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Control of Placental Alkaline Phosphatase Gene Expression in HeLa Cells: Induction of Synthesis by Prednisolone and Sodium Butyrate

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ABSTRACT: HeLa S_3 cells produce an alkaline phosphatase indistinguishable from the enzyme from human term placenta. The phosphatase activity in these cells was induced by both prednisolone and sodium butyrate. Both agents stimulated de novo synthesis of the enzyme. The increase in phosphatase activity paralleled the increase in immunoactivity and biosynthesis of placental alkaline phosphatase. The fully processed phosphatase monomer in control, prednisolone-treated or butyrate-treated cells was a 64.5K polypeptide, measured by both incorporation of L-[^{35}S]methionine into enzyme protein and active-site labeling. The 64.5K polypeptide was formed by the incorporation of additional *N*-acetylneuraminic acid moieties to a precursor polypeptide of 61.5K. However, this biosynthetic pathway was identified only in butyrate-treated cells. In prednisolone-treated cells, the processing of 61.5K to the 64.5K monomer was accelerated, and the presence of the 61.5K precursor could only be detected by either neuraminidase or monensin treatment. Phosphatase mRNA which comigrated with the term placental alkaline phosphatase mRNA of 2.7 kilobases was induced in the presence of either prednisolone or butyrate. Alkaline phosphatase mRNA in untreated HeLa S_3 cells migrated slightly faster than the term placental alkaline phosphatase mRNA. Butyrate also induced a second still faster migrating alkaline phosphatase mRNA. Both prednisolone and butyrate increased the steady-state levels of placental alkaline phosphatase mRNA. Our data indicate that the increase in phosphatase mRNA by prednisolone and butyrate resulted in the induction of alkaline phosphatase activity and biosynthesis in HeLa S_3 cells. Furthermore, both agents induced the expression of different alkaline phosphatase gene transcripts without altering its protein product.

Human term placental alkaline phosphatase (orthophosphoric-monoester phosphohydrolase; alkaline pH; EC 3.1.3.1) is a membrane-bound tissue-specific glycoprotein (Badger & Sussman, 1976; Mulivor et al., 1978a). In addition to the gene locus encoding term placental alkaline phosphatase, there exist at least two other gene loci encoding human alkaline phosphatases. They are the gene locus encoding adult intestinal alkaline phosphatase and the gene locus encoding tissue-unspecific alkaline phosphatase which is the major isozyme expressed in liver, kidney, and bone (Sussman et al., 1968; Gottlieb & Sussman, 1968; Mulivor et al., 1978a,b). A great deal of interest in the term placental alkaline phosphatase has stemmed from the finding that this enzyme was produced ectopically by a lung cancer (Fishman et al., 1968). It was subsequently found that term placental alkaline phosphatase or term placental alkaline phosphatase like enzyme was produced by a variety of trophoblastic and nontrophoblastic tumors and tumor-derived cell lines [for reviews, see Fishman and Stolbach (1979) and Stigbrand et al. (1982)]. Thus, an understanding of the mechanism which controls the expression of term placental alkaline phosphatase gene is likely to yield insight into the transformation process.

Alkaline phosphatase activity in cultured placental and nonplacental cells can be modulated by a variety of hormones and agents (Ghosh et al., 1972; Bulmer et al., 1976; Hamilton et al., 1979; Hanford et al., 1981; Mulkins et al., 1983; Ito

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& Chou, 1984; Ito et al., 1984). Expression of the term placental alkaline phosphatase gene in choriocarcinoma cells (malignant trophoblasts) has been extensively studied in our laboratory (Ito & Chou, 1983, 1984; Ito et al., 1984). We found that both sodium butyrate (Ito & Chou, 1984) and 5-bromo-2′-deoxyuridine (BrdUrd; Ito et al., 1984) induce de novo synthesis of this phosphatase in choriocarcinoma cells. Furthermore, this induction resulted from an increase in the term placental alkaline phosphatase mRNA activity. However, several groups have shown that in HeLa cells the increase of alkaline phosphatase activity by glucocorticoid hormone (Ghosh et al., 1972) or halogenated pyrimidines (Bulmer et al., 1976) is a consequence of altered kinetic properties of the enzyme and not due to new enzyme synthesis. Since HeLa cells synthesize an alkaline phosphatase indistinguishable from the term placental alkaline phosphatase, this suggests that regulation of alkaline phosphatase gene expression in HeLa cells differs from that in choriocarcinoma cells. Singer and Fishman (1975), however failed to detect an alkaline phosphatase with higher catalytic activity in HeLa cells treated with the glucocorticoid analogue prednisolone. Furthermore, Hanford et al. (1981) have demonstrated an increase in the amount of term placental alkaline phosphatase protein in HeLa cells following glucocorticoid treatment. These results, therefore, suggest that the term placental alkaline phosphatase gene in choriocarcinoma and HeLa cells is similarly regulated. In the present work, we have made a detailed study of term placental alkaline phosphatase gene expression and regulation in HeLa cells. We find that glucocorticoid hormone as well as sodium butyrate induce HeLa alkaline phosphatase biosynthesis and this induction results from an increase in the steady-state levels of term placental alkaline phosphatase mRNA.

