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Catabolism of heparan sulfate proteoglycans in *Drosophila* cell lines

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Abstract

We have studied intracellular catabolism of heparan sulfate proteoglycans (HSPGs) in *Drosophila* cell lines, Kc and S2, by a series of pulse-chase experiments using [^{35}S]sulfate as a precursor in metabolic labeling experiments. HSPGs in culture medium and cell layer were separately purified by serial chromatographic procedures using Q-Sepharose and Superose 6 for characterization. Analysis of intact HSPG on Superose 6 chromatography revealed that Kc and S2 cells synthesize one major molecular species with slightly differing in sizes (estimated to be 54 kDa in Kc and 78 kDa in S2 cells). Analysis of glycosaminoglycans for ^{35}S-labeled macromolecules showed that the majority of ^{35}S-labeled macromolecules in Kc and S2 cells are HSPGs (~60% and ~80%, respectively). Results from continuous labeling and 2 h pulse labeling-chase experiments revealed that, in both cell lines, the intact HSPGs were degraded in multiple phases; the degradation of HSPG was rapid in the early phase (with half-lives of ~6 h in Kc and ~3 h in S2 cells) and slow in the later phase (with half-lives >80 h in both Kc and S2 cells). The rapid degradation appeared similar to that observed for glycosylphosphatidylinositol-anchored HSPGs (glypicans) in mammalian cell cultures. While the slow degradation appeared similar to that observed for transmembrane HSPGs (syndecans) in mammalian cell cultures. These experiments suggested that vertebrates and invertebrates shared common mechanisms for intracellular HSPG catabolism.

Keywords: Heparan sulfate proteoglycans; Metabolism; *Drosophila*; Degradation; Pulse-chase experiment

Proteoglycans belong to one of the major classes of extracellular matrix molecules, and they have a general structure of a core protein with one or more covalently linked glycosaminoglycans. Proteoglycans can be classified by the type of their glycosaminoglycan component. Heparan sulfate proteoglycan (HSPG), for instance, has glycosaminoglycan chains composed of disaccharide repeats of alternating glucuronic acid and *N*-acetylglucosamine linked to a core protein through a linkage tetrasaccharide. Heparan sulfate (HS) chains undergo extensive biosynthetic modifications, including N-sulfation, O-sulfation, and epimerization, and are present as complex carbohydrates with diverse structural domains. HSPGs have been shown to play a variety of biological roles in cell activities; well-characterized properties include their binding to a number of growth factors classified as 'heparin-binding growth factors' (e.g., fibroblast growth factors, vascular endothelial growth factor, and platelet derived growth factor) and cytokines (e.g., tumor necrosis factor-α, interferon-γ, and interleukins), and consequent modulation of their biological activities. They are also known to be involved in cell adhesion, cell migration, cell proliferation, etc. [1].

Recently, many critical biological roles of HSPG, especially those during developmental processes, have been clarified using a model organism, *Drosophila melanogaster*. *Drosophila* with genetically mutated enzymes that are involved in HSPG metabolism, such as 'sugarless,' a homolog of UDP-glucose dehydrogenase, 'sulfateless,' a homolog of HS specific *N*-deacetylase/*N*-sulfotransferase, and 'tout-velu,' a homolog of HS copolymerase, were

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used for investigation. These resulted in disturbance in signaling pathways involving Wingless, Hedgehog, Decapentaplegic (a homolog of transforming growth factor-β), and Branchless (a homolog of fibroblast growth factor) during developmental stages [1–3]. These studies provided important clues for understanding how HSPGs regulate growth factor signaling pathways during development in *D. melanogaster*.

The cellular metabolism of HSPG has been studied in several organisms such as rat [4], chicken [5], and human [6]. Results from these studies suggested that basic metabolic pathways of HSPG are shared among those mammalian cells. The study using metabolic labeling of proteoglycans and pulse-chase experiments in rat ovarian granulosa cell culture demonstrated that there were two distinct intracellular catabolic pathways of HSPGs [4]. In the first pathway, internalized HSPGs were rapidly transferred to lysosomes where HS chains were completely degraded into monosaccharides and sulfate. In the second pathway, endocytosed HSPGs from cell surface underwent slow, stepwise degradation, generating catabolic intermediates of HS chains in prelysosomal compartments, and eventually were transported to lysosomes for their final degradation. There has been only one report [7], from our laboratory, which studied turnover of HSPGs in *Drosophila* cell lines, Kc and S2. It demonstrated a relatively slow turnover of cell surface HSPGs and the generation of catabolic intermediates. In the present study, we have extended the previous work and obtained more detailed information about the catabolism of HSPG using metabolic radiolabeling experiments in combination with various pulse-chase protocols.

