Regulation of $\beta$-Galactoside $\alpha$2,6-Sialyltransferase Gene Expression by Dexamethasone*

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The hepatic acute phase response is accompanied by increased levels of Gal$\beta$1-4GlcNAc $\alpha$2,6-sialyltransferase activity in liver and in circulation. Previous studies suggested that cytokines and glucocorticoids mediate the induction of this sialyltransferase activity. In this study the regulation of sialyltransferase expression by dexamethasone in H35 rat hepatoma cells is assessed by Northern hybridization and enzyme activity assays. Exposure of H35 cells to $1 \mu$M dexamethasone for 24 h causes a 3-4-fold enrichment of sialyltransferase mRNA and a corresponding increase in enzymatic activity. The induction of sialyltransferase mRNA begins within 3 h of dexamethasone treatment and reaches a plateau within 24 h. Minimum concentration of dexamethasone necessary for induction is $10^{-8}$ M, and induction was maximal at $10^{-6}$ M. Induction is sensitive to actinomycin D, suggesting that regulation may be exerted by altering the rate of mRNA synthesis. Puromycin and cycloheximide are ineffective in blocking induction, suggesting that de novo protein synthesis is not required for induction. Finally, dexamethasone alone is sufficient for maximum induction of sialyltransferase mRNA. In contrast, maximal induction of $\alpha$1-acid glycoprotein, a well studied hepatic acute phase reactant, requires both dexamethasone and cytokines, implying that different pathways exist for the induction of participants in the acute phase response.

The $\beta$-galactoside $\alpha$2,6-sialyltransferase catalyzes the transfer of sialic acid onto exposed Gal$\beta$1-4GlcNAc termini of N-linked oligosaccharides common to serum and cell surface glycoproteins (1, 2). Although this sialyltransferase has widespread tissue distribution, it is particularly abundant in liver, the major site of serum glycoprotein synthesis (3). However, very little is known about the regulatory mechanisms that dictate the expression of this enzyme, especially within the context of its role in appropriate glycosylation of hepatic and serum glycoproteins. The sialyltransferase exists predominantly in a membrane-bound form within the Golgi and trans-Golgi network (4) where it participates in the post-translational modification of newly synthesized secretory or cell surface glycoproteins. A soluble form of the sialyltransferase exists in the serum (5) and is thought to be derived from the liver (6, 7) by a proteolytic event that liberates the catalytic domain from its membrane anchor (6, 8).

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Elevation in liver and serum $\beta$-galactoside $\alpha$2,6-sialyltransferase activity is one of the hepatic responses to acute systemic injury (6, 9, 10) that include increased serum protein-bound carbohydrate (11, 12) and the induction of a subset of serum glycoproteins, the acute phase reactants (13–15). Because many of the acute phase reactants, most notably $\alpha$1-AGP, fibrinogen, and haptoglobin, are sialylated glycoproteins (15), it is not surprising that enhanced sialyltransferase activity is part of the hepatic acute phase response. Nevertheless, the precise molecular mechanism(s) that coordinate the overall hepatic acute phase response remain far from clear.

Activated monocytes (16), tissue macrophages (17), and growing keratinocytes (18) secrete factors that elicit acute phase protein production in vitro as well as in vivo. In some cases, the "hepatocyte stimulating factors" (HSF) appear to be the systemic lymphokines, interleukin-1 (19) and interleukin-6 (20). For the expression of some acute phase proteins, maximum induction by HSF requires the synergistic cooperation of glucocorticoids (21). It has been proposed that glucocorticoids are required to maintain cells in a state receptive to HSF stimulation (22). Consistent with this view is the observation that dexamethasone induction of $\alpha$1-AGP on the mRNA level requires continued protein synthesis (23). This may suggest the participation of yet unidentified, short-lived protein intermediates in the acute phase response. On the other hand, the existence of sequences residing 5' of the $\alpha$1-AGP structural gene required for the response to glucocorticoid indicates that glucocorticoid may regulate acute phase protein gene transcription directly. There are additional mechanisms by which glucocorticoids could influence hepatic gene expression post-transcriptionally. For example, it was recently reported that dexamethasone facilitates the intracellular transport of secretory glycoproteins in hepatocytes (24).