Materials and Methods

Cell Culture. HeLa S_3 cells were grown at 37 °C in α-modified minimal essential medium supplemented with streptomycin, penicillin, and 4% fetal bovine serum. Cells in logarithmic growth phases were used in this study.

Enzyme Assay and Radioimmunoassay. Cells were harvested by scraping with a rubber policeman, and cell extracts in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4, containing 0.15 M NaCl were prepared as previously described (Ito & Chou, 1984). The cell extracts were used for measurement of alkaline phosphatase activity and immunoactivity.

Alkaline phosphatase activity was measured by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate at pH 10.7 and 37 °C (Ito & Chou, 1984). Protein was determined by the method of Lowry et al. (1951). By definition, 1 unit of enzyme releases 1 μmol of *p*-nitrophenol/min.

The immunoactivity of placental alkaline phosphatase was determined by double-antibody radioimmunoassay (Ito & Chou, 1984). A purified preparation of human term placental alkaline phosphatase was used as the standard and also radioiodinated for use as a radioligand. Sheep antiserum against rabbit γ-globulin was used as the precipitating antibody. The sensitivity of the assay was 0.1 ng.

Immunoprecipitation and Polyacrylamide Gel Electrophoresis. HeLa S_3 cultures in 25 cm^2 flasks were incubated for various intervals with L-[^{35}S]methionine (100 μCi/mL; Amersham Corp., Arlington Heights, IL) as described previously (Ito & Chou, 1983). Polypeptides immunoprecipitated by rabbit antiserum to human term placental alkaline phosphatase (anti-placental alkaline phosphatase precipitable polypeptides) in the cell lysates were isolated by the direct immunoprecipitation assay (Ito & Chou, 1983). The immunoprecipitates were washed as described by Roberts and Roberts (1975), heated for 5 min at 95 °C, and used for electrophoresis in a 10% polyacrylamide slab gel containing sodium dodecyl sulfate (SDS) (Laemmli, 1970). Radioactivity was visualized by fluorography (Bonner & Laskey, 1974). Apparent molecular weights were determined by using the following [^{14}C]methionine-labeled protein standards obtained from Amersham: myosin (200K), phosphorylase *b* (93K), bovine serum albumin (69K), ovalbumin (46K), and carbonic anhydrase (30K).

[^{32}*P*]*Phosphate Binding.* Alkaline phosphatases were labeled by incubation with carrier-free $H_3{}^{32}PO_4$ (New England Nuclear, Boston, MA) at pH 5.0 following the technique of Milstein (1964). The ^{32}P-labeled phosphatases were precipitated with acidified acetone, washed successively with acidified acetone and ether, and dried. The dried pellets were dissolved in 1 mL of phosphate-buffered saline (PBS) containing 1% Triton X-100, 0.1% SDS, and 0.5% sodium deoxycholate. ^{32}P-Labeled alkaline phsophatases were isolated by immunoprecipitation.

Neuraminidase Digestion. Cultures labeled with L-[^{35}S]-methionine (100 μCi/mL) for 180 min were harvested by scraping with a rubber policeman. The cell pellet was resuspended in 1 mL of 50 mM sodium acetate buffer (pH 5.2) containing 0.5% Triton X-100, 500 μg of trypsin inhibitor, 2 μg of leupeptin, and 0.5 mM L-1-(tosylamido)-2-phenylethyl chloromethyl ketone, and the cells were ruptured by sonication. The sonicates were centrifuged at 10000*g* for 15 min; the supernatant solutions of the cell extracts were used for neuraminidase treatment.

The cell extract (0.5 mL) was incubated at 37 °C for 16 h in the presence or absence of neuraminidase from *Clostridium perfringens* (0.5 unit; Worthington Biochemical Co., Freehold, NJ), and the reaction was terminated by the addition of 1 volume of 2 times concentrated lysis solution (2× PBS containing 2% Triton X-100, 1% sodium deoxycholate, and 0.2% SDS). Anti-term placental alkaline phosphatase precipitable polypeptides in the cell extracts were isolated by immunoprecipitation.

Isolation of cDNA Clones for Placental Alkaline Phosphatase. The human placental cDNA library constructed in phage λgt11 was kindly provided by Dr. Frank Gonzales (National Institutes of Health). The cDNA library was screened with a polyclonal antibody against human term placental alkaline phosphatase by method of Young and Davis (1983a,b). The inserted cDNA of the positive clones was digested with *Eco*RI and was subcloned into pUC 18 vector (Yanisch et al., 1985). Plasmid DNA was prepared from transformed HB101 cells.

