Regulation of Hepatocytic Glycoprotein Sialylation and Sialyltransferases by Peroxisome Proliferators*

Barbara E. Fayos and James R. Bartles†

From the Department of Cell, Molecular and Structural Biology, Northwestern University Medical School, Chicago, Illinois 60611

Short-term dietary exposure of rats to a representative member of each of the three classes of peroxisome proliferators was found to elicit: (i) 71-80 and 66-75% reductions in the specific activities of the hepatic β-galactoside α2,6- and α2,3-sialyltransferases, respectively; (ii) a 67-69% reduction in the level of hepatic β-galactoside α2,6-sialyltransferase protein; and (iii) 41-46 and 6-28% reductions in the levels of the hepatic β-galactoside α2,6- and α2,3-sialyltransferase mRNAs, respectively. These changes were found to correlate with a reduction in the sialylation of the N-linked glycans of a prototypical hepatocytic sialoglyconjugate, the integral plasma membrane glycoprotein CE9, as was evident through: (i) a decrease in apparent molecular mass, (ii) a conversion to a more basic distribution of isoelectric points, and (iii) 56-72 and 33-44% decreases in the ability to bind lectins specific for sialic acid in α2,3- and α2,6-linkage, respectively. When assessed by labeling semithin frozen sections of liver tissue with a fluorescent lectin specific for α2,6-linked sialic acid, the reduced sialylation observed for CE9 was found to extend to other hepatocytic glycoconjugates in the livers of peroxisome proliferator-treated rats.

Sialylation is a posttranslational modification of glycoproteins implicated in the regulation of processes as diverse as receptor-mediated endocytosis, protein targeting, cell adhesion, virus-host cell recognition, and hormone signal transduction (e.g., see Ashwell and Harford, 1982; Rutishauser et al., 1988; Lasky, 1992; Weiss et al., 1988; Stockell Hartree and Renwick, 1992). Sialic acids are added to membrane and secretory glycoproteins during their posttranslational processing in the Golgi complex to become the terminal sugars on N- and O-linked oligosaccharides (Kornfeld and Kornfeld, 1985). Two sialyltransferases are responsible for adding sialic acids in a linkage-specific manner to the galactose residues of nascent complex-type N-linked oligosaccharides: the β-galactoside α2,3-sialyltransferase (2,3-ST)1 and the β-galactoside α2,6-sialyltransferase (2,6-ST) (Weinstein et al., 1962b, 1987; Wen et al., 1992). We have investigated the effects of the peroxisome proliferators (PPs) on the activities and/or levels of these sialyltransferases.

The PPs are a structurally diverse group of relatively low molecular weight xenobiotic compounds that includes certain hypolipidemic drugs and phthalate-ester plasticizers (Reddy and Rao, 1986). Dietary administration of PPs to rodents elicits a complex pleiotropic response that is remarkably specific to hepatocytes. The best characterized aspects of the response are the proliferation of hepatocytic peroxisomes and smooth endoplasmic reticulum and the induction of many of their enzymatic constituents, particularly those involved in the oxidation of fatty acids (Reddy and Rao, 1986; Rao and Reddy, 1987; Hawkins et al., 1987). Despite the observation that PPs are nonmutagenic, chronic dietary exposure to these compounds causes hepatocellular carcinoma in rodents with an extremely high efficiency (Rao and Reddy, 1987).

Previously we observed that short-term dietary exposure of rats to PPs caused the hepatocytic plasma membrane glycoprotein CE9 to migrate slightly faster in SDS-gels (Bartles et al., 1990). Encoded by a single gene and mRNA in the rat, CE9 is a widely distributed Type-Ia transmembrane protein and a member of the immunoglobulin superfamily (Nehme et al., 1993). When expressed by the rat hepatocyte, CE9 exhibits an apparent molecular mass of 48 kDa, contains three N-linked glycans, and is concentrated within the basolateral plasma membrane domain (Hubbard et al., 1985; Bartles et al., 1985; Nehme et al., 1993). We determined that the PP-induced difference in the electrophoretic mobility of CE9 could be eliminated by prior chemical deglycosylation, but was not yet apparent when comparing pulse-radiolabeled high-mannose precursors (Bartles et al., 1990). Thus, we tentatively concluded that CE9 experienced an altered pattern of posttranslational glycosylation in the hepatocytes of PP-treated rats. In this article, we demonstrate that dietary exposure to the PPs brings about a reduction in the sialylation of CE9 and other hepatocytic glycoconjugates and that this reduction in sialylation mirrors decreases in the specific activities and/or levels of the relevant hepatic sialyltransferases.

