Effect of Chlorate on the Sulfation of Lipoprotein Lipase and Heparan Sulfate Proteoglycans

SULFATION OF HEPARAN SULFATE PROTEOGLYCANS AFFECTS LIPOPROTEIN LIPASE DEGRADATION

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In plasma, the major enzyme responsible for the hydrolysis of VLDL and chylomicron triglycerides to free fatty acids is lipoprotein lipase (LPL). In recent years it has become evident that posttranslational events may be important in the regulation of LPL. In studies utilizing 3T3-L1 adipocytes (1), it was concluded that regulation of LPL by insulin occurred mainly at posttranscriptional and posttranslational levels. Others (2) similarly report that in response to fasting and feeding, the regulation of LPL occurs mainly at the posttranslational level.

One potential posttranslational mechanism in the regulation of LPL is a modulation of the degradation rate of LPL with little or no change in the synthetic rate. Three laboratories have shown, independently, that in isolated cultured adipocytes, 70-80% of the newly synthesized enzyme is degraded before appearing in the culture medium, and that addition of heparin to the medium reduces the amount of LPL degraded (3-5). Furthermore, Cisar and co-workers (6) have shown that degradation of LPL is dramatically reduced when the adipocyte cell surface heparan sulfate proteoglycans (HSPG) are removed by endoglycosidases. Therefore, modulation of the interaction of LPL with HSPG on the adipocyte plasma membrane may have dramatic effects on the degradation of LPL and may determine the efflux of LPL from cells. To test this possibility, we have decreased the LPL binding capacity of the plasma membrane by decreasing the sulfation density of glycosaminoglycans. Sodium chlorate, a potent inhibitor of sulfate adenylytransferase, reduces protein and carbohydrate sulfation (7). Greve and co-workers (8) concluded that treatment of human fibroblasts with chlorate-reduced proteoglycan sulfation but did not affect proteoglycan chain polymerization. This paper reports the effects of chlorate on the sulfation of HSPG and LPL, and the effects of altered HSPG sulfation on LPL turnover in adipocytes.

EXPERIMENTAL PROCEDURES

RESULTS

Effect of Chlorate on the Sulfation of 35SO4 into HSPG—To determine whether chlorate treatment affected the sulfation of HSPG, 35S04 incorporation into HSPG was measured. In the presence of chlorate, the incorporation of 35S04 into HSPG was increased by 20% in control cells and by 70% in chlorate-treated cells. The increase in 35S04 incorporation into HSPG was accompanied by a decrease in the binding of LPL to HSPG, as measured by enzyme-linked immunosorbent assay (ELISA). These results suggest that chlorate treatment does not alter the binding of LPL to HSPG but does affect the sulfation of HSPG.

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EXPERIMENTAL PROCEDURES

REFERENCES

1. The abbreviations used are: VLDL, very low density lipoprotein; LPL, lipoprotein lipase; HSPG, heparan sulfate proteoglycan; HEPES, N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; CHAPS, (3-[3-cholamidopropyl]dimethylammonio)1-propanesulfonate; SDS, sodium dodecyl sulfate; PAPS, 3'-phosphoadenylylphosphosulfate; LSC, liquid scintillation counting; GcNaC, N-acetylgalactosamine.

2. "Experimental Procedures" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
the incorporation of $^{35}$SO$_4$ into HSPG of cultured adipocytes, cells were pretreated in the presence and absence of 10 mM sodium chlorate, and then incubated with Na$_2$[${}^{35}$SO$_4$] for 48 h in the presence and absence of 10 mM chlorate. At this concentration of chlorate, no change in the amount of protein or DNA/dish was observed. The incorporation of $^{35}$SO$_4$ into heparan sulfate and HSPG was measured by sensitivity to low pH nitrous acid degradation. Table I shows that the incorporation of $^{35}$SO$_4$ into HSPG and heparan sulfate was decreased in all fractions collected. A dramatic decrease to 28 and 20% of control levels was observed in the medium and trypsin-releasable fractions, respectively.