Restriction endonuclease mapping and partial DNA sequencing indicate that the placental alkaline phosphatase cDNA clone TPAP8E [approximately 2.1 kilobase (kb)] contains sequences spanning from the C-terminal to 150 base pairs (bp) upstream of the *Sac*I site of cDNA clone AP27, reported by Kam et al. (1985).

RNA Electrophoresis, Blotting, and Hybridization. Total cell RNA was extracted by the guanidinium thiocyanate method of Chirgwin et al. (1979). RNA in the cell lysates was isolated by sedimentation through cesium chloride. Polyadenylated RNA was obtained by subjecting the total RNA to oligo(dT)–cellulose affinity chromatography (Aviv & Leder, 1972).

RNA samples were dissolved in MOPS buffer [0.2 M 3-(*N*-morpholino)propanesulfonic acid, pH 7.0, 50 mM sodium

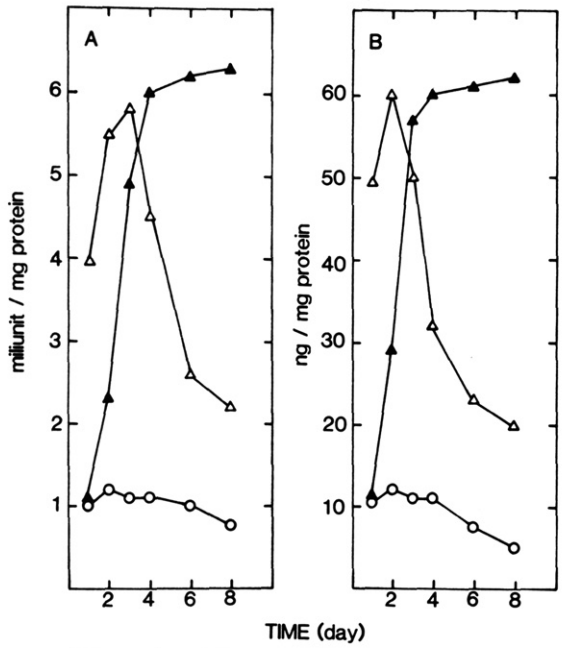

FIGURE 1: Effects of prednisolone and sodium butyrate on alkaline phosphatase activity and immunoactivity. HeLa S_3 cells were grown in the absence or presence of prednisolone (10^{-6} M) or butyrate (3 mM), and the corresponding medium was changed every 2 days. (A) Phosphatase activity. (B) Phosphatase immunoactivity measured by radioimmunoassay. (○) Control; (△) prednisolone; and (▲) sodium butyrate.

acetate, and 1 mM ethylenediaminotetraacetic acid (EDTA), pH 8.0] containing 50% formamide and 2.2 M formaldehyde, heated for 15 min at 60 °C, and electrophoresed on a 1.2% agarose gel in 2.2 M formaldehyde (Lehrach et al., 1977) with MOPS buffer as the circulating electrode solution.

Denatured RNAs were transferred from agarose gels to Zetabind (AMF Cuno, Microfiltration Production Division, Meriden, CT) using the Electro-Blot procedures. Hybridization in the presence of dextran sulfate and washing were performed as described by Wahl et al. (1979) with the following modifications: blots were washed twiced in 2 times concentrated 0.15 M sodium chloride–0.015 M sodium citrate (SSC), pH 7.2, and 0.5% SDS for 30 min each at room temperature, twice in one-tenth concentrated SSC and 0.1% SDS for 30 min each at room temperature, and then 4 times in one-tenth concentrated SSC and 0.1% SDS for 60 min each at 65 °C.

Results

Stimulation of Alkaline Phosphatase Activity and Biosynthesis by Prednisolone and Sodium Butyrate. It has been demonstrated that alkaline phosphatase activity in HeLa cells can be increased by glucocorticoid hormone (Ghosh et al., 1972; Hanford et al., 1981) and sodium butyrate (Griffin et al., 1974; Chou, 1979). We found that the increase in enzyme activity paralleled the increase in immunoreactive enzyme protein (Figure 1). Alkaline phosphatase of HeLa S_3 cells resembled the phosphatase in human term placenta in physicochemical properties. HeLa and term placental alkaline phosphatases were similarly inhibited by L-phenylalanine, L-homoarginine, L-leucine, EDTA, and heat (data not shown). Neither prednisolone nor butyrate altered the physicochemical properties of the HeLa enzyme. Furthermore, alkaline phosphatase produced by HeLa S_3 cells was immunologically indistinguishable from the term placental alkaline phosphatase. The slopes of immunotitration curves for control, prednisolone-treated, or butyrate-treated HeLa alkaline phosphatase

