GLYCOPROTEIN BIOSYNTHESIS: FOLIC ACID EFFECTS ON GLYCOPROTEIN:GLYCOSYL TRANSFERASE ACTIVITIES OF RAT KIDNEY AND LIVER[1]

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Summary Folic acid at 14 μM to 1.4 mM increased the activity of the collagen:glc and fetuin:gal and decreased the activity of the fetuin:NANA glycoprotein:glycosyl transferases of rat liver and kidney in vitro; highest effects were found with 1.4 mM folic acid. 1.4 mM folic acid increased kidney fetuin:gal activity 5-fold and decreased fetuin:NANA activity 3-fold. At 1.4 mM, folinic acid and p-methylaminobenzoic acid were totally inactive toward the transferases, methasquin was moderately active, and homofolic, tetrahydrohomofolic and methotrexate were very active toward the transferases. In all instances, however, the fetuin:gal and collagen:glc transferases were activated while the fetuin: NANA transferase was inhibited. From the data presented, folic acid is viewed as a possible control molecule in the synthesis of glycoprotein.

Folic acid, N-(p-{[(2-amino-4-hydroxy-6-pteridinyl)-methyl] amino}benzoyl)-glutamic acid, is thought to be primarily involved in the transfer of one carbon fragment (1) and as such is intimately related to DNA synthesis in the pathway involving deoxyuridate conversion to thymidylate mediated by folic acid and dihydrofolate reductase (2). Recently (3,4) intravenous injection of folic acid in rats has been found to result in a partial chemical nephrectomy and the morphological and biochemical changes in the kidney as a result of folic acid administration have been studied (3,4). In attempts to further study this effect of folic acid on the kidney we have found that folic acid has a profound effect on glycoprotein synthesis both in vivo (5) and in vitro. The present report gives evidence that folic acid greatly affects in vitro glycoprotein:-glycosyl transferase activity for transferases from either the kidney or the liver of the rat. The results indicate that folic acid may be a key molecule in the regulation of glycoprotein synthesis.

Glycoprotein synthesis (6-9) is thought to occur by the addition of individual monosaccharides to the growing oligosaccharide portion of the

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glycoprotein by the following reaction:

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

where NNP is a nonspecified nucleotide mono- or diphosphate, usually uridine, guanidine, or cytidine; M^{++} is a divalent cation, usually Mg^{++} or Mn^{++}; and the acceptor glycoprotein is an incomplete glycoprotein recognized by the transferase. The glycosyl transferases are usually solubilized with non-ionic detergents and there is some evidence (10-12) that a lipid intermediate in some cases may be involved in the actual transfer of monosaccharide into glycoprotein. The work described herein shows that folic acid *in vitro* can modify glycoprotein:glycosyl transferase activity as much as five fold.

Materials and Methods

CMP-[^{14}C]NANA (specific activity 100 Ci/mole), UDP-[^{14}C]glucose (240 Ci/mole), UDP-[^{14}C]galactose (240 Ci/mole), and UDP-*N*-[^{14}C]acetylglucosamine (40 Ci/mole) were purchased from New England Nuclear Corp. Protein determinations were made by the procedure of Lowry et al. (13). Fetuin (14) and bovine tendon collagen (Sigma) (15,16), and their degraded products were prepared as previously described (7,17-21). Folic acid and other biochemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Various folic acid analogues, derivatives, etc., were kind gifts of Dr. A. Nahas (University of Rochester Medical School).

Male Sprague-Dawley rats (160-200 g) were killed by decapitation. Livers and kidneys were immediately removed and homogenized for 30 strokes in a Ten Broeck homogenizer in 4 volumes of 0.1 M Tris, 0.1% Triton X-100, 0.002 M 2-mercaptoethanol at a pH of 6.8 at 0°C. Homogenates were sonicated for four 5-sec periods in a Branson sonicator at setting 2. The homogenates were centrifuged at 10,000 x *g* for 30 min and the supernatants were utilized as source material for the transferase assay.

All standard transferase assays were conducted at 37°C for 60 min. Reactions were terminated by adding 1% phosphotungstic acid in 0.5 N HCl. After centrifugation for 5 min at 2000 x *g*, precipitates were washed twice with 10% trichloroacetic acid, once with ethanol:ether (1:1), and dissolved in 0.2 ml of 1 M NaOH (18,19). The dissolved pellets were plated onto glass filter discs, dried, dropped into 5 ml toluene-phosphor solution, and counted in a Nuclear Chicago scintillation counter (15-21). Specific activities are expressed as counts per milligram of protein ± estimated standard error of the mean ($\hat{\sigma}_{\bar{x}}$) as computed on an Olivetti Programma 101. Where indicated, *P* values are determined by Student's *t*-test with a minimum of six separate determinations per assay.

