PRODUCTION AND SECRETION OF CHONDROITIN SULFATES AND DERMATAN SULFATE BY ESTABLISHED MAMMALIAN CELL LINES

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Summary

Ten established mammalian cell lines of different origin (lymphoid, epithelial and fibroblastic) were found to produce and secrete a mixture of chondroitin sulfates A, C and dermatan sulfate into the medium. The rates of production and secretion of the total sulfated mucopolysaccharides as well as the proportion of individual mucopolysaccharides differ strikingly among different cell lines.

Introduction

The production and secretion of mucopolysaccharides (MPS) is thought to be one of the properties characteristic of animal connective tissue cells. It has been shown recently that this specific function is present also in other tissues in the early chick embryo (1, 2), suggesting that it may become selectively repressed in some tissues as the embryo matures. The ability to synthesize MPS (mainly, hyaluronic acid) has been reported to be retained by cultured fibroblasts for long periods of time (3, 4, 5). More recently, the non-fibroblastic line (HeLa) as well as the fibroblastic line (L-929) have been shown to be able to produce a mixture of several sulfated MPS (6, 7). These reports have suggested,

then, that the ability to produce MPS may be retained in different cell types and can be demonstrated when sufficiently sensitive methods are applied. The present study was undertaken to determine whether or not the ability to produce and secrete chondroitin sulfates and dermatan sulfate is repressed or expressed in ten established mammalian cell lines of different types of origin.

Materials and Methods

The following cell lines were used in this study: 1) Lymphoid; L1210 and P1534 (murine lymphoma), 2) Epithelial; KB and HeLa (human carcinoma), Ehrlich murine ascites carcinoma, and 3) Fibroblastic; L929 and 3T3 (normal mouse), SV_{40}-transformed 3T3, Don (normal Chinese hamster), murine Sarcoma-180.

All cell lines were grown as monolayers in Falcon plastic culture flasks (250 ml) in Basal medium Eagle in which $MgCl_2$ was substituted for $MgSO_4$, and which was supplemented with 10% fetal calf serum, 5% horse serum and antibiotics. After one-week incubation in this medium in order to deplete the possible intracellular pool for SO_4^{--}, triplicate subcultures were prepared for each cell line . 24 hours later $Na_2{}^{35}SO_4$ (sp. act. 1000 mC/mM) was added to log-phase cells in a concentration of 71.5 μCi per ml of medium. After 24 hours, both medium and cells were collected from two flasks and the number of cells in the third flask was counted. MPS were isolated from medium and from washed cells by adding carrier chondroitin sulfates A and C, followed by treatment with NaOH, pronase, trichloroacetic acid, extensive dialysis and ethanol precipitation, and were analyzed by the enzymic method as previously described (8, 9). Briefly, the final ethanol precipitates were dissolved in water and divided into three aliquots. One aliquot was

enzymatically digested by chondroitinase ABC (Miles Laboratories) for determining amounts of chondroitin sulfate A + B (dermatan sulfate) and chondroitin sulfate C. A second aliquot was treated with testicular hyaluronidase (Sigma) followed by repeated ethanol precipitation and chondroitinase ABC digestion for determining dermatan sulfate. The third aliquot not treated by enzymes is for determining total sulfated MPS. Subsequently, all three aliquots were then subjected to paper chromatography in 1-butyric acid-0.5 N ammonia (5:3, V/V). The origin and the ultraviolet-absorbing areas corresponding to unsaturated disaccharides were cut out from the chromatograms and counted in a Beckman Liquid Scintillation Counter (LS-250). As is conventionally done, the amount of radioactive SO_4^{--} incorporated into chondroitin sulfates or dermatan sulfate was taken as a measure of their synthesis. The results were expressed as radioactivity (cpm) incorporated per 10^6 cells per 24 hours.

Results and Discussion

Figure 1 shows the total amount of sulfated MPS produced by each of the ten different cell lines expressed as the sum of intracellular and extracellular (medium) MPS. It is obvious that while all cell lines examined produced and secreted into the medium some sulfated MPS, there was a definite difference in the rate of production among different cell lines all of which were in log-phase growth. Lymphoid cell lines (L1210, P1534) appear to produce much less MPS per unit of time than cell lines of fibroblastic or epithelial origin, reflecting the in vivo situation and suggesting that the ability to produce sulfated MPS may be largely repressed in the lymphoid cell lines. The differences among the 5 fibroblastic cell lines examined may reflect not only their original ability, but also the overall length of time during which each cell strain has been