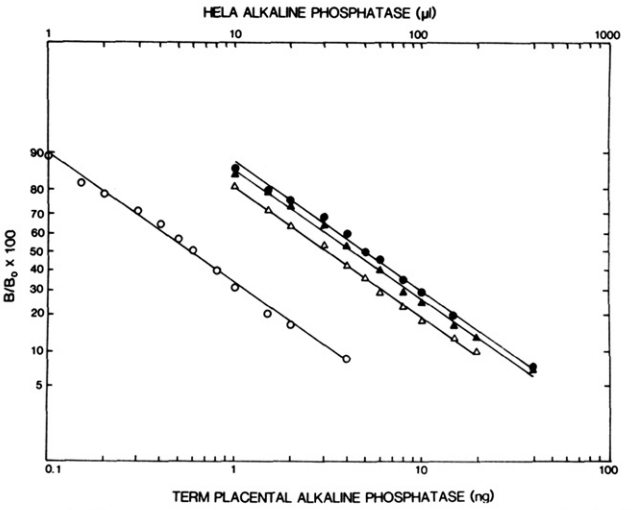

FIGURE 2: Immunotitration curves of alkaline phosphatase synthesized by HeLa S_3 cells in the absence or presence of prednisolone or butyrate: (○) term placenta; (●) HeLa S_3, control; (△) HeLa S_3, prednisolone; (▲) HeLa S_3, butyrate.

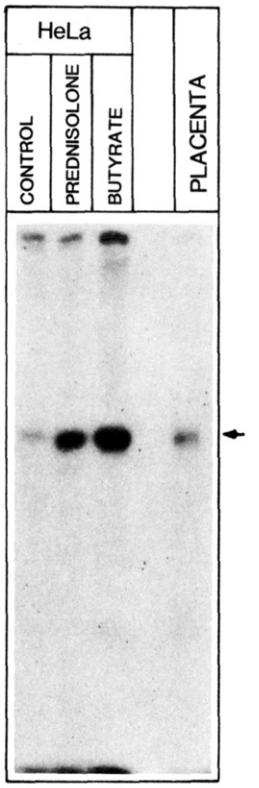

FIGURE 3: [^{32}P]Phosphate labeling of alkaline phosphatases. Binding of [^{32}P]phosphate to human term placental alkaline phosphatase and control, prednisolone-treated, or sodium butyrate treated HeLa alkaline phosphatase was carried out as described under Materials and Methods. ^{32}P-Labeled phosphatases were analyzed by SDS gel electrophoresis and autoradiography. Arrow indicates anti-placental alkaline phosphatase precipitable polypeptides. For HeLa cells, phosphatases from 10^7 cells were applied to each gel.

paralleled those of term placental alkaline phosphatase (Figure 2).

[^{32}P]Phosphate has been shown to bind tightly to the active site of alkaline phosphatase (Milstein, 1964). HeLa alkaline phosphatase in cells grown in the absence or presence of prednisolone or butyrate was labeled with [^{32}P]phosphate and electrophoresed in a polyacrylamide gel containing SDS (Figure 3). HeLa alkaline phosphatase bound phosphate, as did term placental alkaline phosphatase. Furthermore, HeLa phosphatase was composed of just one subunit, which comi-

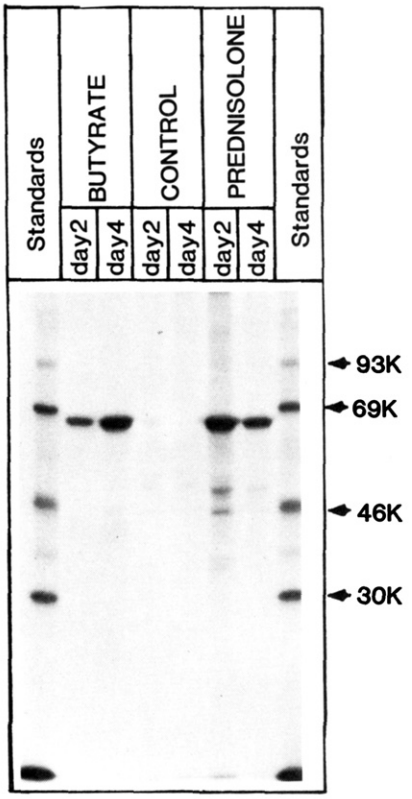

FIGURE 4: Increase of alkaline phosphatase biosynthesis by prednisolone and sodium butyrate. HeLa S_3 cells were grown in the absence or presence of prednisolone (10^{-6} M) or sodium butyrate (3 mM), and the corresponding medium was changed every 2 days. At indicated times, cells were labeled with L-[^{35}S]methionine for 3 h, and anti-placental alkaline phosphatase precipitable polypeptides in the cells lysates were analyzed by SDS gel electrophoresis and fluorography. Polypeptides from 4×10^6 cells were applied to each gel.