Materials and methods

Materials. Fetal bovine serum and a specially formulated 'sulfate-free' Schneider's *Drosophila* medium were from Gibco/BRL (Grand Island, NY). Triton X-100 was from Sigma (St. Louis, MO). Sephadex G-50 (fine), Superose 6, and Q-Sepharose (Fast Flow) were from Amersham Biosciences (Uppsala, Sweden). Heparitinase (*Flavobacterium heparinum*) and chondroitinase ABC (*Proteus vulgaris*) were from Seikagaku (Tokyo, Japan). $Na_2[^{35}S]SO_4$ was obtained from New England Nuclear (Boston, MA). Other reagents used were of the highest grades commercially available.

Cell culture. Drosophila cell lines, Kc [8] and S2 [9], were cultured at 25 °C in 10 cm diameter plastic dishes (Falcon) with 10 ml Schneider's *Drosophila* medium supplemented with 10% fetal bovine serum and 4.6 mM Na_2CO_3. Sulfate concentration of the medium was adjusted to 0.15 mM by the addition of solid $MgSO_4$.

Metabolic labeling of cell culture and extraction of proteoglycans. Cells ($\sim 1 \times 10^6$ cells/ml) were metabolically labeled with $Na_2[^{35}S]SO_4$ at the concentration of 100 or 500 μCi/ml at 25 °C for periods indicated in the text. After labeling, cells were separated from the labeling medium by centrifugation at 500*g* at 25 °C for 7 min and extracted with 1 ml of 4 M guanidine–HCl containing 2% (v/v) Triton X-100 by vigorous vortexing for 2 min. Solid guanidine–HCl was added to the medium fraction to make it 4 M in guanidine–HCl. The extracted cell layer and medium fractions were further processed for isolation of proteoglycans.

Isolation of proteoglycans. Proteoglycans were isolated essentially as described previously [10]. Briefly, the extracted cell layer and medium fractions were chromatographed on a Sephadex G-50 column (4-ml bed volume) equilibrated with 8 M urea, 0.2 M NaCl, and 50 mM sodium acetate, pH 6.0, containing 0.5% (v/v) Triton X-100. Excluded fractions containing macromolecules were collected and small aliquots were counted for radioactivity to measure the amount of the total ^{35}S-incorporation. Labeled macromolecules were analyzed further by Q-Sepharose chromatography as follows: Q-Sepharose gel (0.5 ml) equilibrated with 8 M urea, 0.2 M NaCl, and 50 mM sodium acetate, pH 6.0, containing 0.5% (v/v) Triton X-100 was added to each sample and gently shaken for 1 h at room temperature. The slurry was then packed into a 10 ml disposable serological pipette and washed with 5 ml of the same urea buffer. Bound molecules were eluted with a linear NaCl gradient (0.2–1.5 M) in the 8 M urea, 50 mM sodium acetate, pH 6.0, containing 2% (v/v) Triton X-100 at 12 ml/h flow rate. Eluted fractions (1 ml each) were collected and measured for radioactivity and conductivity [11]. Aliquots (3000–5000 cpm) from radioactive peaks indicated in the text were analyzed by Superose 6 chromatography using an FPLC system (Amersham Biosciences, Uppsala, Sweden) [12]. Superose 6 chromatography was performed in 4 M guanidine–HCl, 0.1 M sodium acetate, pH 7.0, containing 0.5% (v/v) Triton X-100 at 24 ml/h flow rate. Fractions of 0.4 ml were collected, mixed with 3 ml of scintillation cocktail, 'Opti-Phase HiSafe 3' (Wallac, Turku, Finland), and counted with a liquid scintillation counter (Beckman, Fullerton, USA).