In order to determine if the decreased $^{35}$SO$_4$ incorporation into HSPG was caused by a decrease in the mass or a decrease in the sulfation of HSPG, the $^{35}$SO$_4$ radioactivity and the mass of uronic acid were determined for the glycosaminoglycans in the trypsin-releasable pool. Glycosaminoglycans were isolated by gel permeation chromatography after digestion of the trypsin-releasable fraction with thermolysin. The amount of $^{35}$SO$_4$/mass of uronic acid in heparan sulfate and in chondroitin sulfate/dermatan sulfate was reduced to the trypsin-releasable fraction with thermolysin. The amount of uronic acid in the trypsin-releasable HSPG was not different in control and chlorate-treated cells (0.89 ± 0.37 and 0.87 ± 0.19 μg/5 dishes, respectively). This indicates that the primary effect of chlorate was to decrease proteoglycan sulfation, rather than to decrease the mass. The effect of chlorate on the incorporation of $^{35}$SO$_4$ into glycosaminoglycans was more pronounced for chondroitin sulfate/dermatan sulfate than for heparan sulfate. To determine if chlorate altered the average length of heparan sulfate chains in control and chlorate-treated cells, the trypsin-releasable fraction was digested with Pronase, and the glycosaminoglycans were precipitated. Heparan sulfate was obtained by digestion of glycosaminoglycans with chondroitinase ABC followed by incubation with alkaline borohydride. The chains were sized on a calibrated Sephacryl S-300 column. The results show that chlorate treatment increased the median size of heparan sulfate chains from 61,790 ± 1,710 in control cells to 90,100 ± 260 in the chlorate-treated cells.

**TABLE I**

| Heparan sulfate proteoglycan        | Medium           | Trypsin-releasable | Intracellular |
|-------------------------------------|------------------|-------------------|---------------|
| Control                             | 78,900 ± 19,000  | 34,000 ± 3,400    | 33,300 ± 8,400 |
| Chlorate                            | 21,700 ± 3,800   | 6,900 ± 2,200     | 19,700 ± 7,500 |

* $p < 0.05$  
$^b$ $p < 0.002$

**TABLE II**

Effect of chlorate on the degree of sulfation of glycosaminoglycans in chicken adipocytes

Adipocytes were incubated with Na$_2$[${}^{35}$SO$_4$] and 0 or 20 mM sodium chlorate for 48 h after an overnight pretreatment with or without sodium chlorate. The intracellular (IC), trypsin-releasable (TR), and media (M) pools were collected. The TR pool was successively dialyzed against 10 mM Na$_2$SO$_4$ and water, was digested with thermolysin or Pronase, and the products of digestion were separated by gel permeation chromatography. The percentage of heparan sulfate (HS) or chondroitin sulfate and dermatan sulfate (CS/DS) labeled with $^{35}$SO$_4$ was determined by digesting the glycosaminoglycans with heparitinase or chondroitinase ABC, respectively. After digestion, the digestion products were separated by gel permeation chromatography. The material resistant to digestion by chondroitinase ABC was used to measure the mass and radioactivity of HS, while the low molecular weight products of the chondroitinase ABC digestion were used for the analysis of CS/DS. Uronic acid was measured using glucuronic acid as a standard. Values are means ± S.D. for three pools of 5-60-mm dishes or from one pool for the determination of the percent of HS in the total glycosaminoglycans.

|                      | Control          | Chlorate         |
|----------------------|------------------|------------------|
| Total radioactivity in glycosaminoglycans (cpm/6 dishes) | 2,303,400 ± 744,600 | 412,100 ± 199,000$^*$ |
| % of $^{35}$SO$_4$ in specified glycosaminoglycan |                  |                  |
| HS                   | 35%             | 71%             |
| CS/DS                | 69 ± 2%         | 22 ± 1%$^*$     |
| Radioactivity/mass (cpm/μg uronic acid) |                  |                  |
| HS                   | 524,600 ± 79,600 | 239,700 ± 129,000$^*$ |
| CS/DS                | 836,000 ± 180,000 | 46,000 ± 16,800$^*$ |

$^*$ $p < 0.05$.  
$^b$ $p < 0.001$. 