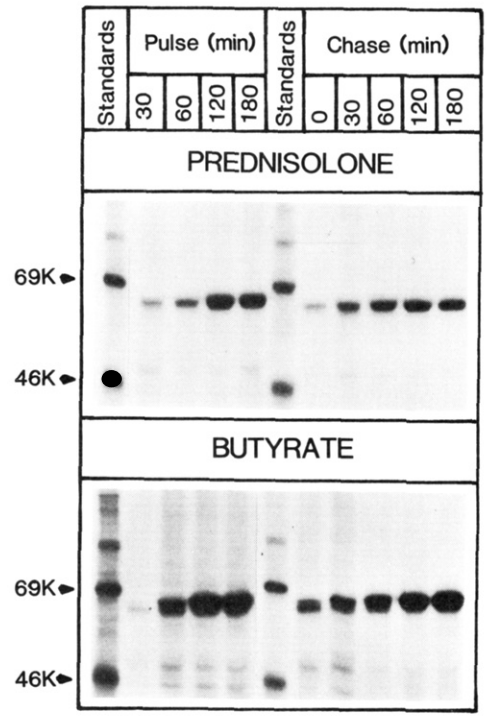

FIGURE 5: Pulse and pulse–chase labeling of alkaline phosphatase in the presence of prednisolone or sodium butyrate. HeLa S_3 cells were grown in the presence of prednisolone (10^{-6} M) or sodium butyrate (3 mM) for 4 days, and the medium was changed every 2 days. Pulse experiment: Cells were labeled with L-[^{35}S]methionine for various intervals as indicated. Chase experiment: Cells were labeled with L-[^{35}S]methionine for 30 min and then subjected to chase with fresh medium for various intervals. Anti-placental alkaline phosphatase precipitable polypeptides were analyzed by SDS gel electrophoresis and fluorography. Polypeptides from 4×10^6 cells were applied to each gel.

grated with the subunit of term placental alkaline phosphatase. The apparent molecular weight of the phosphatase subunit was 64.5K (Figure 3). Both prednisolone and butyrate increased the catalytic activity of this enzyme without altering its electrophoretic mobility.

Labeling experiments were performed to confirm that the observed increases in phosphatase activity and immunoreactive protein resulted from increase in placental alkaline phosphatase biosynthesis. HeLa S_3 cells grown in the absence or presence of inducers were labeled with L-[^{35}S]methionine, and cell lysates were examined for anti-placental alkaline phosphatase precipitable polypeptides. In the absence of inducers, HeLa cells synthesized low levels of a phosphatase immunoprecipitated by antiserum to term placental alkaline phosphatase (Figure 4). The amount of newly synthesized enzyme was greatly increased in the presence of either prednisolone or butyrate. The apparent molecular weight of the fully processed, newly synthesized HeLa alkaline phosphatase either in the presence or in the absence of inducers was also 64.5K. Neither prednisolone nor butyrate increased the incorportion of L-[^{35}S]methionine into total trichloroacetic acid precipitable materials (data not shown).

Biosynthesis and Processing of Alkaline Phosphatase in HeLa Cells. Although HeLa S_3 cells synthesize an alkaline phosphatase indistinguishable from the enzyme in human term placenta, it was not possible to study its biosynthetic pathway because of the low levels of phosphatase in HeLa cells. However, biosynthesis and processing of alkaline phosphatase in HeLa cells could be examined in the presence of either prednisolone or butyrate. Pulse and pulse–chase labeling of prednisolone-treated HeLa cells demonstrated that a polypeptide of 64.5K was the only anti-placental alkaline phosphatase precipitable product synthesized (Figure 5). The continued increase in radioactivity incorporated into this enzyme in the absence of added precursor in the pulse–chase experiment suggests that the pool size of methionine in HeLa cells is rather large.

Pulse labeling of butyrate-treated HeLa cells, however, demonstrated that a polypeptide of 61.5K was synthesized first (Figure 5). An additional polypeptide of 64.5K appeared after pulses of 60 min or longer. The precursor–product relationship of these two polypeptides was demonstrated by pulse–chase experiments (Figure 5): the radioactivity in the 61.5K polypeptide could be chased into the 64.5K polypeptide.

Characterization of Placental Alkaline Phosphatase in HeLa S_3 Cells. The fully processed alkaline phosphatase monomer in either prednisolone- or butyrate-treated HeLa cells was a 64.5K polypeptide. Furthermore, the unglycosylated HeLa alkaline phosphatase monomer, synthesized in the presence of the protein glycosylation inhibitor tunicamycin (Struck & Lennarz, 1977), had the same apparent molecular weight (58K) in the presence of either butyrate (Figure 6) or prednisolone (data not shown). Thus, the failure to detect the 61.5K precursor polypeptide in prednisolone-treated cells may indicate that processing of alkaline phosphatase in the presence of prednisolone was accelerated. To examine whether the biosynthetic pathways of placental alkaline phosphatase in the presence of either prednisolone or butyrate were the same, we tested the sensitivity of the 64.5K polypeptide synthesized in the presence of either agent toward neuraminidase digestion (Figure 6). In both cases, the 64.5K polypeptide was sensitive to neuraminidase digestion. In the presence of neuraminidase,