All glycoprotein:glycosyl transferase assays were conducted in a total volume of 81 µl, which included: 10 µl of 0.1 M $MnCl_2$, 10 µl of 0.2 M Tris (pH 6.8), 10 µl of appropriate sugar nucleotide (CMP-[^{14}C]sialic acid, 3 µCi/ml; UDP-[^{14}C]glucose, 5 µCi/ml; UDP-[^{14}C]galactose, 3 µCi/ml), 10 µl of the 10,000 x *g* supernatant containing 12-16 mg/ml of protein except for sialic

Table 1. Activity of Rat Kidney Glycoprotein:Glycosyl Transferases in the Presence of Various Folic Acid Derivatives

Data are cpm per mg enzyme protein for experiments as described in the text. All concentrations are final assay concentration of compounds. Values are means from six independent observations.

Condition	Glycoprotein:glycosyl transferase			
	Collagen:Glc	Fetuin:gal		Fetuin:NANA
		Endogenous	Exogenous	
Complete	765	555	3941	6627
14 μM Folic acid	---*	725	6791	5287
140 μM Folic acid	---	1118	14477	5059
1.4 mM Folic acid	2784	1601	29581	2163
2.8 mM Folic acid	2792	1587	27421	2093
1.4 mM Hydrolyzed folic acid**	682	464	4060	5479
1.4 mM Homofolic acid	1464	1340	14144	3902
1.4 mM Tetrahydrohomofolic acid	1183	1157	15078	3366
1.4 mM Methasquin	876	1039	9555	3013
1.4 mM Folinic acid†	768	768	5916	6338
1.4 mM p-Methylaminobenzoic acid	744	562	4121	6488
1.4 mM Methotrexate§	1492	1818	39416	5279

*Experiment not performed.

**Folic acid hydrolyzed 6 hours at 100°C in 6 N HCl.

†5-formyl-5,6,7,8-tetrahydrofolic acid.

§N-(p-{[2,4-diamino-6-pteridinyl methyl]methylamino}glutamic acid)4-amino-10-methylfolic acid.

acid transferases where a lower protein concentration (8-10 mg/ml) was used. Any additions of biochemicals were made in 1 or 2 μl of 0.9% NaCl adjusted to pH 8.8-9.0 with 1 N NaOH; appropriate amounts of the same 0.9% saline solution were added to the control incubations.

Endogenous galactosyl transferase was determined by measuring radioactivity incorporated into acid insoluble material derived from the 10,000 x g supernatant without added protein acceptor. The collagen:glc transferase assay contained 400 μg of acceptor protein, and the collagen:gal transferase was assayed in the presence of 500 μg of acceptor protein (determined by the Lowry method [13]). The fetuin:NANA transferase contained as acceptor 200 μg of fetuin minus sialic acid, and the fetuin:gal transferase assay, 200 μg of fetuin minus sialic acid minus galactose. Appropriate substrate and enzyme blanks were subtracted from the total assay to permit calculation of exogenous transferase activity. Endogenous acceptor protein levels were low for all sugar nucleotides except UDP-[^{14}C]galactose.

Results

Folic acid and analogue effects on kidney glycoprotein:glycosyl transferases. The data of Table 1 clearly indicate that folic acid had a profound effect on kidney glycoprotein:glycosyl transferases. Folic acid in concentrations of 14 μM to 1.4 mM final concentration greatly enhanced the activity of

the collagen:glc transferase and the fetuin:gal transferase while the activity of the fetuin:NANA transferase was dramatically decreased in the presence of the folic acid. Highest effects of the folic acid occurred at 1.4 mM and a dose-response curve was generated between lower concentrations and 1.4 mM folic acid; higher concentrations of folic acid did not increase the response found with the 1.4 mM concentration. Hydrolysis of folic acid (final concentration, 1.4 mM) completely abolished the enhancement of the collagen:glc and fetuin:gal transferase activity and completely abolished the inhibition of the fetuin:NANA transferase activity. The folic acid derivatives investigated were of extreme interest: when tested at 1.4 mM, folinic acid and p-methylaminobenzoic acid had essentially no effect on acceleration or inhibition of glycoprotein:glycosyl transferase activity; methasquin was moderately active, and homofolic acid, tetrahydrohomofolic acid and methotrexate were very active in activating the collagen:glc and fetuin:gal transferases while inhibiting the fetuin:NANA transferase. None of the analogues produced as great an effect as the 1.4 mM folic acid except methotrexate, which elevated the fetuin:gal transferase to ten times its control activity (Table 1). It should be noted that in every instance the compound either had no effect at all on the transferase or increased the activity of the collagen:glc and fetuin:gal transferases but inhibited the activity of the fetuin:NANA transferase.