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levels in the intracellular and cell surface compartments were effects of 20 mM chlorate and 10 unit/ml heparin on LPL measured. Chlorate was compared to heparin, a glycosaminoglycan which exerts its action on LPL secretion by decreasing the interaction of LPL with the cell surface, and thereby decreases LPL degradation. The effect of heparin was consistent with the earlier observation that heparin decreases the LPL degradation rate (3, 6). With heparin, LPL in the medium was increased 6-fold. LPL levels on the cell surface and in the cell were decreased; this decrease in the cell-associated pool size is consistent with a lower amount of LPL binding to cell surface HSPG and decreased LPL internalization. Total amounts of LPL recovered in all three compartments were increased 2.4-fold. The effect of chlorate treatment on LPL levels was similar to heparin, although the effect on LPL secretion was not as dramatic. LPL secretion was increased 3.3-fold, and intracellular and cell surface-associated LPL levels were decreased to 85 and 62% of control levels, respectively. Total amounts of LPL recovered in all three compart-

Fig. 1. Molecular weight distribution of $^{35}$SO₄ radioactivity associated with heparan sulfate chains from control (A) and chlorate-treated (B) cultures. Total glycosaminoglycans were isolated from the trypsin-releasable fraction of cells and digested with chondroitinase ABC and alkaline borohydride as described under "Experimental Procedures." The molecular weight distribution (C) and the integral distribution curve (D) were determined by gel chromatography on a calibrated column of Sephacryl S-300. Each value represents the mean ± S.D. for three separate molecular weight determinations. Where error bars cannot be seen, the S.D. is smaller than the symbol.

$\pm 0.20 \mu g$ of LPL/60-mm dish (mean ± S.D., $p = 0.0011$). The association constant of LPL binding was not significantly different between control and chlorate-treated cultures ($1.83 \times 10^{-9} M \pm 0.70 \times 10^{-9} M$ and $2.67 \times 10^{-9} M \pm 1.69 \times 10^{-9} M$, respectively). Thus, chlorate treatment of adipocytes resulted in a decreased maximum binding of LPL to the adipocyte cell surface.

Effect of Chlorate on LPL in Cultured Adipocytes—Since chlorate altered the degree of sulfation of HSPG and reduced the binding of $^{125}$I-LPL to the cell surface, it was important to assess how these changes affected the levels of LPL in adipocytes. The secretion of LPL in adipocytes was measured by pretreating cells with increasing concentrations of chlorate and then incubating the cells with the indicated chlorate concentrations for 5 h. LPL was measured in the medium by ELISA. Fig. 3 shows data from one such experiment. Incubation of cells with graded levels of chlorate resulted in a dose-dependent increase in LPL secretion. At 20 mM chlorate concentration, a 3.9-fold increase in LPL secretion was observed. Total cellular protein did not change as a result of chlorate treatment at concentrations up to 50 mM chlorate, and increased LPL secretion was observed at concentrations up to 30 mM chlorate (data not shown). All further experiments utilized either 10 or 20 mM chlorate, since these concentrations consistently produced increased LPL secretion levels without any decrease in cellular protein or DNA levels.

Table III presents the results of an experiment where the effects of 20 mM chlorate and 10 unit/ml heparin on LPL levels in the intracellular and cell surface compartments were measured. Chlorate was compared to heparin, a glycosami-
TABLE III
Effects of 20 mM chlorate and 10 units/ml heparin on lipoprotein lipase levels in adipocytes

Adipocytes were pretreated with heparin and/or chlorate for 18 h. The media were changed, keeping constant the chlorate and heparin concentrations, and the cells were incubated for 2 h to release cell surface LPL prior to a 5-h incubation. LPL levels after 5 h were measured by ELISA in the medium, heparin washes, and cell extracts. Values are the mean ± S.D. from three pools of 3-60 mm dishes. Shown is a representative of three experiments.

| Lipoprotein lipase | Media | Cell-surface | Intracellular | DNA |
|--------------------|-------|--------------|---------------|-----|
| Concentration (ng/dish) | | | | |
| Chlorate          | 2.26 ± 0.32 | 2.47 ± 0.16 | 3.63 ± 0.29 | 15.9 ± 0.6 |
| Heparin           | 19.6 ± 1.5* | 0.87 ± 0.05* | 1.67 ± 0.20* | 13.3 ± 0.9* |
| Chlorate/heparin | 16.5 ± 2.1* | 0.89 ± 0.13* | 1.93 ± 0.34* | 12.9 ± 0.6* |

*p < 0.05, significantly different from control.