EXPERIMENTAL PROCEDURES

Materials—The following were obtained from the designated sources: Na125I, CMP-[1-C14]NeuAc, [α-32P]ATP, and [γ-32P]ATP (Amersham Corp., Arlington Heights, IL); N-acetylactosamine, Dowex 1-X8, Trition CF-54, polyvinylpyrrolidone (average M, 40,000) and Clostridium perfringens neuraminidase (type X) (Sigma); lacto-N-tetraose (Oxford GlycoSystems, Inc., Rosedale, NY); ampholytes (Bio-Rad); protein A and random primer pd(N)10 (Pharmacia LKB Biotechnology Inc.); Athrobacter ureafaciens neuraminidase and digoxigenin-labeled lectin (Boehringer Mannheim); natural N-glycanase (Genzyme Co., Cambridge, MA); fluorescently labeled Maackia amurensis agglutinin (MAA) and Sambucus nigra agglutinin (SNA) (Vector Laboratories, Inc., Burlingame, CA); rhodaminated goat anti-rabbit immunoglobulin G (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA); Klenow and T4 poly-

1 The abbreviations used are: 2,3-ST, β-galactoside α2,3-sialyltransferase; 2,6-ST, β-galactoside α2,6-sialyltransferase; PP, peroxisome proliferator; DEHP, di-(2-ethylhexyl) phthalate; MAA, Maackia amurensis agglutinin; SNA, Sambucus nigra agglutinin.

* This work was supported by Grant CA53997 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of CMS Biology, Northwestern University Medical School, Ward Bldg., 303 East Chicago Ave., Chicago, IL 60611. Tel.: 312-503-1845; Fax: 312-503-7912.

2151
Electrophoresis was quantitatively immunoprecipitated from Clostridium perfringens and Athrobacter ureafaciens. CE9 was treated (v/v) of Nonidet P-40, 0.16% (w/v) of SDS, 3 mM sodium azide, pH 8.6, for collecting the beads by microcentrifugation, the partially desialylated CE9 eluted from the beads by heating in 0.5% (w/v) of SDS at 100 °C for 3 min. The eluted protein was then treated with 2 M NaCl and either frozen and stored in liquid nitrogen for Northern blotting, stored at −90 °C for enzyme assays, or homogenized in 50 mM sodium phosphate buffer, pH 6.8, followed by immunoprecipitation or electrophoresis in SDS-gels.

Immune precipitation, Glycosylase Treatments, and Two-dimensional Electrophoresis—CE9 was quantitatively immunoprecipitated from Triton X-100-insoluble or SDS-insoluble fractions of rat liver homogenates in the presence of protease inhibitors using either mouse monoclonal or rabbit polyclonal anti-CE9 IgG-Sepharose with equal results (Bartles et al., 1987). Complete desialylation of the immunoprecipitated CE9 required sequential incubation with neuraminidases isolated from Clostridium perfringens and Akrobacter ureafaciens. CE9 was treated first with 2 units/ml of C. perfringens neuraminidase in 50 mM sodium acetate, 10 mM calcium acetate, 3 mM sodium azide, pH 7.4, containing the protease inhibitors phosvitin, antipain (1 μg/ml), and leupeptin (1 μg/ml) and stored at −90 °C in preparation for immunoprecipitation or electrophoresis in SDS-gels.