Induction of sialyltransferase activity by glucocorticoids (7) as well as by HSF (25) has been reported. Owing to the lack of nucleic acid probes, these investigators have relied solely on enzyme assays to measure sialyltransferase gene expression. Consequently, the molecular pathways that coordinate sialyltransferase expression with the induction of other acute phase proteins remain unclear. In this report, we utilize a probe complementary to the coding region of the liver $\beta$-galactoside $\alpha$2,6-sialyltransferase mRNA to examine the regulation of this sialyltransferase in hepatoma and primary hepatocyte cell cultures. We report that dexamethasone induces sialyltransferase activity by elevating the cellular level of sialyltransferase mRNA. Furthermore, induction is not dependent on continuing protein synthesis but does require ongoing transcription. Together the data support the model.

The abbreviations used are: $\alpha$1-AGP, $\alpha$1-acid glycoprotein; DMEM, Dulbecco's modified Eagle's medium; HSF, hepatocyte stimulating factor; PMA, phorbol 12-myristate 13-acetate.
whereby glucocorticoids mediate the direct induction of sialyltransferase expression at the transcriptional level.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dexamethasone, cycloheximide, puromycin, actinomycin D, all-trans-retinoic acid, N\(^{\text{2}}\)O\(^{\text{2}}\)-O-dibutyryl adenosine 3',5'-cyclic monophosphate (cAMP), phorbol 12-myristate 13-acetate (PMA), asialofetuin, and collegenase type I were purchased from Sigma. Collagen (Vitrogen 100) was provided by Collagen Co. (Palo Alto, CA). Restriction enzymes were purchased from Bethesda Research Laboratory, New England Biolabs, and Boehringer Mannheim. [\(\alpha\^{32}\text{P}\)]dATP (3000 Ci/ml) and CMP [\(^{14}\text{C}\)]NeuAc (18 mCi/ml) were purchased from Amersham Corp. and Du Pont-New England Nuclear, respectively. The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, glutamine, pyruvate, penicillin, and streptomycin were purchased from GIBCO Laboratories. All other biochemicals were of the highest quality commercially available, and the chemicals were of reagent grade or higher.

**Conditioned Media**—Colo-16 conditioned medium was unfractionated medium from Colo-16 (human squamous carcinoma) cells, which constitutively produce hepatocyte-stimulating factors (26). This conditioned medium was generously provided by Dr. H. Baumann, Roswell Park Memorial Institute, Buffalo, NY.

**Cell Cultures**—A rat liver hepatoma cell line (H35) (27), a gift from Dr. H. Baumann, was grown in monolayer culture in DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, 100 units/ml of penicillin, and 100 \( \mu \)g/ml of streptomycin, and incubated at 37 °C in 5% CO\(_2\). Cells were plated at a density of approximately 6 \( \times \) 10\(^5\) cells/cm\(^2\). Stock solutions of dexamethasone and retinoic acid were diluted in ethanol to a concentration of 10\(^{-3}\) M. PMA (0.33 \( \mu \)g/ml) and cAMP (0.1 M) were dissolved in dimethyl sulfoxide and neutralized with sodium bicarbonate, respectively. Drugs were added to the culture 24 h later. Control cultures contained equivalent amounts of solvent.

**Primary Culture of Hepatocytes**—Primary hepatocytes were isolated by collagenase perfusion of male rat (Sprague-Dawley, 250-300 g body weight) liver and in primary hepatocyte culture upon stimulation by dexamethasone and specific cytokines (25). To examine the molecular basis of this induction, we used a probe complimentary to the distal two-thirds of the coding region of the rat liver \(\alpha\,2\,6\) sialyltransferase mRNA to assess expression on the RNA level. To determine if induced sialyltransferase activity is accompanied by increased steady-state mRNA, total cellular RNA was isolated from H35 cells that express nearly all major acute phase reactants in response to HSF stimulation (26). Northern blot analysis revealed that the H35-derived sialyltransferase mRNA migrated as a single band consistent with the 4.7-kilobase mRNA observed in rat liver (8) (Fig. 1, lane 1). Exposure of H35 cells to 1 \( \mu \)M dexamethasone for 24 h resulted in a 4-fold increase in the steady-state level of the sialyltransferase mRNA (Fig. 1, lane 2).

