LYSOLECITHIN ENHANCEMENT OF GLYCOPROTEIN:GLYCOSYL TRANSFERASE ACTIVITY

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1. Introduction

The addition of individual monosaccharides in the synthesis of glycoproteins is thought to be catalyzed by glycoprotein: glycosyl transferases in mammalian tissues according to the following reaction:

$$\text{Acceptor} + \text{NNP-monosaccharide} \xrightarrow[M^{2+}]{\text{glycosyl transferase}} \text{acceptor-monosaccharide} + \text{NNP}$$

where the acceptor is an incomplete glycoprotein, NNP-monosaccharide is a nonspecific mono- or diphosphonucleotide monosaccharide, the glycosyl transferase has a high degree of specificity, and M^{2+} is a divalent cation (usually Mn^{2+} or Mg^{2+}) but is not always required [1–10]. In general, it is well established that the glycosyl transferases are membrane bound and require detergent solubilization for full expression of activity. Of late, however, two lines of evidence suggest that the addition of monosaccharides to glycoproteins may not occur as simply as related in the equation above but may involve other factors. The first is the implication that lipids or glycolipids may function in the transfer of monosaccharides either as intermediates or by undefined mechanisms [10–15], perhaps in analogy with the role of lipids in the biosynthesis of the bacterial cell envelope [16]. The second is the report [17] that folic acid may be intimately involved in the above reaction. The present report provides evidence that lysolecithin in the presence of folic acid can specifically and significantly increase the activity of fetuin:galactosyl transferase from rat kidney. The results are of importance because they substantiate the concept that the addition of monosaccharide to glycoproteins may not be a simple one-step reaction. The definition of the mechanisms by which glycoprotein synthesis occurs is of timely interest because of the attention given to glycoproteins as cell membrane macromolecular constituents with respect to lectin, electrokinetic, immunologic, and cell receptor phenomena.

2. Materials and methods

Details of the fetuin:galactosyl transferase enzyme properties and assay have been given elsewhere [5, 17]. The acceptor utilized in these studies is fetuin minus sialic acid, galactose (terminal residue *N*-acetylglucosamine). Lipids were purchased from Applied Science (Philadelphia, Pa.) and Serdary Research Laboratory (London, Ontario). All lipids were adjusted to a concentration of 5 mg/ml in chloroform or benzene as purchased. Appropriate volumes were added to assay tubes and solvent evaporated in vacuo. The enzyme source was rat kidney homogenized in 0.1 M Tris–0.1% Triton X-100–2 mM 2-mercaptoethanol (pH 6.8) as described [17]. Three fractions of this homogenate were used for the experiments described here: a 10 000 *g* supernatant of the crude homogenate, a Sephadex G-75 fraction of the supernatant, and a Bio-Gel P-200 fraction of the Sephadex fraction.

The complete assay system contained the desired amount of lipid plus 10 μl of 0.2 M Tris–HCl (pH 6.8), 10 μl of 0.1 M $MnCl_2$, 200 μg of acceptor protein

Table 1

Effects of lipids on the activity of the variously purified fetuin:galactosyl transferases of rat kidney in the presence of absence of folic acid.

Addition to complete system		Enzyme source					
		10 000 *g* Supernatant		Sephadex G-75 fraction		Bio-Gel P-200 fraction	
		Without folic acid	With folic acid	Without folic acid	With folic acid	Without folic acid	With folic acid
None		2 300	6 000	2 300	31 000	3 000	54 000
Lysolecithin (egg)	25 μg	4 100	11 000		68 000	4 100	91 900
	50 μg	4 100	11 000	5 500	80 000	3 700	85 200
	125 μg	4 800	12 000		84 000	–	–
Lysolecithin (liver)	25 μg	3 900	12 000	–	–	–	–
	50 μg	4 400	13 000	–	–	–	–
Lecithin (bovine)	500 μg	–	–	–	–	2 700	37 800
Lysophosphatidyl-ethanolamine	50 μg	2 400	7 000	1 900	38 000	–	–
Phosphatidyl-ethanolamine	250 μg	2 700	5 500	1 800	35 000	2 700	33 600
Phosphatidyl-ethanolamine plasmalogen	50 μg	1 600	5 500	–	26 000		–
Lysophosphatidyl-ethanolamine plasmalogen	150 μg	2 200	6 000	–	2 800	–	–
Phosphatidyl serine	250 μg	–	–	–	–	3 300	37 800
Bovine brain extract	Type III	–	–	–	–	2 400	26 000
	Type V	1 100	5 200		–	3 000	39 500