Sialyltransferase Assays—All enzyme assays were performed under conditions that were established empirically to give a linear or near-linear response as a function of input over the concentration range of interest (e.g., see Bartles et al., 1991). Protein samples resolved in one- or two-dimensional SDS-gels were transferred electrophoretically to nitrocellulose and blots were labeled sequentially with affinity-purified rabbit polyclonal antibodies directed against either rat CE9 or rat liver 2,6-ST and 2,3;6-ST protein A, and the relative levels of 2,3-ST protein A binding were determined using an Pharmacia LKB Ulroscan XL laser densitometer to scan autoradiograms (Bartles et al., 1990). Subsequently quantifying the 2,6-ST on Western blots, samples containing nearly equal amounts of immunoprecipitated CE9, as determined by Western blotting, Total RNA was isolated from frozen chunks of liver (Chomczynski and Sacchi, 1987). Samples containing 35 μg of RNA were electrophoresed in formaldehyde-denaturing 1% agarose gels, transferred to nitrocellulose by capillary action, and labeled with randomly primed [32P]DNA (Sambrook et al., 1989) encoding the 2,3-ST or 2,6-ST. The relative levels of bound [32P]DNA were determined using a Pharmacia LKB Ulroscan XL laser densitometer to scan the corresponding autoradiograms and were normalized to the level of 28 S RNA as determined subsequently on the same blot using an end-labeled 32P-labeled oligonucleotide probe (Sambrook et al., 1989).

RESULTS

When examined in two-dimensional isoelectric focusing SDS-gels, the hepatocytic plasma membrane glycoprotein CE9 present in the liver homogenate of control rats was observed to focus as a constellation of seven to nine partially resolved spots mirroring the relative potencies of these compounds at eliciting pronounced other aspects of the pleiotropic response (Reddy and Lalwani, 1983). Conventional, quantitative, and more precise methods were also employed to examine the liver homogenates for 2,6-ST activity, a PP caused the constellation to become more basic, yet comparable numbers of 2,6-ST activity were noted for normal rats (Paulson et al., 1989). Lectin Fluorescence and Immunofluorescence—1.5-μm-thick frozen sections were obtained from livers fixed with 2% paraformaldehyde/lysine/periodate by perfusion and labeled according to the procedure outlined by Bartles et al. (1990), substituting 2% (w/v) polyvinylpyrrolidone (average Mr, 40,000) for gelatin as the blocking agent when labeling with the lectins. For lectin fluorescence, the sections were labeled with 10 μg/ml of fluoresceinated SNA or MAA. For immunofluorescence, mouse polyclonal antibody to the rat liver 2,6-ST or nonimmune rabbit IgG followed by rhodamine goat anti-rabbit IgG. The specimens were examined and photographed using a Leitz Diaplan fluorescence microscope.
Fig. 1. Two-dimensional electrophoretic analysis of CE9 with and without pretreatment with neuraminidases (NEUR) or N-glycanase (N-GLY). Rats were fed a control diet (CON, a, e, and i) or a diet containing ciprofibrate (C10, b, f, and j), DEHP (D10, c, g, and k), or Wy-14,1643 (W10, d, h, and l) for 10 days. Samples containing CE9 were resolved in two-dimensional isoelectric focusing SDS-gels, and the CE9 was revealed by Western blotting. The direction of isoelectric focusing was from left to right (-, cathode; +, anode), and the direction of SDS-gel electrophoresis was from top to bottom. i-l, homogenates resolved in a pH 4–6 gradient. e-h, CE9 immunoprecipitates desialylated by sequential treatment with neuraminidases from C. perfringens and A. ureafaciens neuraminidases revealed by Western blotting. The direction of isoelectric focusing was from left to right (−, anode; +, cathode), and the direction of SDS-gel electrophoresis was from top to bottom. i-l, homogenates resolved in a pH 5–7 gradient. a-d, CE9 immunoprecipitates deglycosylated by treatment with N-glycanase resolved in a pH 5–7 gradient. Although only the relevant region of each blot autoradiogram is shown, equivalent regions are presented in a-d, e-h, and i-l to facilitate comparison.

of immunoprecipitated CE9 with C. perfringens and A. ureafaciens neuraminidases (Fig. 1, e–h), suggesting that the shift to more basic isoelectric points was due to a reduction in the level of sialylation of CE9 in the livers of the PP-treated rats. Patterns consisting of three common spots were also observed when the CE9 immunoprecipitated from the livers of control and PP-treated rats were stripped of their N-linked glycans by enzymatic deglycosylation using N-glycanase (Fig. 1, a–d). The persistence of multiple spots upon enzymatic desialylation or deglycosylation most likely reflects the differential phosphorylation of the CE9 protein.2