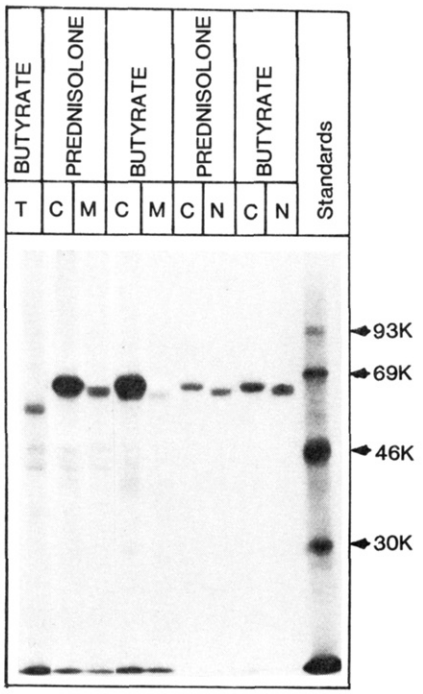

FIGURE 6: Effects of neuraminidase, monensin, and tunicamycin on HeLa alkaline phospahtase processing. HeLa S_3 cells were grown in the presence of prednisolone (10^{-6} M) or sodium butyrate (3 mM), and the corresponding medium was changed every 2 days. Neuraminidase (N) treatment: Cells were treated with prednisolone or butyrate for 4 days and were labeled with L-[^{35}S]methionine for 3 h on day 4. Cell extracts were treated with neuraminidase as described under Materials and Methods. Monensin (M) treatment: Cells were treated with prednisolone or butyrate for 3 days, and monensin (1 mM) was added on day 3. Cultures were incubated for an additional 24 h in the presence of prednisolone plus monensin or butyrate plus monensin and were labeled with L-[^{35}S]methionine for 3 h on day 4. Tunicamycin (T) treatment: cells were treated with sodium butyrate for 4 days and were labeled with L-[^{35}S]methionine for 3 h in the presence of tunicamycin (2 μg/mL) and butyrate. Before being labeled cells were preincubated with tunicamycin and butyrate for 4 h. Anti-placental alkaline phosphatase precipitable polypeptides were analyzed by SDS gel electrophoresis and fluorography.

the 64.5K polypeptide disappeared while a 61.5K polypeptide which comigrated with the precursor form of placental alkaline phosphatase monomer appeared. This indicates that the 64.5K polypeptide in both prednisolone- and butyrate-treated HeLa cells contained additional *N*-acetylneuraminic acid moieties, as was demonstrated in choriocarcinoma alkaline phosphatase (Ito & Chou, 1984).

To further demonstrate that prednisolone changed the rate of alkaline phosphatase processing without altering its biosynthetic pathway, HeLa S_3 cells grown in the presence of either prednisolone or butyrate were treated with monensin for 24 h before being labeled with L-[^{35}S]methionine (Figure 6). In the presence of monensin, the only anti-placental alkaline phosphatase precipitable product in either prednisolone- or butyrate-treated cells was the 61.5K precursor polypeptide.

Effects of Prednisolone and Sodium Butyrate on Placental Alkaline Phosphatase mRNA Levels. Polyadenylated RNA preparations isolated from control, prednisolone-treated, and butyrate-treated cells were electrophoresed and hybridized with a human term placental alkaline phosphatase cDNA fragment, TPAP8E. This cDNA fragment was isolated by screening a phage λgt11 expression library with antiserum to human term placental alkaline phosphatase. Results of the Northern blot hybridization are shown in Figure 7. In agreement with Kam et al. (1985), alkaline phosphatase mRNA of human term placenta migrated as a 2.7-kb band. The alkaline phosphatase

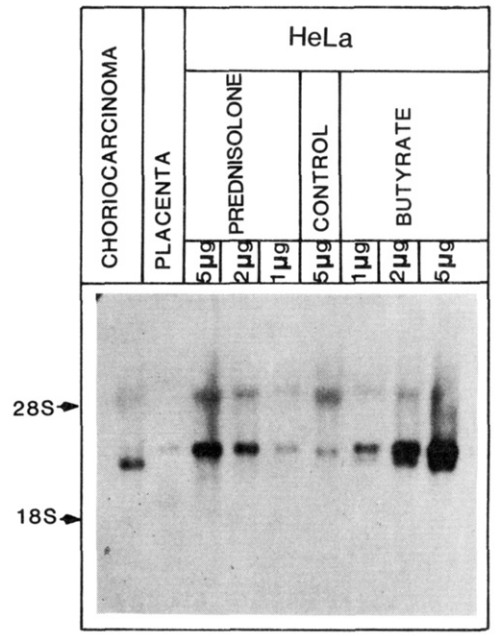

FIGURE 7: Northern blot hybridization of polyadenylated RNAs with placental alkaline phosphatase cDNA. Polyadenylated RNAs isolated from JEG-3 choriocarcinoma cells, human term placenta, and control, prednisolone-treated, or sodium butyrate treated HeLa S_3 cells were separated by electrophoresis on 1.2% agarose gels containing formaldehyde and transferred to a Zetabind membrane as described under Materials and Methods. RNA was hybridized to the ^{32}P-labeled phosphatase cDNA fragment TPAP8E.