*p < 0.01, significantly different from control.

Incorporation of Tran35S-Label into lipoprotein lipase

Adipocytes were pretreated with or without 10 mM chlorate for 5 or 17 h, when cells were pulsed for 15 min with Tran35S-Label (100 μCi/dish) in RPMI-1640 containing 5 μM (experiments 1 and 3) or no methionine (experiment 2). [35S]LPL was immunoadsorbed from cell extracts and separated by SDS-PAGE. After fluorography, bands representing LPL were excised from the gel, dried, and the radioactivity determined by LSC. To calculate the relative rate of LPL synthesis, [35S]LPL was divided by total [35S]-labeled protein and expressed as a percentage. Results are expressed as the mean ± S.D. from three pools of 8 dishes.

| Exp. | Pretreatment time | Control | Chlorate |
|------|------------------|---------|----------|
|      |                   |         | (cpm/8 dishes (relative rate)) |
| 1    | 5 h              | 3050 ± 260 | 3800 ± 560 |
| 2    | 5 h              | 1370 ± 150 | 1660 ± 290 |
| 3    | 17 h             | 390 ± 90  | 880 ± 160 |

TABLE IV
Effects of Chlorate on Sulfation in Adipocytes

Adipocytes were pretreated with or without 10 mM chlorate and heparin for 18 h. The media were changed, keeping constant the chlorate and heparin concentrations, and the cells were incubated for 2 h to release cell surface LPL prior to a 5-h incubation. LPL levels after 5 h were measured by ELISA in the medium, heparin washes, and cell extracts. Values are the mean ± S.D. from three pools of 3-60 mm dishes. Shown is a representative of three experiments.

| Time (minutes) | Media CPM | Cell-surface CPM | Intracellular CPM |
|---------------|-----------|-----------------|-------------------|
| 0             | 1000      | 0               | 0                 |
| 10            | 560       | 260             | 240               |
| 20            | 290       | 130             | 150               |
| 30            | 150       | 70              | 40                |
| 40            | 50        | 20              | 10                |
| 60            | 20        | 10              | 10                |

*The Effect of Chlorate on LPL Synthesis.—To evaluate the effect of chlorate on enzyme synthesis, cultures were pretreated in the presence and absence of 10 mM chlorate for 5 or 17 h at 37 °C and then pulsed for 15 min with Tran35S-Label (100 μCi/dish). Whether cells were pretreated for 5 or 17 h with chlorate, there was no statistical difference in the rate of incorporation of the label into control or chlorate-treated cells (Table IV).

The Effect of Chlorate on LPL Turnover in Cultured Adipocytes.—Since chlorate did not significantly change LPL synthesis, the increase in enzyme secretion can be explained only by a decreased rate of enzyme degradation. Enzyme degradation was measured in three separate experiments by a pulse-chase protocol (25). Cells were pretreated with and without 20 mM chlorate for 15 h and then pulsed with Tran35S-Label for 1 h to label the intracellular pool. Cells were then chased for up to 1 h with medium containing excess unlabeled methionine. [35S]LPL was measured following immunoadsorption, and LPL protein was measured by ELISA. Values for intracellular, cell surface-associated, and medium LPL radioactivity for one experiment are presented in Fig. 4. Loss of LPL radioactivity in the top panel is due to both degradation and appearance of labeled enzyme on the cell surface and medium. For the three experiments, chlorate treatment reduced the radiolabeled cell surface associated LPL to 17-46% of control levels at the time points measured (Fig. 4, center panel). After 60 min of chase, radiolabeled LPL in the medium (Fig. 4, bottom panel) was 2.1-fold higher in chlorate-treated cells than in control cells. Cell surface-associated LPL levels decreased 4.5-fold with chlorate treatment. Values for one experiment were 7.3 ± 1.0 ng/100-mm dish for control cells, and 1.6 ± 0.7 ng/100-mm dish for chlorate-treated cells (p < 0.0001). In the other experiments, cell surface-associated LPL levels were undetectable in the chlorate-treated cells.