To assess the dosage dependent stimulation of sialyltransferase in H35 cells, total RNA isolated from cells that were exposed to increasing concentrations of dexamethasone was analyzed on Northern blots. The sialyltransferase mRNA signal was quantitated by \( \alpha\,2\,6\) sialyltransferase activity was determined in liver and in primary hepatocyte culture upon stimulation by dexamethasone and specific cytokines (25). To examine the molecular basis of this induction, we used a probe complimentary to the distal two-thirds of the coding region of the rat liver \(\alpha\,2\,6\) sialyltransferase mRNA to assess expression on the RNA level. To determine if induced sialyltransferase activity is accompanied by increased steady-state mRNA, total cellular RNA was isolated from H35 cells that express nearly all major acute phase reactants in response to HSF stimulation (26). Northern blot analysis revealed that the H35-derived sialyltransferase mRNA migrated as a single band consistent with the 4.7-kilobase mRNA observed in rat liver (8) (Fig. 1, lane 1). Exposure of H35 cells to 1 \( \mu \)M dexamethasone for 24 h resulted in a 4-fold increase in the steady-state level of the sialyltransferase mRNA (Fig. 1, lane 2). The minimum concentration of dexamethasone required for hormonal induction of sialyltransferase is 10\(^{-9}\) M, and optimal stimulation occurs at 10\(^{-6}\) M. The induction of sialyltransferase mRNA appears to be rapid, with half-maximal induction being achieved approximately 6 h after the addition of dexamethasone (Fig. 3A). By 24 h, the mRNA level was close to maximal. The time course of induction was also assessed by determination of sialyltransferase enzymatic activity (Fig. 3B). As expected, the increase in enzymatic activity lagged behind the rise in the mRNA level, although close to maximum sialyltransferase activity was achieved within 24 h. Overall, exposure of H35 cells to dexamethasone resulted in a reproducible 3-4-fold induction of sialyltransferase gene expression on both the mRNA and enzymatic levels.

**RESULTS**

**Sialyltransferase Induction in H35 Cells Is Induced by Dexamethasone**—Previous reports have demonstrated that \(\beta\)-retinoic acid, an \(\alpha\,2\,6\) sialyltransferase activator is eliminated in the liver and in primary hepatocyte culture upon stimulation by dexamethasone and specific cytokines (25). To examine the molecular basis of this induction, we used a probe complimentary to the distal two-thirds of the coding region of the rat liver \(\alpha\,2\,6\) sialyltransferase mRNA to assess expression on the RNA level. To determine if induced sialyltransferase activity is accompanied by increased steady-state mRNA, total cellular RNA was isolated from H35 cells that express nearly all major acute phase reactants in response to HSF stimulation (26). Northern blot analysis revealed that the H35-derived sialyltransferase mRNA migrated as a single band consistent with the 4.7-kilobase mRNA observed in rat liver (8) (Fig. 1, lane 1). Exposure of H35 cells to 1 \( \mu \)M dexamethasone for 24 h resulted in a 4-fold increase in the steady-state level of the sialyltransferase mRNA (Fig. 1, lane 2).

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**Protein and RNA Synthesis on Sialyltransferase Induction**—Heightened sialyltransferase mRNA levels in dexamethasone-stimulated H35 cells may result from increased mRNA synthesis or stabilization of existing message. We tested the ability of H35 cells to maintain the appropriate level of sialyltransferase mRNA in the

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\[ X\cdot C. \text{ Wang, G. Hart, and J. T. Y. Lau, unpublished data.} \]
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FIG. 1. Effect of dexamethasone on sialyltransferase mRNA levels. Total cellular RNA was extracted from H35 cells cultured for 24 h without (lane 1) or with 1 μM dexamethasone (lane 2). RNA (5 μg) was electrophoresed in a 0.8% agarose gel in the presence of formaldehyde, electrotransferred to Zetabind filter, and hybridized to 32P-labeled 780-base pair sialyltransferase cDNA fragment (see “Experimental Procedures”). The blot was exposed to x-ray film with an intensifying screen for 24 h.