mRNA in untreated HeLa S_3 cells migrated slightly faster than the phosphatase mRNA of human term placenta but slower than the messenger in choriocarcinoma cells (Figure 7). Prednisolone increased the steady-state levels of alkaline phosphatase mRNA in HeLa cells approximately 5-fold, and this mRNA comigrated with the phosphatase mRNA of human term placenta. Sodium butyrate also increased the steady-state levels of alkaline phosphatase mRNA, approximately 10-fold. In butyrate-treated HeLa cells, there were two mRNAs for alkaline phosphatase; the major one comigrated with the term placental alkaline phosphatase mRNA; the minor one migrated faster than the mRNA in control HeLa cells but slower than the mRNA in choriocarcinoma cells (Figure 7).

Discussion

The alkaline phosphatase from human term placenta is an oncotrophoblastic protein which reappears in adult tissue only under certain pathological conditions. The synthesis of this enzyme by the cervical carcinoma cell line HeLa has stimulated interest in examining mechanisms controlling the expression of term placental alkaline phosphatase in tumor cells. Alkaline phosphatase activity in HeLa cells can be modulated by various hormones and agents, such as glucocorticoid hormone (Ghosh et al., 1972; Hanford et al., 1981), sodium butyrate (Griffin et al., 1974; Chou, 1979), halogenated pyrimidines (Bulmer et al., 1976), and methotrexate (Speeg et al., 1976). In addition, the activity is affected by modification of the cellulr microenvironment (Nitowsky et al., 1963). Stimulation of enzyme activity may result in the following ways: first, an increase in the amount of enzyme protein; second, an increase in the catalytic activity of the enzyme by alteration of the existing enzyme molecules; and third, an increase in the amount of enzyme protein and its catalytic activity. The results presented in this study demonstrated that prednisolone and sodium butyrate increased placental alkaline

phosphatase activity by inducing de novo synthesis of this enzyme. Neither prednisolone nor butyrate altered the physicochemical or immunological properties of HeLa alkaline phosphatase. The increase in placental alkaline phosphatase protein paralleld the increase in enzyme activity. In addition, we have demonstrated that induction of phosphatase biosynthesis resulted from an increase in the steady-state levels of mRNA for term placental alkaline phosphatase. The increase in alkaline phosphatase mRNA levels paralleled the increase in enzyme biosynthesis and activity.

The strong correlation in the increase in alkaline phosphatase mRNA levels, its biosynthesis and activity, established the order of events in alkaline phosphatase induction in HeLa cells by prednisolone and butyrate. However, at least three forms of alkaline phosphatase mRNA were identified in HeLa cells. These and the fastest migrating phosphatase mRNA found in choriocarcinoma cells [see Figure 7 and Ovitt et al. (1986)] demonstrated the polymorphism of alkaline phosphatase at the messenger RNA level. It was well established that human term placental alkaline phosphatase is a polymorphic enzyme (Boyer, 1961; Robson & Harris, 1965; Beckman et al., 1966). Despite the polymorphic nature of alkaline phosphatase mRNA and the enzyme, we have been unable to detect polymorphism among the synthesized alkaline phosphatases. In HeLa cells, the 64.5K polypeptide was the only phosphatase monomer synthesized by control, prednisolone-treated, or butyrate-treated cells. A similar polypeptide was the phosphatase monomer synthesized by choriocarcinoma cells in the absence (Ito & Chou, 1983) or presence of butyrate (Ito & Chou, 1984) or BrdUrd (Ito et al., 1984). Analysis of the mRNA and genomic structure of placental alkaline phosphatase should yield information as to its polymorphism.

We have previously demonstrated that in choriocarcinoma cells, both butyrate and BrdUrd induce term placental alkaline phosphatase biosynthesis. The induction was due to an increase in term placental alkaline phosphatase mRNA activity. Our results in HeLa and choriocarcinoma cells suggest that regulation of term placental alkaline phosphatase gene expression in both types of tumor cells is similar. However, choriocarcinoma phosphatase activity was not affected by glucocorticoid hormone (Speeg et al., 1979). Thus, a direct comparison for glucocorticoid-mediated induction between these two type cells is not possible.