Intracellular LPL radioactivities in both the control and chlorate-treated cultures decreased by an apparent first order process (Fig. 4, top panel). The first order rate constants were 0.0213 ± 0.0065 min⁻¹ (r = 0.98 ± 0.02) and 0.0279 ± 0.003 min⁻¹ (r = 0.99 ± 0.01), respectively, for the control and chlorate-treated cultures. These first order rate constants were not statistically different. Intracellular LPL remained relatively constant during the chase. The coefficient of variation for intracellular LPL measured by ELISA varied between experiments from 5 to 8.6% and from 5 and 9.9% for control and chlorate treated cultures, respectively. Synthesis rates were calculated as described previously by Cusar et al. (3) as the product of the average intracellular pool size over 60 min, and the first order rate constant of the decrease in intracellular radioactivity (Fig. 4, top panel). Table V shows that the synthesis rate in control cells, 9.0 ± 1.3 ng/h, is similar to the rate in chlorate-treated cells, 10.0 ± 2.1 ng/h. These results confirm the conclusions drawn from the synthesis experiments shown in Table IV.

The degradation rate constant during the chase can be estimated as follows by the method described by Cusar et al. (25).

\[ K = \frac{A_0 - A_{40}}{\int p Cdt} \]

where \( A_0 \) and \( A_{40} \) are the total radioactive LPL at \( t = 0 \) and \( t \).
TABLE V  
Effects of 20 mM chloride on lipoprotein lipase turnover  
in cultured adipocytes  

LPL synthesis rate was calculated as the product of the first order rate constant of the decrease in intracellular LPL radioactivity (Fig. 4, top) and the average intracellular LPL concentration. Degradation rates were obtained by multiplying the measured degradation rate constant by the average intracellular pool of LPL for a given set of dishes. The rates of LPL release to the medium were calculated by subtracting degradation rates from the synthesis rates.

| Lipoprotein lipase turnover        | Release to medium |
|-----------------------------------|-------------------|
| Synthesis                        | Degradation       | ng/h/100 mM dish |
| Exp. 1 Control                   | 10.0              | 6.2 (64)         | 3.8 |
| Chlorate                         | 10.1              | 3.9 (38)         | 6.2 |
| Exp. 2 Control                   | 9.5               | 7.8 (82)         | 1.7 |
| Chlorate                         | 12.0              | 4.2 (35)         | 7.8 |
| Exp. 3 Control                   | 7.5               | 6.3 (84)         | 1.2 |
| Chlorate                         | 7.9               | 4.2 (53)         | 3.7 |

* % of synthesized amount.

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Lipoprotein lipase (LPL) synthesis rate was calculated as the product of the first order rate constant of the decrease in intracellular LPL radioactivity (Fig. 4, top) and the average intracellular LPL concentration. Degradation rates were obtained by multiplying the measured degradation rate constant by the average intracellular pool of LPL for a given set of dishes. The rates of LPL release to the medium were calculated by subtracting degradation rates from the synthesis rates.

TABLE VI

Effect of chlorate on the incorporation of \(^{35}S\)O\(_4\) into lipoprotein lipase

Adipocytes were pretreated with control media (30 dishes) or media containing 10 mM chloride (30 dishes) for 15 h. Cells were then incubated with 0.15 mCi/ml Na\(_2\)\(^{35}S\)O\(_4\) sulfate-free media, with and without chloride, for 12 h. The radiolabeled LPL from cells and media was immunoadsorbed and separated by SDS-PAGE. After fluorography, bands representing LPL were excised from the gel, digested, and the radioactivity determined by LSC. LPL protein was determined by ELISA in aliquots of cell extracts and media.

|                 | Cpm/30 dishes | LPL Protein μg/30 dishes |
|-----------------|---------------|--------------------------|
| Control Cell    | 235           | 0.430                    |
| Media           | 87            | 6.412                    |
| Chlorate Cell   | 2             | 0.351                    |
| Media           | 0             | 1.032                    |

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**Fig. 5.** The effect of the sulfate moiety of lipoprotein lipase on the binding to cultured adipocytes. Concentration-dependent binding studies of sulfated and unsulfated LPL were performed using cell extracts which had been metabolically labeled with Tran\(^3S\)S-Label\(^a\) in the absence (sulfated) and presence (unsulfated) of 20 mM sodium chloride. Control or chlorate-treated cells were incubated with the labeled cell extracts for 2 h at 4 °C. The media were collected, and heparin washes were performed to release the bound LPL. The amount of free and bound LPL was determined after isolation of LPL by immunoadsorption, SDS-PAGE, and fluorography (see "Experimental Procedures"). Shown are binding studies performed with total cell extracts (A), and partially purified cell extracts (B).