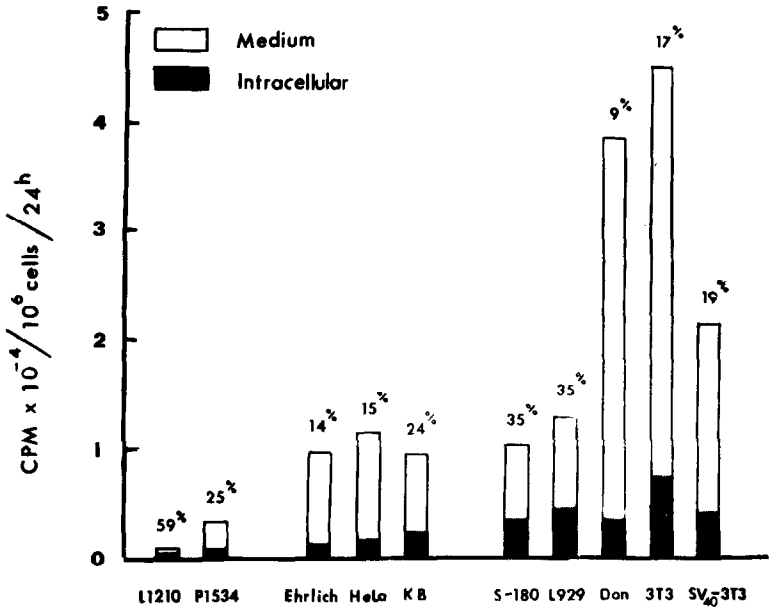

Figure 1 Sulfated MPS production and secretion by 10 established cell lines. Each column represents the mean of duplicate flasks. Figure above each column represents the amount of intracellular MPS as % of the total MPS.

grown in vitro, since the loss of the ability to produce hyaluronate in some very old fibroblastic cell lines has been reported (5). It is also clear that the ratio of intracellular to extracellular (medium) MPS varied in different cell lines, the intracellular fraction accounted for anywhere from 9% to 59% of the total, suggesting that each cell line has a different rate of secretion. In this regard, it must be remembered that a small part of the intracellular MPS may be due to the uptake of MPS by the cells, as shown in previous reports from this laboratory concerning the incorporation of the extracellular chondroitin sulfates into cultured mammalian cells (10, 11). It is noteworthy that SV_{40}-transformed 3T3 cells showed the diminished production of sulfated MPS compared with non-transformed 3T3 cells, although the fraction secreted

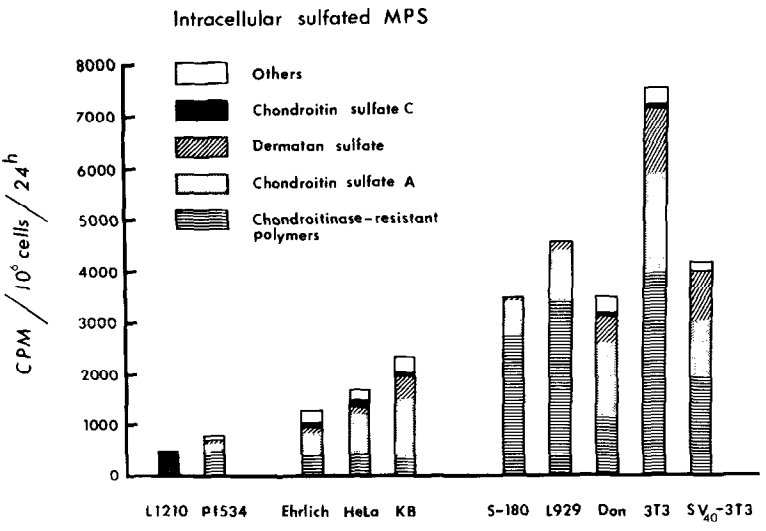

Figure 2 Analysis of intracellular sulfated MPS. Each value is the mean of duplicate flasks. White column ("Others") represents some minor heterogenous components of chondroitin sulfates (9). Chondroitinase-resistant polymers are probably heparin-related MPS (6) and/or keratan sulfate.

is the same. This is similar to the decreased rate of hyaluronate synthesis already reported in SV_{40}-transformed 3T3 cells (5).

The distribution of chondroitin sulfates and dermatan sulfate in sulfated MPS is presented in figure 2. All the cell lines produced a mixture of chondroitin sulfate A (ChS A), chondroitin sulfate C (ChS C), dermatan sulfate (DS) and chondroitinase-resistant sulfated MPS, but in differing proportions. ChS C represents 85% of sulfated MPS produced by L1210 cells, but only 0.3% by Sarcoma-180 cells. 3T3 cells produced 21% DS, whereas L929 cells produced only 4%. The pattern of MPS types secreted by the cells is similar to that retained intracellularly. These data emphasize striking qualitative as well as quantitative differences in sulfated MPS production and secretion among different cell lines.

The present results concerning sulfated MPS production and

secretion together with extensive data on collagen production in various types of cultured cells by Green et al. (12, 13), suggest that these specific functions of connective tissue cells are maintained at different levels (repressed or derepressed ?) in different cell types. The present system may offer a broader model for exploring the regulation of a specific cellular function in cell culture, since qualitative differences in the molecular species involved can be more easily documented.

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