Two hour pulse labeling-chase experiments. Cells were labeled with 500 μCi/ml $Na_2[^{35}S]SO_4$ at 25 °C for 2 h. The cell layer and labeling medium were separated by centrifugation at 500*g* at 25 °C for 10 min, washed twice with radioisotope-free medium, and chased in radioisotope-free medium for periods indicated in the text. At the end of each chase time, cell layer and medium were separated by centrifugation at 500*g* at 25 °C for 7 min as above. Cell layer and medium fractions were then extracted and subjected to isolation of proteoglycans as described above.

Identification of glycosaminoglycans in Drosophila cells. Radioactive peaks identified by Q-Sepharose chromatography were pooled as indicated in the text and dialyzed against 0.1 M Tris, 0.1 M sodium acetate, pH 7.3, at 4 °C for 24 h. Samples were then treated with chondroitinase ABC (0.1 U/ml) or heparitinase (0.01 U/ml) at 37 °C for 1 h. Enzyme reaction was stopped by the addition of 4 M guanidine–HCl, 0.1 M sodium acetate, pH 7.0, containing 0.5% Triton X-100 (0.4 ml) and samples were analyzed by Superose 6 chromatography as described above.

Results

Modification of sulfate concentration of Schneider's Drosophila medium for metabolic labeling

Sulfate concentration of Schneider's *Drosophila* medium (originally 15 mM [9]) was modified to 0.15 mM in $MgSO_4$ to enhance radioactive incorporation. Preliminary experiments showed no significant alterations in cell morphology (size and shape) and proliferation by the reduced sulfate concentration (data not shown).

Incorporation of [^{35}S]sulfate into macromolecules in Drosophila cells

Kc and S2 cells were labeled with 100 μCi/ml [^{35}S]sulfate in Schneider's medium at 25 °C for various times: 4,

24, and 48 h. Cell layer and medium were separated and extracted with 4 M guanidine–HCl buffer. ^{35}S-Labeled macromolecules were isolated by Sephadex G-50 chromatography and analyzed by Q-Sepharose anion exchange chromatography as described in Materials and methods (a typical analysis is shown in Fig. 2). About 80% of the total ^{35}S-labeled macromolecules in Kc and S2 cell layer bound to Q-Sepharose column, and all the sulfated proteoglycans were present in this fraction [10]. The remaining 20% of the ^{35}S-labeled macromolecules, which did not bind to Q-Sepharose, were mostly sulfated glycoproteins [10] and were not studied further in the present investigation. Analyses of molecules bound to Q-Sepharose by Superose 6 chromatography revealed the presence of ^{35}S-labeled macromolecules in several molecular size classes. In Kc cell layer, three major ^{35}S-labeled peaks were detected: peaks A, B, and C with K_d values 0.03, 0.35, and 0.71, respectively (Figs. 1A–C). Based on the elution behavior of the glycosaminoglycan molecular weight standards on Superose 6 [10], molecular sizes of ^{35}S-labeled macromolecules in peaks A, B, and C in Kc cell layer were estimated to be approximately >190, 54, and 14 kDa, respectively. The amount of peak A material was small and relatively constant in the cell layer throughout labeling times (Figs. 1A–C). The molecule