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amounts. The \(^{35}S\)LPL bound to the cell surface or in the media at equilibrium was measured following immunoadsorption. The maximum concentration of free LPL in the medium was 20 ng/ml. Previous binding experiments with iodinated LPL showed that the nonspecific binding component in this range is negligible. Fig. 5 shows the results of experiments performed with total cell extracts (A), or partially purified
LPL (B). There was no difference in the binding of sulfated versus unsulfated LPL to the adipocyte cell surface. However, the binding of both sulfated and unsulfated $^{35}$S-labeled LPL to chlorate-treated cells was less than the binding to control cells. This is consistent with the results of the binding studies performed with $^{125}$I-LPL.

**Effect of Chlorate on the Lipolytic Activity of LPL in Cultured Adipocytes**—Since chlorate treatment of cultured adipocytes provides a convenient method of producing unsulfated LPL it was of interest to determine if the sulfate moiety of LPL is important for catalytic activity. The specific activity of lipoprotein lipase in control chlorate and chlorate-treated cells was calculated by dividing the triacylglycerol hydrolyase activity by the enzyme mass for four pools of four dishes. The specific activity of lipoprotein lipase was 9.77 ± 1.18 and 12.09 ± 5.14 nmol of free fatty acid released/ng LPL/h for control and chlorate-treated cells, respectively. These differences were not considered statistically significant ($p = 0.082$).

**DISCUSSION**

The results of this study show that the sulfation of the cell surface HSPG plays an important role in the binding, secretion, and degradation of LPL in cultured adipocytes. Chlorate treatment of adipocytes was used to inhibit the formation of PAPS, the activated form of sulfate. Treatment of adipocytes with chlorate resulted in reduced incorporation of $^{35}$SO$_4$ into HSPG and LPL, reduced binding of $^{125}$I-LPL to the adipocyte cell surface, increased LPL secretion, and reduced LPL degradation.

These data support the following hypothesis proposed by Cisar and co-workers (6). In control cells, LPL is transported to the cell surface where it binds to high affinity sites on HSPG. If the enzyme is not released from the cell surface HSPG, it is internalized as a LPL-HSPG complex and is then either degraded or recycled to the surface. The net release of LPL from the surface would be determined by the association constant of LPL for the HSPG-binding site, the number of HSPG-binding sites, the residence time of HSPG-LPL complex on the surface, and the presence of soluble molecules which bind LPL and compete with the binding of LPL to HSPG. In the absence of competing molecules, 76% of the synthesized enzyme is degraded; the addition of heparin reduced the degradation rate to 21% of the synthetic rate (3). Treatment with heparinase and heparitinase, endoglycosidases which specifically cleave HSPG and therefore reduce the number of HSPG-binding sites, resulted in reduced LPL binding and degradation (6). Alternatively, it remains possible that sulfation of a cellular protein other than LPL and HSPG is inhibited by chlorate and that this factor contributes to the reduced LPL degradation observed in chlorate-treated adipocytes.