Absence of transcription. H35 cells were stimulated with dexamethasone in the presence or absence of 10 μg/ml actinomycin D for 12 h. While stimulation with dexamethasone for 12 h normally caused a 3-4-fold increase in sialyltransferase mRNA (Fig. 5, compare lanes 1 and 2), actinomycin D prevented dexamethasone-dependent induction of sialyltransferase activity (compare lanes 5 and 6).

To determine if de novo protein synthesis is required for sialyltransferase induction, the effects of puromycin and cycloheximide inhibition were tested. H35 cells were cultured in the presence of either 60 μg/ml puromycin or 10 μg/ml cycloheximide; concentrations previously demonstrated as sufficient to block the dexamethasone-mediated induction of α1-AGP (23). Inhibition of protein synthesis by puromycin did not effect the steady-state level of sialyltransferase mRNA (Fig. 5, lane 3). More importantly, dexamethasone-mediated induction at the mRNA level was not blocked by puromycin (Fig. 5, lane 4). Identical results were obtained when 10 μg/ml cycloheximide was used in place of puromycin (data not shown).

Comparison of Induction of α1-AGP and Sialyltransferase—While previously published data indicated that de novo protein synthesis is required for α1-AGP induction (23), our data indicate that sialyltransferase induction by dexamethasone does not require ongoing protein synthesis (see above). To assess the differences that exist between the regulation of sialyltransferase and α1-AGP, conditions known to stimulate α1-AGP expression were examined for their stimulatory effects on sialyltransferase expression. As mentioned in the Introduction, maximal α1-AGP induction requires glucocorticoid as well as hepatocyte stimulating factor (21). Consistent with these previous observations, a low level of α1-AGP expression was achieved when H35 cells were exposed to dexamethasone alone (Fig. 6, lane 2) or to a conditioned medium known to contain HSFs (26) (Fig. 6, lane 3). Combined stimulation by both glucocorticoid and conditioned medium elicited maximal α1-AGP induction (Fig. 6, lane 4). In contrast, sialyltransferase expression is induced by dexamethasone (Fig. 6, lane 3) but remain unaffected by conditioned medium (Fig. 6, lane 4). Furthermore, the combined exposure of H35 cells to dexamethasone and conditioned
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A. 12345678

20

40

60

80

Time (hour)

0

20

40

60

80

Time (hour)

FIG. 3. Kinetics of dexamethasone-induced sialyltransferase mRNA and enzyme activities in H35 cells. A, time course of dexamethasone induction of sialyltransferase mRNA in H35 cells. Cells were exposed to 1 \( \mu M \) dexamethasone for the indicated times. Total cellular RNA was isolated and aliquots were analyzed as described in the legend to Fig. 2. B, time course of dexamethasone induction of sialyltransferase activity in H35 cells. Parallel dishes of cells were washed twice with ice-cold phosphate-buffered saline and the cells scraped from them in 1 ml of cold phosphate-buffered saline. The cells were resuspended in 100 \( \mu l \) of homogenization buffer (50 mM imidazole-HCl, pH 7.0, 1% Triton X-100 detergent, 2.5 mM MnCl\(_2\)) and sonicated (W-225; Ultrasonics, Inc.). 10 \( \mu l \) of cellular homogenate was assayed for sialyltransferase activity and normalized using variations in total cellular protein concentrations as described under "Experimental Procedures." Each point represents the mean of three experiments.

medium resulted in a level of sialyltransferase expression that was not substantially different from treatment with dexamethasone alone (Fig. 6, lane 4).