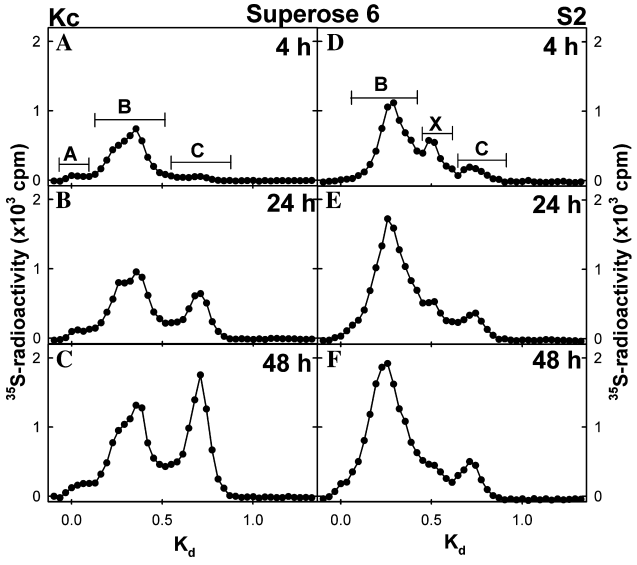

Fig. 1. Superose 6 chromatography of ^{35}S-labeled macromolecules in cell layer of Kc and S2 cells with different labeling times. After indicated labeling periods, cell layer was separated from medium and extracted with 4 M guanidine–HCl containing 2% Triton X-100. ^{35}S-Labeled macromolecules were isolated by sequential chromatography with Sephadex G-50 and Q-Sepharose. Pooled ^{35}S-labeled peaks from Q-Sepharose chromatography (a representative analysis was shown in Fig. 2) were analyzed by gel filtration chromatography on Superose 6. (A–C) Profiles for Kc cell layer labeled for 4, 24 or 48 h, respectively. (D–F) Profiles for S2 cell layers labeled for 4, 24 or 48 h, respectively. Bars and letters indicate peaks of ^{35}S-labeled macromolecular species collected for further analyzes (see below).

with the same elution position, $K_d = 0.03$, recovered in the medium fraction and accumulated with labeling time (data not shown). After labeling for 4 h (Fig. 1A), the majority of ^{35}S-labeled macromolecules eluted in peak B, while peak C was hardly detected. With labeling times longer than 4 h, the peak B gradually increased while peak C increased rapidly throughout labeling periods (Figs. 1B and C).

Three major ^{35}S-labeled peaks were also detected in S2 cells on Superose 6 profiles (Figs. 1D–F) and they were designated as peaks B, C, and X, eluting at $K_d = 0.26$, 0.71, and 0.50, respectively. Their estimated molecular masses against glycosaminoglycan molecular weight standards were 78, 14, and 30 kDa, respectively. When cell cultures were labeled for 24 or 48 h, the accumulation of peaks B and C materials was observed while the amount of peak X remained relatively unchanged.

Identification of glycosaminoglycan species in Drosophila cells

Glycosaminoglycan species in peaks observed in Superose 6 profiles in Kc and S2 cells were identified by digestion with specific glycosaminoglycan degrading enzymes. In a separate experiment, macromolecules obtained from cell cultures labeled with [^{35}S]sulfate (500 μCi/ml) at 25 °C for 24 h were sequentially analyzed by Sephadex G-50 and Q-Sepharose chromatography (Fig. 2) as above. In the analysis of Kc cell layer sample, proteoglycans eluted in two separated peaks in Q-Sepharose chromatography; peaks C_k-I and -II with peak position at 0.36 and 0.66 M NaCl, respectively (Fig. 2A). In S2 cell layer fraction, three peaks were identified; peaks C_s-I, -II, and -III in elution fractions between 0.5 and 0.8 M NaCl (Fig. 2C). For medium samples, three peaks in Kc cells (M_k-I, -II, and -III, Fig. 2B) and two peaks in S2 cells (M_s-I and -II, Fig. 2D) were identified in elution fractions between 0.3 and 0.6 M NaCl. To identify glycosaminoglycan species in each peak, peak fractions indicated by bars in Fig. 2 were pooled separately, dialyzed against enzyme buffer, treated with or without heparitinase or chondroitinase ABC, and analyzed by Superose 6 chromatography (Figs. 3A–J). Only a small amount (~10%) of radioactivity in C_k-I peak was digested by heparitinase or chondroitinase ABC (Fig. 3A), while most of peak C_k-II was susceptible to heparitinase with only a small amount (<10%) digested by chondroitinase ABC (Fig. 3B). A previous study [7] indicated that peak B and peak C represented intact HSPG and free HS chains, respectively. Small amounts of ^{35}S-labeled molecules in peaks M_k-I and M_k-III were digested by chondroitinase ABC but not by heparitinase. In M_k-II fraction, most of the ^{35}S-labeled molecules in peak A (82%) was found to be digested by chondroitinase ABC with only a small amount (7%) digested by heparitinase (Fig. 3D). In S2 cells, most