According to the current model for HSPG biosynthesis, unsulfated glycosaminoglycans are first elongated on the protein core and are then subsequently modified and sulfated (28). Chlorate has been used to inhibit the sulfation of proteoglycans by several groups. In bovine endothelial proteoglycans (29), 30 mM chlorate treatment reduced the sulfation to 35% of control levels, as measured by ratios of $^{35}$SO$_4$ to $[^3H]$glucosamine incorporation into HSPG. Another study utilizing human skin fibroblasts (8) showed that chlorate reduced the sulfation of proteoglycans but did not affect the degree of chain polymerization. This was measured by the effect of chlorate on $[^3H]$glucosamine incorporation into HSPG. It is interesting to note that the sulfate moiety of LPL did not contribute to the binding of LPL to the adipocyte cell surface. The most proximal N-acetylg glucosamine (GlcNAc) residue of the complex oligosaccharide conjugated at Asn-45 contains the sulfate moiety of LPL (26). Consensus sequences for heparin-binding domains have been postulated by comparisons of known heparin-binding domains of apolipoproteins B-100 and E (31). The consensus sequences for these domains are B$_1$-B$_2$-X-X-B$_3$ and B$_1$-B$_2$-X-B$_3$, where B represents a basic and X a hydrophobic amino acid. In chicken LPL, these sequences occur at amino acids 148–153, and 281–284 (32). It was of interest to consider the effect of sulfation of LPL on its binding to cell surface HSPG since the native LPL characterized by GlcNAc conjugated to Asn-45 may be in close proximity to the postulated HSPG-binding regions.

Chlorate is an effective inhibitor of sulfation and is a useful tool to investigate the role of sulfate moieties in macromolecules. However, chlorate treatment of cells has no physiological relevance. Since sulfation may play a significant role in the binding properties of heparan sulfate chains, it will be important to identify physiological modulators of sulfation. There is very limited information on the effect of hormones on glycosaminoglycan sulfation. Shishiba and co-workers (33) reported that thyroid-stimulating hormone (10 milliunits/ml) increased the incorporation of $[^3H]$glucosamine and $^{35}$SO$_4$ into HSPG of the cell layer of rat thyroid cells by 7- and 3-fold, respectively. This would result in an alteration of the sulfation state of HSPG. The same report also noted that thyroid-stimulating hormone enhanced the degradation of cell-associated proteoglycans. Perhaps other hormones which are relevant to adipocytes will be found to influence the sulfation of HSPG.

The present study has shown that chlorate treatment decreases the sulfation of both LPL and the adipocyte HSPG. The decreased sulfation of HSPG reduces the number of binding sites for LPL on the adipocyte cell surface. Consistent with the model that binding of LPL to cell surface HSPG is necessary for degradation of LPL to occur, LPL degradation is reduced in chlorate-treated cultures. The decreased sulfation of LPL does not affect the affinity of LPL for cell surface
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HSPG, nor does the decreased sulfation affect the specific catalytic activity of LPL.

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Supplemental Material

Effect of chlorate on the sulfation of lipoprotein lipase and heparan sulfate proteoglycans: Sulfation of heparan sulfate proteoglycans affects lipoprotein lipase degradation.

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**EXPERIMENTAL PROCEDURES**

**MATERIALS AND Methods**

In experiments utilizing metabolically labeled LPL, cells were preincubated for 4 h with RPMI-1640 containing methyl cellulose. Eighty percent of the cells were then precipitated by rotation at 30,000 rpm for 1 h prior to the beginning of the 4 h incubation. At the beginning of the 4 h incubation, the cells were washed with 2 ml/dish PBS and 2 ml/dish medium containing 105 cpm/ml methionine or no methionine. Cells were removed from the dishes by trypsinization and washed twice with 2 ml of complete RPMI-1640. L-glutamine, and penicillin, streptomycin, and amphotericin B were present in all media. Barium nitrite and sodium nitrite were obtained from Boehringer Mannheim Biochemicals. Enlightening, nonradioactive LPL, and the sample mixed by rotation. The acetone powder was separated between two 50 ml polypropylene tubes. The cells were pelleted by centrifugation at 30,000 rpm for 1 h at 4°C. The supernatant was used for binding experiments.

In binding experiments utilizing metabolically labeled LPL, cells were preincubated for 4 h with RPMI-1640 containing 10% chlorate. Cells were then washed with 2 ml/dish PBS and 2 ml/dish medium containing 105 cpm/ml methionine and the indicated concentrations of chlorate.