Since H35 cells appropriately express major acute phase proteins in response to glucocorticoid and HSF stimulation (26), we sought to demonstrate that primary hepatocytes exhibit the same pattern of regulated sialyltransferase expression observed in H35 cells. As shown in Fig. 7, the presence of 1 \( \mu M \) dexamethasone caused identical elevations of sialyltransferase mRNA levels in H35 cells (lanes 1 and 2) and in primary cultures of hepatocytes (lanes 3 and 4). In addition, conditioned medium, whether alone or in conjunction with dexamethasone, has no further effect on the level of sialyltransferase mRNA in primary hepatocyte cultures (data not shown).

[Figures 4, 5, and 6 are shown in the text.]

FIG. 4. Effects of retinoic acid, cAMP, and PMA on the hormone-dependent elevation of sialyltransferase mRNA levels in H35 cells. H35 cells were incubated for 24 h in the absence (odd numbered lanes) or in the presence of 1 \( \mu M \) dexamethasone (even numbered lanes). In addition, 1 \( \mu M \) retinoic acid (lanes 3 and 4), 1 mM cAMP (lanes 5 and 6), or 0.4 \( \mu M \) PMA (lanes 7 and 8) was included in the incubation. Total cellular RNA was extracted and analyzed by Northern blot hybridization with sialyltransferase cDNA probe as described in the legend to Fig. 1.

FIG. 5. Effects of RNA or protein synthesis inhibitors on the hormone-induced enrichment of sialyltransferase mRNA in H35 cells. H35 cells were incubated in the absence (odd numbered lanes) or the presence of 1 \( \mu M \) dexamethasone (even numbered lanes) for 12 h. In addition, puromycin (60 \( \mu g/ml \)) was added 2 h prior to hormone treatment (lanes 3 and 4); actinomycin D (10 \( \mu g/ml \)) was added at the same time as dexamethasone (lanes 5 and 6). After 12 h incubation, total cellular RNA was isolated, electrophoresed, blotted, and hybridized to the sialyltransferase cDNA sequence as already described.

FIG. 6. Dexamethasone induction of sialyltransferase (ST) and \( \alpha \)-AGP mRNA in H35 cells. Parallel dishes of H35 cells were incubated in the absence (lanes 1 and 2) or presence of one-third diluted Colo-16 conditioned medium (lanes 3 and 4). In addition, 1 \( \mu M \) dexamethasone was included in cells represented by lanes 2 and 4. After 24 h, total cellular RNA was isolated and analyzed by Northern blot hybridization and probed with sialyltransferase and \( \alpha \)-AGP cDNA sequence.
results in further induction of α1-AGP but not sialyltransferase. In addition, retinoic acid, cAMP, and phorbol ester, either alone or in concert with dexamethasone alone were ineffective in further stimulation of sialyltransfase gene expression.

Our observations contrast with those of Woloski et al. (25), who reported elevated sialyltransferase activity in response to a hepatocyte stimulating factor produced by human peripheral blood monocytes (25). In our hands, exposure of cells to conditioned medium derived from human squamous carcinoma cells (Colo-16) was ineffective in stimulating sialyltransferase mRNA levels, yet Colo-16 medium did promote the expression of α1-AGP, an acute phase protein. A possible explanation for the discrepancy is that an HSF that stimulates α2,6-sialyltransferase expression is produced by peripheral blood monocytes but not by squamous carcinoma cells. This is a plausible possibility, since the hepatic acute phase response is apparently mediated by multiple HSFs, each with a slightly different range of target specificities (22). The regulation of sialyltransferase gene expression by different factors has been postulated by Jamieson et al. (38) based on the response kinetics of sialyltransferase, α1-AGP, and albumin when animals were challenged by a monokine derived from peripheral monocytes. However, the 3–4-fold elevation in sialyltransferase mRNA achieved by dexamethasone alone is consistent with reported increases in sialyltransferase activity using both in vivo and cell culture models that mimic the acute phase response (6, 7). Thus our data support a model whereby the increased sialyltransferase activity present in inflamed liver is the result of mRNA synthesis, and that this induction is dictated by the concentration of circulating glucocorticoids.

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