Although both glucocorticoid and butyrate induce the biosynthesis of HeLa alkaline phosphatase, the mode of induction appears to be different. In the presence of glucocorticoid hormone, the 61.5K precursor polypeptide can be detected only either by blocking alkaline phosphatase processing with monensin or by neuraminidase treatment. On the other hand, the 61.5K polypeptide was easily detectable in the butyrate-treated cells. Tokumitsu (1984) has suggested that the induction mechanisms for HeLa alkaline phosphatase by prednisolone and butyrate were different. This author demonstrated that placental alkaline phosphatase induced with prednisolone was mainly localized in the well-developed Golgi apparatus and the plasma membrane, with very little of the enzyme occurring in the endoplasmic reticulum. On the other hand, in butyrate-treated cells, induced placental alkaline phosphatase was localized in Golgi apparatus, plasma membrane, and endoplasmic reticulum. Tokumitsu further demonstrated that alkaline phosphatase became detectable in the endoplasmic reticulum of glucocorticoid-induced cells following monensin treatment. Our results agree with the findings of Tokumitsu. The failure to detect the 61.5K precursor polypeptide in prednisolone-treated HeLa cells in the present study suggests that the transport of placental alkaline phosphatase was rapid in the presence of glucocorticoid. The 61.5K polypeptide after synthesis was immediately processed to the 64.5K monomer.

Registry No. $PrCO_2H$, 107-92-6; alkaline phosphatase, 9001-78-9; prednisolone, 50-24-8.

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Oligomerization and Ring Closure of Immunoglobulin E Class Antibodies by Divalent Haptens†

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ABSTRACT: Cross-linking of antibodies constitutes a widespread initiation signal for their respective effector functions. Cross-linking IgE-class antibodies provide the triggering signal to mast cells for their degranulation process. To obtain a quantitative insight into these cross-linking processes, the interactions between a DNP-specific monoclonal antibody of the IgE class and a series of divalent DNP haptens with spacers of different length and flexibility have been studied by fluorescence titration experiments. These were analyzed by employing the theoretical model developed by Dembo and Goldstein [Dembo, M., & Goldstein, B. (1978) *J. Immunol. 121*, 345-353] in a fitting procedure. Equilibrium constants that describe the aggregation and ring-closure processes caused by divalent hapten binding have been used as free parameters. The intrinsic binding constants were determined by fluorescence titrations with corresponding monovalent haptens. The main results are the following: (1) The divalent haptens with a short and flexible spacer [i.e., N^{α},N^{ϵ}-di-(DNP)-L-lysine, *meso*-bis[(DNP-β-Ala)amino]succinate, and bis[(DNP-tri-D-Ala)amino]heptane, having a maximal DNP–DNP distance of Γ = 14, 21, and 45 Å, respectively] effect aggregation of the antibodies mainly into closed dimers. (2) The divalent hapten family with long and rigid oligoproline spacers di-(DNP)-Ahx-Asp-$(Pro)_n$-Lys with n = 24, 27, and 33 (i.e., Γ = 100, 110, and 130 Å) causes aggregation of the antibodies predominantly into closed dimers and trimers. The corresponding equilibrium constants of the respective ring-closure processes decrease significantly with longer spacer length. (3) Evidence was found that intramolecularly monomeric ring closure of the IgE antibodies is caused by haptens containing oligoproline spacers with n = 37 or 42 (Γ = 130–150 Å). The equilibrium constant of the ring-closure process increases with spacer length. This increase in stability indicates a difference in the imposed strain. Furthermore, the latter results imply that the distance between the two binding sites of the IgE molecule lies in the range dictated by the rigid oligoproline part of the respective hapten's spacer, i.e., 115–130 Å. (4) Nearly all oligomeric ring-closure processes proceed relatively slowly with an approximate lower limit of a half-life of 5–10 s. This slowing down of the aggregation and ring-closure processes most probably reflects steric factors.

Antibodies of the IgE[1] class are responsible for the immunological pathway leading to degranulation of mast cells and basophils, thus initiating reactions of the immediate type of hypersensitivity. The first step of this process involves antigen-induced aggregation of cell-bound IgE antibodies (Ishizaka & Ishizaka, 1969; Segal et al., 1977; Kulczycki et al., 1974; Schlessinger et al., 1976; Menon et al., 1985, 1986a,b). One of the earliest ensuing events which this aggregation effects is the opening of Ca^{2+} channels, raising transiently the cytosolic free Ca^{2+} concentration (Foreman et al., 1977; Mazurek et al., 1983; Corcia et al., 1986). Further steps in this biochemical cascade lead to the eventual degranulation and release of the stored, preformed mediators such as histamine and serotonin and synthesis of new mediators, derivatives of arachidonic acid (Schwartz & Austen, 1984).

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[1] Abbreviations: DNP, 2,4-dinitrophenyl; IgE, immunoglobulin E; IgG, immunoglobulin G; Lys, L-lysine; Gly, glycine; Ahx, 6-aminohexanoate; But, γ-aminobutyric acid; Asp, L-aspartate; $(Pro)_n$, polypeptide containing n L-proline(s); RMS, root mean square.

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