**Experiments: culture conditions**

In experiments designed to examine the effects of chlorate or chlorate on lipoprotein lipase or activity, cultured cells were pretreated with control medium or chlorate-containing medium for 4 h prior to the beginning of the 4 h incubation. At the beginning of the 4 h incubation, the cells were washed with 2 ml of complete RPMI-1640 containing 105 cpm/ml methionine or no methionine. Cells were removed from the dishes by trypsinization and washed twice with 2 ml of complete RPMI-1640 containing methionine. The proteins were precipitated by the addition of 30 ml acetone (-20°C) and 10 ml of ethyl ether (10 ml each). The acetone powder was separated between two 50 ml polypropylene tubes. The cells were pelleted by centrifugation at 30,000 rpm for 1 h at 4°C. The supernatant was used for binding experiments.
The gel was washed twice with 12 ml 0.5 M NaCl, 18 BSA, 10 mM Phosphate, pH 7.2, twice with 5 ml 12 BSA, 10 mM Phosphate, pH 7.2, once with 5 ml 0.18 M NaCl, 0.1% Triton, 0.1% SDS, and 10 ml PBS. Following this incubation at 37°C for 30 min, the sample was dialyzed against 0.15 M NaCl, 10% glycerol, 10 mM Phosphate, pH 7.2. Protein Pak-60 columns connected in series. The buffer used was 0.14 M NaCl, 1% BSA. The sample was applied to the column and elution buffer was added to the column. The column was washed with 10 ml 1% BSA. 10% glycerol, 10 mM Phosphate, pH 7.2, by rotation at 4°C for 1 h. The protein was eluted by adding 0.5 ml elution buffer and rotation at 4°C for 30 min. The sample was dialyzed against 0.15 M NaCl, 10% glycerol, 10 mM Phosphate, pH 7.2 for 2 h.

Equilibrium binding of LPL to isolated adipocytes
The effect of chlorate treatment on the binding of [35S]-LPL to the adipocyte cell surface was examined. Highly purified LPL was iodinated as described (20). Equilibrium binding experiments were performed and non-specific binding was calculated as described (1). Briefly, media containing 0.28 kBq in addition to the normal constituents, following this incubation at 3°C, media were placed on ice and samples were collected. No. 24 ml of media containing 0.28 kBq were added to the dishes, followed by the addition of [35S]-LPL. Cells were isolated by 4°C on ice for 1 h, with shaking. Media were collected and the cells were rinsed 3 times with 0.14 M NaCl-PBS. Bound [35S]-LPL was released with 2 heparin washes (10 ng/ml heparin). [35S]-LPL in the media and heparin wash was precipitated as described (10). In experiments #1 and #2, 10% of the [35S]-LPL in the [35S]-LPL preparation was TCA-precipitable; therefore TCA precipitations on individual samples were not performed.

The effect of the sulfate moiety of LPL on binding to the adipocyte cell surface was examined. Salts of unlabeled cell extracts were metabolically labeled and prepared as described above. The binding studies were carried out as described (1), except that increasing amounts of radio-labeled cell extracts or partially-purified LPL were added to the dishes rather than [35S]-LPL. Media and heparin washes were collected after a 2 h incubation at 4°C on ice. The LPL radioactivity was measured in each media and heparin wash sample by immunoadsorption of LPL followed by SDS-PAGE with a 10% gel, using the procedures described (11). The flow rate of the chromatography using a Waters Protein Pak-125 was performed using 0.005 M Na acetate, 1 M NaCl, 0.25 M Na acetate, 0.2 M Tris-acetate, pH 7.0, according to Fisher et al. (13). The binding of heparin to LPL was performed at a concentration of 0.005 (1/2) heparin in PBS. The products of digestion were dried using a speed-vac (Savant) and were separated by gel. Protein Pak-600 g columns connected in series. The buffer used was 0.14 M NaCl, 1% BSA, 0.2% Triton X-100, 0.8 M NaCl, 0.1 M Na acetate, 0.2 M Tris-acetate, pH 7.0, according to Fisher et al. (13). The binding of heparin to LPL was performed at a concentration of 0.005 (1/2) heparin in PBS. The products of digestion were dried using a speed-vac (Savant) and were separated by gel. Protein Pak-600 g columns connected in series. The buffer used was 0.14 M NaCl, 1% BSA, 0.2% Triton X-100, 0.8 M NaCl, 0.1 M Na acetate, 0.2 M Tris-acetate, pH 7.0, according to Fisher et al. (13). The binding of heparin to LPL was performed at a concentration of 0.005 (1/2) heparin in PBS. The products of digestion were dried using a speed-vac (Savant) and were separated by gel.