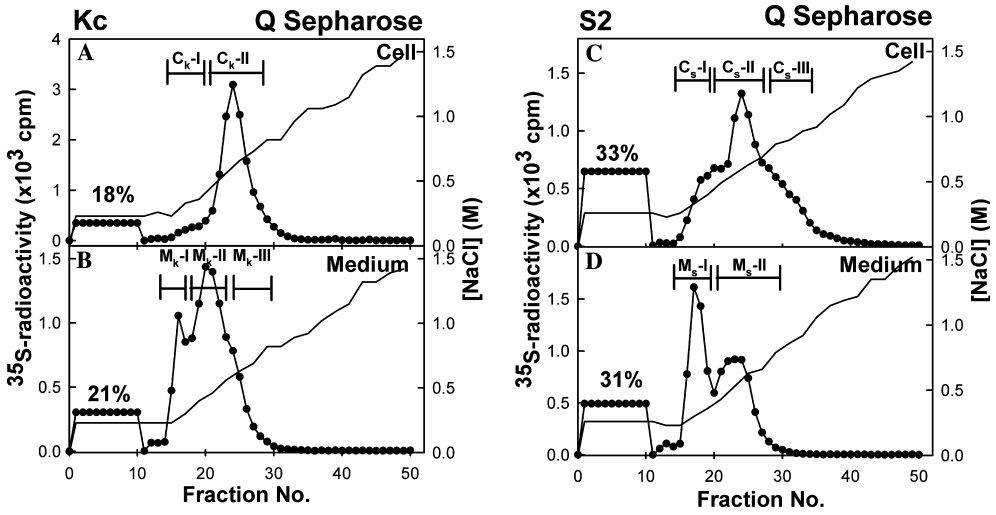

Fig. 2. Q-Sepharose chromatography of cell layer and medium extracts from Kc and S2 cell cultures. Cell cultures were labeled with [^{35}S]sulfate for 24 h. After removing unincorporated isotope with Sephadex G-50 chromatography, ^{35}S-labeled macromolecules in cell layer and medium from Kc cells (A and B, respectively) and S2 cells (C and D, respectively) were analyzed with Q-Sepharose ion exchange chromatography in 8 M urea buffer with a NaCl gradient (0.2–1.5 M). Bars and letters indicate pooled peaks of each sample for further analysis.

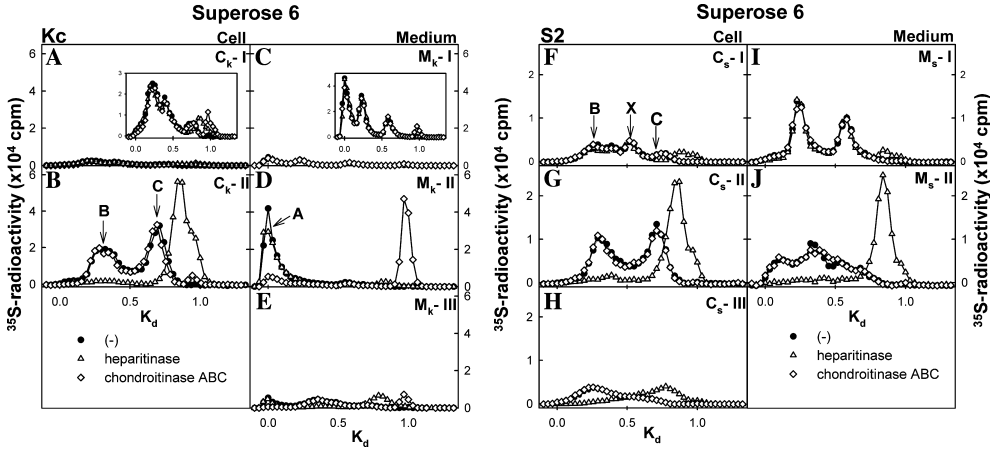

Fig. 3. Superose 6 analysis of Q-Sepharose fractions for glycosaminoglycan identification. Q-Sepharose peaks as indicated in Fig. 2 were separately digested with either heparitinase or chondroitinase ABC at 37 °C for 1 h and analyzed by Superose 6 chromatography in 4 M guanidine–HCl. Superose 6 profiles without enzyme treatment (●), heparitinase treatment (△), and chondroitinase ABC treatment (◊) were superimposed, Kc cell layer (A and B) and medium (C–E) and S2 cell layer (F–H), and medium (I and J). Peaks identified in Fig. 1 were indicated with the same alphabetic codes. The area under each profile is proportional to the total amount of radioactivity recovered in the respective fraction. Insets show chromatographic profiles in expanded scales.