Lectin blotting was used to quantify the levels of sialic acid on CE9 isolated by immunoprecipitation from the livers of control and PP-treated rats. The α2,3-linked and the α2,6-linked sialic acids most commonly found as part of complex-type N-linked oligosaccharides (Kornfeld and Kornfeld, 1985) were quantified using the linkage-specific lectins MAA (Wang and Cummings, 1988) and SNA (Shibuya et al., 1987), respectively. As reported previously (Bartles et al., 1990), the CE9 obtained from the livers of PP-treated rats was observed to migrate slightly faster in SDS-gels (Fig. 2). When normalized on the basis of CE9 protein, the binding of MAA was decreased to 28 ± 6%, 44 ± 8%, and 37 ± 4% of controls, and the binding of SNA was decreased to 56 ± 10%, 67 ± 11%, and 67 ± 12% of controls in the livers of rats fed the ciprofibrate, DEHP, or Wy-14,1643 diets, respectively (Fig. 2). In each case, the binding of the lectin was shown to be specific for sialic acid, because neither lectin bound to CE9 following enzymatic desialylation with C. perfringens and A. ureafaciens neuraminidases (data not shown).

To help determine whether the PP-mediated decrease in sialylation observed for CE9 might extend to other hepatocytic glycoconjugates, fluorescently tagged versions of these same two sialic acid-binding lectins were used to label semithin frozen sections of liver. When sections obtained from the livers of control rats were labeled with fluorescein- and rhodamine-labeled lectins, specific fluorescence signals were found at the surfaces of both hepatocytes and sinusoidal-lining cells (Fig. 3, a and b). With the resolution afforded by immunofluorescence, it was not possible to distinguish the relative labeling contributions of plasma membrane glycoconjugates per se from those present within the surrounding extracellular matrix or subplasmalemmal cytoplasm. Both the basal (sinusoidal) and apical (bile canicular) surfaces of hepatocytes were labeled brightly relative to the lateral surfaces between adjacent hepatocytes, perhaps as a result of limited access. In addition, there was bright specific labeling at certain intracellular sites within hepatocytes that generally exhibited the size, shape, and localization expected for elements of the Golgi complex (Roth et al., 1986). When fixation, processing, labeling, and photography were carried out in parallel under identical conditions, the binding of fluoresceinated SNA to sections obtained from the livers of ciprofibrate-treated rats was found to be decreased, both at the hepatocyte surface and at intracellular sites within hepatocytes (Fig. 3, c and d). Such a decrease was not observed for the surfaces of the sinusoidal-lining cells (Fig. 3, arrowheads), which are generally thought not to be affected by the PPs (Reddy and Rao, 1986). Similar overall results were obtained when examining sections obtained from the livers of rats treated with DEHP or Wy-14,1643 (data not shown). These observations suggested that decreased derivatization with α2,6-linked sialic acid may extend to a variety of cell surface and intracellular glycoconjugates in the hepatocytes of PP-treated rats. Unfortunately, the levels of labeling observed using fluoresceinated MAA were too low to allow such a comparison to be made for α2,3-linked sialic acids (data not shown).

To examine the basis of the effects of PPs on the sialylation of CE9 and other hepatocellular glycoconjugates, rats were fed diets containing one of the three PPs for 10 days, and the specific activities of the two major hepatic glycoprotein sialyltransferases were assayed. The specific activity of the hepatic 2,3-ST was found to be reduced to 25–34% of control levels in the livers of rats fed the PPs (Table I). Likewise, the specific activity of the hepatic 2,6-ST was found to be reduced to 20–29% of control levels in the livers of PP-treated rats (Table I). When assayed as a function of time after initiating dietary treatment with ciprofibrate, 2–5 days were required for the decreases in the specific activities of the sialyltransferases to reach one-half those observed after 10 days of treatment (data not shown).

To determine whether the decreases in the specific activities of the sialyltransferases observed upon PP treatment reflected decreased levels of enzyme, Western blotting was used to quantify the levels of 47-kDa 2,6-ST protein (Weinstein et al., 1987).
in the livers of control and PP-treated rats. When normalized on the basis of total homogenerate protein, the level of 2,6-ST protein was found to be decreased to 31–33% of control levels in the livers of PP-treated rats (Table I). To our knowledge, there are no antibodies available that would allow similar quantification of the levels of the rat liver 2,3-ST.

