I. and Winzler, R.J. J. Biol. Chem. 244, 658, 1969.
6. Li Yu-Teh, Li Su-Chen and Shetlar, M.R. J. Biol. Chem. 243, 656, 1968.
7. Lawford, G.R. and Schachter, H. J. Biol. Chem. 241, 5408, 1966.
8. Augustyniak, J.Z., Martin, W.G. Can. J. Biochem. 46, 983, 1968.
9. Mookerjea, S. Can. J. Biochem. 44, 1064, 1966.
10. Mookerjea, S., Jeng, D. and Black, J. Can. J. Biochem. 45, 825, 1967.
11. Mookerjea, S. Can. J. Biochem. 47, 125, 1969.
12. Mookerjea, S. and Chow, A. Biochem. Biophys. Acta. 184, 83, 1969.
13. Park, C.E. and Mookerjea, S. Fed. Proc. 28, 369, 1969.

14. Kennedy, E.P. and Weiss, S.B. J. Biol. Chem. 222, 193, 1956.
15. Wilgram, G.F., Holoway, C.F. and Kennedy, E.P. J. Biol. Chem. 235, 37, 1960.
16. Bjornstad, P. and Bremer, J.J. J. Lipid Res. 7, 38, 1966.
17. Thompson, W., MacDonald, G. and Mookerjea, S. Biochem. Biophys. Acta. 176, 306, 1969.