^{35}S-labeled macromolecules were digested by heparitinase, especially in peaks C_s-II and M_s-II (Figs. 3F–J). Materials in C_s-I and M_s-I, including peak X, could not be digested either by heparitinase or chondroitinase ABC, indicating that they were neither HS nor chondroitin sulfate (CS) (Figs. 3F and I). Table 1 summarizes glycosaminoglycan constituents in Q-Sepharose peaks based on enzyme susceptibility. In each cell line, about 90% of the Q-Sepharose-bound, ^{35}S-labeled macromolecules in cell layer fraction was HS with only a small amount (7% in Kc cells and none in S2 cells) of CS and the rest probably corresponded to other ^{35}S-labeled macromolecules, such as sulfated glycoproteins or sulfated glycolipids. Since the majority of HS in cell layer fraction was present in the pool II of Q-Sepharose chromatography eluting in fractions between 0.6 and 0.7 M NaCl for samples from both cell lines, this peak was used to study the cellular degradation of HSPGs in 2 h pulse labeling-chase experiments hereafter. Medium compartment contained similar amounts of ^{35}S-labeled macromolecules as those found in cell layer fractions (Table 1). However, unlike in cell layer fraction, about two-thirds of total ^{35}S-labeled macromolecules in Kc medium was CS, while the remaining one-third was HS (Fig. 3D). Small amounts of ^{35}S-labeled macromolecules (approximately 9%) in Kc medium fraction

Table 1
Analysis of glycosaminoglycans for ^{35}S-labeled macromolecules recovered in Q-Sepharose peaks after 24 h labeling

		Q-Sepharose peak	Peak position (NaCl, M)	% of the total ^{35}S-radioactivity			
				HS	CS	other	Total
Kc	Cell layer	Pool I	0.36	1	1	ND	2
		Pool II	0.66	49	3	2	54
	Medium	Pool I	0.27	ND	ND	ND	0
		Pool II	0.46	2	23	3	28
		Pool III	0.63	11	4	1	16
	Total			63	31	6	100
S2	Cell layer	Pool I	0.45	4	ND	1	5
		Pool II	0.60	30	ND	2	32
		Pool III	0.79	12	ND	3	15
	Medium	Pool I	0.30	3	1	3	7
		Pool II	0.53	37	1	3	41
	Total			86	2	12	100

ND, not detectable. HS, heparan sulfate; CS, chondroitin sulfate.

resisted enzyme digestions. S2 conditioned medium contained similar constituents to those from the cell layer fraction, in which majority (83%) of the ^{35}S-labeled macromolecules was HSPG.

Two hour pulse labeling-chase experiment

To clarify kinetics of HSPG degradation in each cell line, a 2 h pulse labeling-chase experiment was done. After labeling with 500 μCi/ml [^{35}S]sulfate for 2 h, cells were washed twice with isotope-free medium and chased for various times: 0, 10 min, 1, 4, 8, and 24 h. Cell layer and medium fractions at each chase time were separately extracted in 4 M guanidine–HCl. Proteoglycans from extracted cell layer and medium fractions were purified as above. Fig. 4 shows the amounts of ^{35}S-labeled HSPGs identified by Q-Sepharose chromatography at various chase times. The result indicated disappearance of ^{35}S-labeled HSPGs in multiple phases; it was initially rapid and then significantly slowed down. During the first 1 h chase, approximately 14% and 21% of the total ^{35}S-labeled HSPGs at chase time 0 were rapidly lost in Kc (with an initial $t_{1/2} \sim 6$ h, Fig. 4A) and S2 cells (with an initial $t_{1/2} \sim 3$ h, Fig. 4B), respectively. When cell cultures were chased longer than 1 h, the degradation of ^{35}S-labeled HSPGs significantly slowed, approaching $t_{1/2}$ values of >80 h in both Kc (Fig. 4A) and S2 cells (Fig. 4B). About 4% (in Kc cells) and 10% (in S2 cells) of the total amount of ^{35}S-labeled HSPGs at chase time 0 were secreted into medium during the early chase period (Figs. 4A and B, filled circle). The amounts of ^{35}S-labeled HSPGs disappeared from cell but not accounted for by the secretion, 10% in Kc and 11% in S2, indicating cellular degradation of HSPG to small molecules. Radiolabeled molecules at each chase time were further analyzed by Superose 6 chromatography (Fig. 5) as above. During chase 0–1 h, peak B (containing intact HSPG) was observed in Kc cell layer (Figs. 5A–C) and a part of it rapidly decreased, while almost none of the peak C (corresponding to free HS chain) was observed. Later during chase time between 4 and 24 h (Figs. 5D–F), the total amount of intact HSPG slowly decreased and a small peak containing free HS chains emerged (Fig. 5F). In S2 cells, intact HSPG and a small peak of free HS chains were detected at chase time 0 and throughout the chase periods (Figs. 5G–L). Similar to Kc cells, free HS peak slowly increased while intact HSPG peak gradually decreased with chase times in