Immunofluorescence was used to compare the localization of the 2,6-ST in the livers of the control and PP-treated rats. Semithin frozen sections of liver were prepared, immunostained with a rabbit serum against CE9, and subsequently stained with a FITC-labeled secondary antibody. The sections were viewed under a fluorescence microscope, and the intensity of labeling was found to be reduced for those sections obtained from the livers of the PP-treated rats, thus causing the foci to also appear somewhat smaller (cf. Fig. 4, a and c). There was, however, no evidence of a gross redistribution of the 2,6-ST protein in the hepatocytes of rats fed the PPs.

By Northern blotting, the decrease in the specific activity of the 2,6-ST and the decrease in the level of the 2,6-ST protein were found to reflect consistent decreases in the level of hepatic 2,6-ST mRNA. Following normalization to the level of 28S rRNA, the level of 4.3/4.7-kilobase 2,6-ST mRNA (Paulson et al., 1989) was found to be decreased to 54–59% of control levels in total RNA preparations isolated from the livers of rats fed the PPs (Table I). In contrast to the consistent decrease observed for the 2,6-ST mRNA, the effect of the PPs on the level of the 2,3-ST mRNA was smaller and considerably more variable. Following normalization to the level of 28S rRNA, the level of 2.5-kilobase 2,3-ST mRNA (Wen et al., 1992) was found to be decreased to 72–94% of control levels in total RNA preparations isolated from the livers of PP-treated rats (Table I).

DISCUSSION

Three possible explanations for the observed PP-mediated decrease in the sialylation of CE9’s N-linked glycans are: (i) a decrease in the number of glycans, (ii) a failure to complete the addition of terminal sugar residues, or (iii) a decrease in the branching of the glycans.

On the basis of cDNA sequence, there are three Asn-X-Ser/Thr consensus sites for N-linked glycosylation predicted to reside within the extracellular domain of CE9 (Nehme et al., 1993). The observation of three products upon partial deglycosylation with N-glycanase substantiates the existence of three N-linked glycans on rat hepatocytic CE9 (Nehme et al., 1993). It appears as though three N-linked glycans must also be present on CE9 in livers of PP-treated rats, because pulse-labeled high-mannose precursors of CE9 from livers of control and PP-treated rats were observed to comigrate in SDS-gels (Bartles et al., 1990). Edlund et al. (1986) observed a 31–34% decrease in the rate of sialylation of endogenous proteins by dolichol monophosphate-mediated UDP-glucosaminyl- and GDP-mannosyltransferases in microsomal fractions prepared from the livers of rats fed a diet containing DEHP for 2 weeks. Our data suggest that such a change does not translate into a reduction in the number of N-linked glycans, at least in the case of the plasma membrane protein CE9. As for the possibility of incomplete processing, surplus terminal galactose residues were not detected on CE9 isolated from the livers of PP-treated rats using a sensitive digoxigenin-Ricinus communis agglutinin-I-binding assay. Furthermore, CE9 isolated from the livers of control and PP-treated rats proved to be resistant to digestion by endoglycosidase H, suggesting that the N-linked glycans of both forms of the protein have been processed significantly beyond their high-mannose precursors. On the basis of these additional observations, the most likely explanations are that either: (i) the terminal processing of some of the branches of CE9’s N-linked glycans is aborted in the livers of PP-treated rats, but prior to the addition of galactose, or (ii) there is less branching of CE9’s N-linked glycans in the livers of PP-treated rats. An intervention in the terminal processing prior to the addition of galactose would be expected to yield glycans with terminal N-acetylgalcosamine (Kornfeld and Kornfeld, 1985). The existence of such truncated glycans may explain the observation that CE9 isolated from the livers of PP-treated rats binds disproportionately larger amounts of wheat germ agglutinin, a lectin specific for both sialic acid and N-acetylgalcosamine (Bartles et al., 1990).

Fig. 2. Binding of the sialic acid-specific lectins MAA and SNA to CE9. Rats were fed a control diet (CON) or a diet containing ciprofibrate (C10), DEHP (D10), or Wy-14,443 (W10) for 10 days, and CE9 was quantitatively immunoprecipitated from nonionic detergent extracts of liver homogenates. Samples of immunoprecipitates containing CE9 were dissociated in SDS and analyzed by Western blotting (A) or followed by 125I-protein A (B). Following color development or autoradiography, the levels of lectin binding were determined by densitometry. The levels of specific binding of MAA (D) and SNA (E) were calculated after normalization on the basis of CE9 content as determined from Western blotting and are plotted as mean ± S.D. (duplicate determinations on three rats of each type) relative to values of 1.0 for the corresponding control.