The complete system was as given in the text with the various lipids added as given. Folic acid was added to the assay to a final concentration of 0.7 mM [17]. Data are mean cpm/mg protein.

and water to a vol of 60 μl. If folic acid was to be added, it was added at this point in a vol of 1 μl. This was incubated at 37°C for 5 min, cooled, and 10 μl of UDP-[^{14}C]galactose (specific activity 240 Ci/mole, 3 μCi/ml) and 10 μl of enzyme protein (10–60 μg as protein) were added to a final vol of 81 μl. This assay mixture is referred to as the complete system and was incubated 30 min at 37°C; protein-bound radioactivity was determined as given elsewhere [3, 5, 9, 10, 17]. Attempts to extract a radioactive product into an organic or lipid phase were unsuccessful.

3. Results

The data presented in table 1 illustrate that folic acid had a profound stimulatory effect on fetuin:galactosyl transferase activity and that in the presence of 0.7 mM folic acid lysolecithin specifically accelerated this transferase activity. It is evident that column chromatography in the absence of folic acid caused little change in specific activity, but in the presence of 0.7 mM folic acid each of the chromatographed fractions demonstrated increased specific activity compared to the crude homogenate. Furthermore, lysolecithin at 25–120 μg per assay increased the activity of the 10 000 *g* supernatant fetuin:galactosyl transferase about 2-fold in the presence and absence of folic acid. However, with the purified Sephadex G-75 fetuin:galactosyl transferase, 50 μg of lysolecithin in the presence of folic acid increased the activity more than 2.5-fold while in the absence of folic acid the activity with lysolecithin increased 1.7-fold. Finally, with the Bio-Gel P-200 fraction, the elevation in fe-

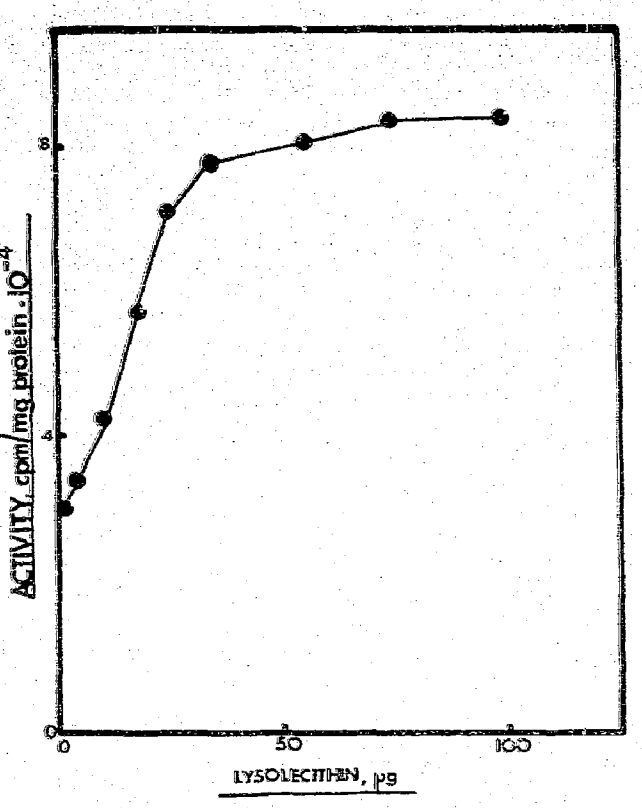

Fig. 1. Effect of lysolecithin concentration on the activity of the fetuin:galactosyl transferase in the presence of folic acid (0.7 mM final concn.). Enzyme source for these experiments was the Sephadex G-75 purified enzyme. Complete system for assay was as given in the text.