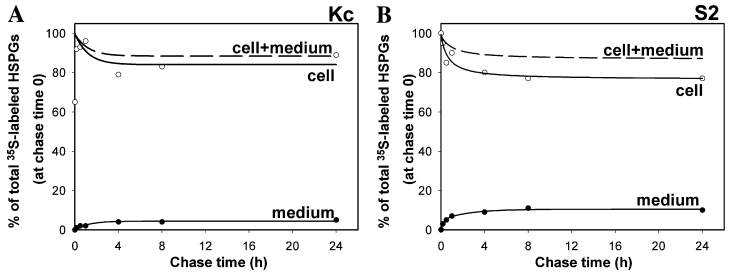

Fig. 4. Chase experiment after 2 h labeling with [^{35}S]sulfate. Cells were labeled with [^{35}S]sulfate for 2 h and chased for different periods after wash. HSPGs were extracted from cell layer and medium fractions, and purified by Sephadex G-50 and Q-Sepharose chromatography. ^{35}S-Labeled HSPGs from Kc (A) and S2 cells (B) were quantified by Q-Sepharose chromatography.

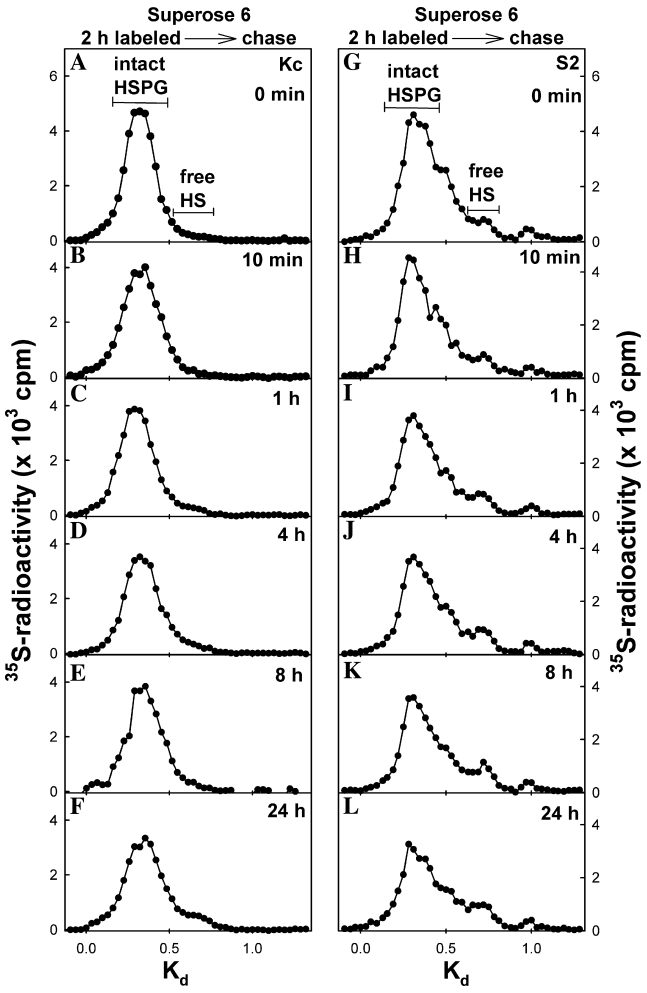

Fig. 5. Superose 6 profiles of ^{35}S-labeled molecules from Kc and S2 cell layers in a 2 h pulse-label/chase experiment. C_k-II peak in Kc cell layer and C_s-II peak in S2 cell layer at various chase times were analyzed by Superose 6 chromatography. (A–F) Analyses for Kc cells and (G–L) those for S2 cells.