3 B. E. Fayos and J. R. Bartles, unpublished data.
**Fig. 3.** Binding of fluorescently labeled SNA to semithin frozen sections of liver. Rats were fed a control diet (a and b) or a diet containing ciprofibrate (c and d) for 10 days, their livers were fixed with paraformaldehyde/lysine/periodate by perfusion, and semithin frozen sections were prepared, labeled with fluoresceinated SNA, and photographed in parallel under identical conditions, c and d, phase, S, examples of sinusoidal lumina. Single arrows, examples of bile canaliculi. Single arrowheads, examples of sinusoidal-lining cells viewed in tangential section. Double arrowheads, examples of sinusoidal-lining cells viewed in transverse section. Magnification bar in d, 10 μm.

**Table 1**

Effects of PPs on the specific activities, protein levels, and mRNA levels of the sialyltransferases

Rats were fed a control diet or a diet containing ciprofibrate (C10), DEHP (D10), or Wy-14,643 (W10) for 10 days. To determine the specific activities of the 2,3-ST or 2,6-ST, nonionic detergent extracts of liver homogenates were assayed using the exogenous substrates lacto-N-tetraose or N-acetyllactosamine as described under "Experimental Procedures." The data were normalized on the basis of total homogenate protein and are reported as mean ± S.D. (triplicate determinations on three rats of each type) relative to values of 100% for the corresponding controls. To determine the level of 2,6-ST protein, samples of liver homogenates were resolved in SDS-gels, and the resultant blots were labeled sequentially with affinity-purified anti-2,3-ST antibody and 125I-protein A. The levels of antibody binding were determined by densitometry, were normalized on the basis of total homogenate protein, and are reported as mean ± S.D. (duplicate determinations on three rats of each type) relative to values of 100% for the corresponding controls.

| Treatment | Specific activity | Protein level | mRNA level |
|-----------|------------------|---------------|------------|
| C10       | 2,3-ST 25 ± 8     | 20 ± 4        | ND*        |
|           | 2,6-ST 25 ± 8     | 20 ± 4        | 31 ± 2     |
| D10       | 2,3-ST 34 ± 8     | 24 ± 4        | ND*        |
|           | 2,6-ST 34 ± 8     | 24 ± 4        | 33 ± 2     |
| W10       | 2,3-ST 25 ± 7     | 27 ± 9        | ND*        |
|           | 2,6-ST 25 ± 7     | 27 ± 9        | 31 ± 2     |

* Protein levels were not determined because anti-2,3-ST antibodies were not available.

CE9 is a basolateral plasma membrane protein of hepatocytes, both in control and in PP-treated rats (Bartles et al., 1990). Yet the decrease in labeling by fluoresceinated SNA (Figs. 3c) was found to apply to both the basal and apical surfaces of hepatocytes as well as to their intracellular compartments. Therefore, the decrement in sialylation noted for CE9 appears to extend to other hepatocellular glycoconjugates in the hepatocytes of PP-treated rats. Even though CE9 is synthesized at 1.7 times the normal rate and is induced 1.8-fold in the livers of ciprofibrate-treated rats (Bartles et al., 1990), there is no evidence of an intracellular accumulation of CE9 by immunofluorescence (Bartles et al., 1990). This suggests that transport through the hepatocytic secretory pathway is neither blocked nor slowed to a great extent by treatment with PPs. Additional support comes from the observations that six other hepatocytic plasma membrane proteins continue to be sent to their correct surface domains in PP-treated rats (Bartles et al., 1990) and that there is no drastic change in the localization of the hepatocytic 2,6-ST (Fig. 4, c and d).