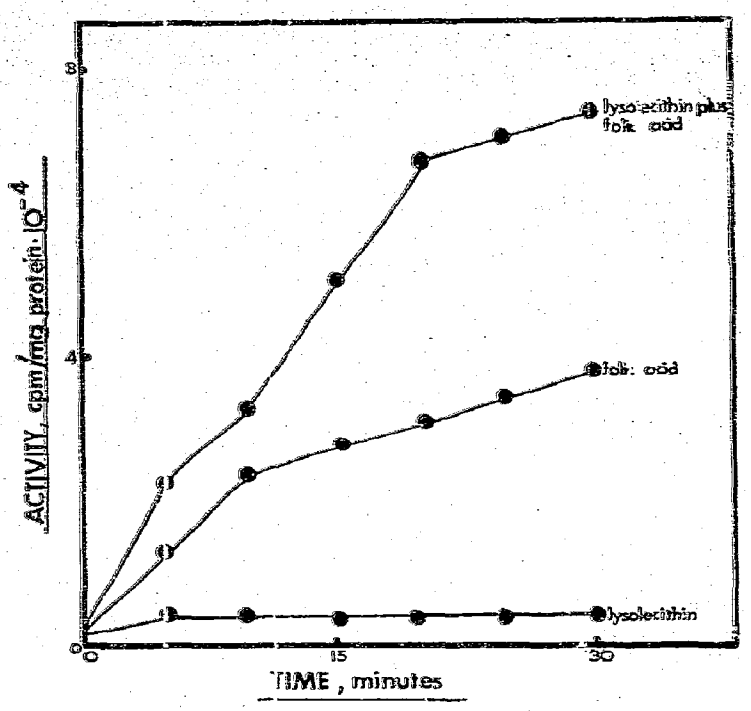

Fig. 2. Effect of folic acid and lysolecithin on the time course of reaction of the fetuin:galactosyl transferase of rat kidney. Enzyme source for the experiments was the Sephadex G-75 purified enzyme. Complete system for assay was as given in the text. '+ Lysolecithin' refers to 50 µg of lysolecithin in the assay; '+ folic acid' refers to 0.7 mM folic acid final concn. in the assay; and '+ lysolecithin + folic acid' refers to 50 µg of lysolecithin and 0.7 mM folic acid present in the assay.

tuin:galactosyl transferase activity with 50 µg of lysolecithin was 1.6-fold with, and 1.2-fold without, added folic acid. Thus, lysolecithin, when included in the folic acid stimulated assay system, is capable of further enhancing activity approx. 2-fold. The specificity of the activation with lysolecithin is clear from the data in table 1 for closely related compounds such as lecithin or other lyso-compounds, such as lysophosphatidylethanolamine; indeed, none of the other lipids tested, including the crude brain extracts, had a stimulatory effect on any of the various fractions of fetuin:galactosyl transferase.

The curve in fig. 1. indicates that the activity of the Sephadex G-75 fetuin:galactosyl transferase in the presence of folic acid was very sensitive to the amount of lysolecithin in the assay, especially in the 0–50 µg lysolecithin range. The curves in fig. 2 indicate that the interaction of the enzyme with either folic acid or lysolecithin and folic acid was characteristic of an enzyme reaction and activation of an enzyme with respect to time of incubation.

4. Discussion

The results show that folic acid and lysolecithin activate the reaction of rat kidney fetuin:galactosyl transferase. Several possibilities exist for the lysolecithin activation: a) The lysolecithin acts as a detergent, causing increased solubilization and availability of the enzyme; this seems unlikely since there is a non-ionic detergent, Triton X-100, in the assay system, since other lyso-compounds also with detergent properties are inactive, and since the lysolecithin has an equal effect on the purified enzyme compared to the crude enzyme extract; b) The lysolecithin acts to

form a glycolipid intermediate similar to the glycosyl carrier lipids known to occur in bacterial systems [16] and mammalian systems [11—15]; this, although likely, remains tentative since it was impossible in this study to isolate a glycolipid intermediate, as had been done with the CDP-choline [11] and dolichol phosphate [12, 15] systems. The present system contains the further complication that folic acid is necessary for the full expression of the lysolecithin activation; c) The lysolecithin may cause conformational or configurational changes in the acceptor or enzyme that facillitate the reaction. If this is the case, it may be that in previous work [9] in which substantial purification of glycoprotein:glycosyl transferase has been reported, the detergents used to solubilize these enzymes partially fulfilled this function. It is likely that either explanation b or c obtains for the interaction of lipids, folic acid, and glycoprotein:glycosyl transferases. The demonstration that lipid addition to the assay of these transferases has a significant effect on their activity has serious implications for glycoprotein synthesis in general and in particular for those instances such as neoplastic transformation in which such synthesis is thought to be altered.

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