S2 cells. These results suggested that free HS chains were metabolically derived from intact HSPG in both cell lines.

Discussion

In the present study, the cellular degradation of HSPGs in *Drosophila* cell lines was characterized by metabolic labeling and pulse-chase experiment using [^{35}S]sulfate as a precursor. The intact HSPGs appear to consist of one major molecular size species in Kc and S2 cells when analyzed by Superose 6 chromatography (peaks at $K_d = 0.35$ and 0.26 for Kc and S2 cells, respectively) (Fig. 5). The approximate molecular sizes estimated by Superose 6 chromatography suggested that intact HSPG in Kc cell is slightly smaller than that in S2 cell (54 and 78 kDa, respectively). In 2 h pulse labeling-chase experiment in both cell lines, intact HSPGs were degraded in multiple phases; in the early phase of chase, HSPG disappeared rapidly (with initial half-lives of ~6 h for Kc and ~3 h for S2 cells) and later, it disappeared slowly (with half-lives >80 h in both Kc and S2 cells) (Fig. 4). The results of Superose 6 chromatographic analysis for 2 h pulse labeling-chase experiment of Kc cells (Figs. 5A–C) indicated that, during the initial chase period, little accumulation of free HS chain (peak C) was observed. Therefore, most of the intact HSPGs should have been degraded into small molecules (probably to free sulfate [4]) without appreciable accumulation as free HS chains during this initial period. This mode of HSPG degradation is similar to that observed for glycosylphosphatidylinositol (GPI)-anchored HSPGs (glypicans) in mammalian cells [13]. Indeed, in the Nakanishi's study, about 30% of the cell surface HSPG in Kc cells was found to be GPI-anchored based on its susceptibility to phosphatidylinositol-specific phospholipase C [7]. In the later chase period, intact HSPGs were slowly degraded mostly into free HS chains (Fig. 5F). The degradation of intact HSPG in S2 cells in early phase was more complex; a small amount of free HS chain was already present at chase time 0, and therefore, its conversion from intact HSPG was not clearly demonstrated in this particular experiment. However, the Nakanishi's study showed that the generation of free HS chains was from the intact HSPG by a shorter pulse labeling-chase experiment [7]. Nevertheless, the degradation of intact HSPG to small molecules was rapid in this early phase as in Kc cell culture as shown in Fig. 4D. The degradation pathway observed during the later chase period in both cell lines, in which intact HSPGs are degraded into free HS chains before their final degradation into monosaccharides and sulfate, may be analogous to that observed for transmembrane HSPGs (syndecans) in mammalian cells [4]. In Kc cells, the amount of free HS chain (peak C) observed in continuous 24 and 48 h labeling experiments (Figs. 1B–C) appeared much more than that expected from the results of 2 h pulse labeling-chase experiments (Fig. 5F). This suggested a possibility that some HSPG species removed by the wash procedure at the end of 2 h pulse labeling period may be involved in the generation of free HS chains as well.

The nature of overall catabolic pathways of HSPG in *Drosophila* appears to be similar to those of HSPG extensively studied in mammalian cells [4,14]. One notable difference observed is that the gene for the heparan sulfate-specific endo-β-glucuronidase, heparanase [15], which cleaves HS chains into intermediate oligosaccharides, is lacking in the genome of *Drosophila*. This is consistent with the results of Superose 6 analysis of free HS chains (peak C) observed in the slow degradation phase, i.e., the molecular size of HS chains were not altered from that in the intact HSPG (data not shown).

Further information about the catabolism of HSPG in *Drosophila*, the intracellular trafficking, and the metabolism of different HSPG species, i.e., GPI-anchored HSPGs (dally [16] and dally-like [17]) and a transmembrane HSPG (D-syndecan [18]), need to be separately obtained since their catabolic pathways may differ like those in mammalian cells [14].

The catabolism of HSPG elucidated in *Drosophila*, together with many genetic studies in this organism revealing critical roles of HSPG in development, would shed light on general biological functions of HSPG in higher organisms as well.

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