A likely explanation for these defects in the sialylation of CE9 and other hepatocytic glycoconjugates lies in the observation that the PPs, irrespective of class, were found to elicit substantial reductions in the specific activities of the two major sialyltransferases involved in the terminal processing of hepatocytic N-linked oligosaccharides (Table I). In the case of the 2,6-ST, these reductions in specific activity could be completely accounted for by comparable decreases in the level of 2,6-ST protein (Table I). Given the precedent for the regulation of 2,6-ST activity through changes in the level of its corresponding mRNA (Wang et al., 1989, 1990; Svensson et al., 1990; Shah et al., 1992; Grollman et al., 1993), the observed reduction in the level of 2,6-ST mRNA (Table I) is most likely responsible for the decreases in the level and specific activity of the 2,6-ST in the livers of PP-treated rats. It is presently unclear how the PPs might act to alter the level of 2,6-ST mRNA. Regrettably, the magnitude of the effect at the level of the mRNA is sufficiently small so as to make it difficult to distinguish between the options of decreased transcription and decreased mRNA stability by experimental means, especially without the benefit of a model cell culture system. While the information available concerning the so-called PP-activated receptor suggests that PPs may directly increase the rate of transcription of certain genes (Isserman and Green, 1990; Kliewer et al., 1992), no instances of the PPs eliciting a decrease in the rate of transcription have yet been documented. A subset of hepatocytic plasma membrane proteins is also known to be expressed at a lower level in the livers of PP-treated rats (Bartles et al., 1990). Our observations reinforce the notion that many profound physi-
ological and biochemical changes are occurring in the livers of PP-treated rats. These changes may reflect a shift in cellular emphasis toward the biosynthesis of peroxisomal constituents at the expense of the biosynthesis and maintenance of the secretory pathway and its organelles.

Although the PPs may exert their effects on the specific activity and level of the 2,6-ST by affecting the rate of transcription or stability of the hepatocytic 2,6-ST mRNA, it would seem to be considerably more difficult to invoke such an explanation in the case of the 2,3-ST. The reductions observed in the levels of 2,3-ST mRNA were not only more modest, but were found to vary considerably among the PPs (Table I), despite the uniformity and magnitude of the effects of these compounds on the specific activity of the 2,3-ST (Table I) and the binding of MAA to CE9 (Fig. 2). Thus, although the net effects of the PPs on the specific activities of the two sialyltransferases were similar, there is a distinct possibility that these agents will prove to affect the 2,3-ST and 2,6-ST, and hence the sialylation of hepatocytic glycoproteins, by alternate pathways. The elucidation of the basis for the PP-mediated reduction in the specific activity of the hepatic 2,3-ST awaits further experimentation. In preliminary experiments, we have failed to detect a direct inhibitory effect of ciprofibrate on the activity of the 2,3-ST when assayed at final concentrations as high as 0.3 mM in nonionic detergent extracts of rat liver homogenate.\(^3\) But this by no means rules out the possibility that some metabolite of the PPs or some cellular change brought about by exposure to the PPs might somehow affect the activity or stability of the 2,3-ST protein.

Regardless of their mechanism of action, the data reported here indicate that short-term dietary exposure to the PPs can bring about significant changes in the sialylation of hepatocytic glycoconjugates and that these changes reflect decreases in the specific activities and/or levels of expression of the hepatocytic glycoprotein sialyltransferases. Given the pivotal roles identified for the sialic acid residues of glycoproteins, this newly described aspect of the pleiotropic response to dietary PPs may prove to have a profound influence on the activities, localizations, and/or stabilities of the affected hepatocytic membrane and secretory glycoproteins.

Acknowledgments—We thank Dr. Karen Colley for providing the affinity-purified anti-2,6-ST antibody and the plasmid encoding the 2,6-ST, Dr. James Paulson for providing the plasmid encoding the 2,3-ST, Dr. Janardan Reddy for providing the PPs, V. Subbarao for help in caring for the rats, and D. Chagnovich for help with Northern blotting.

REFERENCES

Ashwell, G., and Harford, J. (1982) Annu. Rev. Biochem. 51, 531–554

Bartles, J. R., Braiterman, L. T., and Hubbard, A. L. (1985) J. Cell Biol. 106, 1126–1138

Bartles, J. R., Feracci, H. M., Stieger, B., and Hubbard, A. L. (1987) J. Cell Biol. 105, 1241–1251

Bartles, J. R., Khoun, S., Lin, X., Zhang, L., Reddy, J. K., Rao, M. S., Issoye, S. T., Nehme, C. L., and Fayos, B. E. (1989) Cancer Res. 50, 669–676

Bartles, J. R., Zhang, L. Q., Verheyen, E. M., Hospedar, K. S., Nehme, C. L., and Fayos, B. E. (1991) Dev. Biol. 143, 256–270

Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159

Djuric, C., Ganning, A., and Dallner, G. (1986) Chem. Biol. Interactions 57, 255–270

Grollman, E. F., Saji, M., Shimura, Y., Lau, J. T. Y., and Ashwell, G. (1993) J. Biol. Chem. 268, 3604–3609

Hawkins, J. M., Jones, W. E., Bonner, F. W., and Gibson, G. G. (1987) Drug Metab. Rev. 18, 441–515

Hubbard, A. L., Bartles, J. R., and Braiterman, L. T. (1985) J. Cell Biol. 106, 1115–1125

Isemann, I., and Green, S. (1990) Nature 347, 645–650

Kliewer, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) Nature 358, 771–774

Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664

Lasky, L. A. (1992) Science 258, 964–969

Markwell, M., Haas, A. K., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 296–210

Nehme, C. L., Cesario, M. C., Myles, D. G., Koppel, D. E., and Bartles, J. R. (1993) J. Cell Biol. 120, 687–694

O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021

Paulson, J. C., Weinstein, J., and Schauer, A. (1989) J. Biol. Chem. 264, 10931–10934

Rao, M. S., and Reddy, J. K. (1987) Carcinogenesis 8, 631–636

Reddy, J. K., and Lahwani, N. D. (1983) CRC Crit. Rev. Toxicol. 12, 1–58

Reddy, J. K., and Rao, M. S. (1986) Trends Pharmacol. Sci. 7, 438–443

Roth, J., Taatjes, D. J., Luozuo, J. M., Weinstein, J., and Paulson, J. C. (1985) Cell 43, 297–305

Rutishauser, U., Acheson, A., Hall, A. K., Mann, D. M., and Sunshine, J. (1988) Science 240, 53–57

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A Laboratory Manual, 2nd Ed., Vols. 1–3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Shah, S., Lance, P., Smith, T. J., Berenson, C. S., Cohen, S. A., Horvath, P. J., Lau, J. T. Y., and Baumann, H. (1992) J. Biol. Chem. 267, 10652–10658

Shibuya, N., Goldstein, I. J., Broekaert, W. F., Nsimba-Luhaki, M., Peeters, B., and Peumans, W. J. (1987) J. Biol. Chem. 262, 1596–1601

The diagram shows immunofluorescent labeling of 2,6-ST on semithin frozen sections of liver. Rats were fed a control diet (a and b) or a diet containing ciprofibrate (c and d) for 10 days, their livers were fixed with paraformaldehyde/lysine/periodate by perfusion, and semithin frozen sections were prepared, labeled sequentially with affinity-purified anti-2,6-ST antibody and rhodaminated goat anti-rabbit IgG, and photographed in parallel under identical conditions. a and c, fluorescence; b and d, phase. S, examples of sinusoidal lumina. Magnification bar in d, 10 μm.
Regulation of Sialyltransferases by Peroxisome Proliferators

Stockel Hartree, A., and Renwick, A. G. C. (1992) Biochem. J. 287, 665–679
Svensson, E. C., Soreghan, B., and Paulson, J. C. (1990) J. Biol. Chem. 265, 20863–20868
Wang, W., and Cummings, R. D. (1986) J. Biol. Chem. 261, 4576–4585
Wang, X., O’Hanlon, T. P., and Lau, J. T. Y. (1989) J. Biol. Chem. 264, 1854–1860
Wang, X., Smith, T. J., and Lau, J. T. Y. (1990) J. Biol. Chem. 265, 17849–17853
Weinstein, J., de Souza-e-Silva, U., and Paulson, J. C. (1982b) J. Biol. Chem. 257, 13835–13844
Weinstein, J., de Souza-e-Silva, U., and Paulson, J. C. (1983b) J. Biol. Chem. 257, 13845–13853
Weinstein, J., Lee, E. U., McEntee, K., Lai, P.-H., and Paulson, J. C. (1987) J. Biol. Chem. 262, 17725–17733
Weiss, W., Brown, J. H., Cusack, S., Paulson, J. C., Skehel, J. J., and Wiley, D. C. (1998) Nature 333, 426–431
Wen, D. X., Livingston, B. D., Medzihradetzky, K. P., Kelm, S., Burlingame, A. L., and Paulson, J. C. (1992) J. Biol. Chem. 267, 21011–21029