Folic acid effects on liver and kidney glycoprotein:glycosyl transferases. In order to determine whether the effects of folic acid were limited to the kidney glycoprotein:glycosyl transferases, experiments were performed on liver transferases as shown in Table 2. It is evident that 1.4 mM folic acid enhanced the liver transferase activity of the collagen:glc and fetuin:gal transferases while the same concentration of folic acid inhibited the liver fetuin:NANA transferase.

The data of Table 3 demonstrate that the effect of the folic acid was on the actual transferase reaction since the activity was dependent on enzyme, acceptor, and non-heat denatured enzyme for the reaction. Furthermore, the addition of 1.4 mM (final concentration) folic acid to control reactions after the one hour reaction had no effect on the activity, indicating the necessity of the presence of the folic acid during the incubation period at 37°C.

Time course of glycoprotein:glycosyl transferase reactions in the presence of folic acid. The data in Table 4 indicate that for each of three transferases studied in the presence of 1.4 mM folic acid, activity increased with time of incubation and was indicative of normal enzyme kinetics.

Table 2. Activity of Rat Liver and Rat Kidney Glycoprotein:Glycosyl Transferases in the Presence of 1.4 mM Folic Acid

Data are cpm per mg protein for experiments as described in text. P values are derived from Student's t-test.

Transferases	Liver			Kidney		
	Control	Folic acid (1.4 mM)	P	Control	Folic acid (1.4 mM)	P
Collagen:gal	---*	---	---	668	1172	= 0.005
Collagen:glc	1385	2946	< 0.005	1025	3826	< 0.001
Fetuin:gal						
Endogenous	1158	3700	< 0.005	568	1627	< 0.005
Exogenous	8308	24061	< 0.001	3868	23372	< 0.001
Fetuin:NANA	16061	6461	< 0.001	5162	1884	< 0.001

* Experiment not performed.

Table 3. Control Activities of Rat Kidney Glycoprotein:Glycosyl Transferases in the Presence or Absence of Folic Acid

Experiments were performed as given in Materials and Methods and are expressed as cpm per mg protein; means from 8 independent determinations. All volume adjustments were made with glass-distilled water or appropriate buffer.

Condition	Glycoprotein:glycosyl transferase					
	Fetuin:gal		Collagen:glc		Fetuin:NANA	
	Control	Folic acid (1.4 mM)	Control	Folic acid (1.4 mM)	Control	Folic acid (1.4 mM)
Complete	3940	29500	760	2780	6630	2160
Minus acceptor	555	1600	152	336	127	130
Minus enzyme plus boiled enzyme*	43	48	71	61	100	134
Minus enzyme, acceptor plus boiled enzyme*	42	45	43	65	62	69
Minus enzyme source	42	58	65	60	--**	--
Subsequent folic acid†	3939	4210	752	810	6480	6380

* Enzyme source boiled 10 min before assay.

** Experiment not performed.

† Folic acid was added at the end of the 60-min incubation period, prior to addition of phosphotungstic acid in 0.5 N HCl to terminate the reaction.

Table 4. Time Course of Reaction of Kidney Glycoprotein:-Glycosyl Transferases in the Presence of 1.4 mM Folic Acid

Data are cpm per mg protein and are means from 8 independent observations.

Time (min)	Glycoprotein:glycosyl transferase		
	Fetuin:gal	Collagen:glc	Fetuin:NANA
0	43	81	65
15	15,775	1686	1290
30	20,400	2201	1506
60	29,987	3111	2325

Discussion

The results clearly demonstrate that folic acid may act as a pivotal control molecule in the synthesis of glycoproteins. It is not entirely clear from the present data whether the folic acid acts directly as a cofactor-inhibitor in the transferase reaction, whether the folic acid functions in formation of an intermediate--possibly a lipid; or whether the interaction is more subtle, for example involving conformational changes, feedback mechanisms or complexing of other factors involved in the reaction. Since glycoprotein:glycosyl transferase activity levels are thought to be altered (22-27) in the neoplastic state and since methotrexate is used extensively in the chemotherapy of neoplasia, the effects of folic acid on glycoprotein:-glycosyl transferases of neoplastic cells, including those transformed by oncogenic viruses, deserve to be studied.

The two outstanding differences between NANA and the other monosaccharides occurring in glycoproteins are its structure with the dissociable acid group with pK $\sim$2.7 and the fact that in glycoproteins sialic acid almost always occurs as a terminal residue. Since as a terminal residue of glycoproteins NANA functions as a recognition molecule (for example, to determine when a given glycoprotein should be cleared by the liver from the circulation), as a molecule imparting charge and electrokinetic properties to cell surfaces and as a molecule allowing passage of glycoproteins through certain membranes, it may be that the addition of NANA to glycoproteins (and indeed catabolism, i.e., by neuraminidase) may be under a much different control than addition of internal monosaccharides to glycoproteins and thus the markedly different response of the transferases to folic acid.

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