Stimulation by Concanavalin A of Cartilage-Matrix Proteoglycan Synthesis in Chondrocyte Cultures*

Weiqun Yan, Kazuhisa Nakashima, Masahiro Iwamoto§, and Yukio Kato§

From the Departments of Biochemistry and Radiology, Faculty of Dentistry, Osaka University, 1-8, Yamadaoka, Suita, Osaka 565, Japan

The effect of concanavalin A on proteoglycan synthesis by rabbit costal and articular chondrocytes was examined. Chondrocytes were seeded at low density and grown to confluency in medium supplemented with 10% fetal bovine serum, and then the serum concentration was reduced to 0.3%. At the low serum concentration, chondrocytes adopted a fibroblastic morphology. Addition of concanavalin A to the culture medium induced a morphologic alteration of the fibroblastic cells to spherical chondrocytes and increased by 3- to 4-fold incorporation of [35S]sulfate and [3H]glucosamine into large chondroitin sulfate proteoglycan that was characteristically found in cartilage. The stimulation of incorporation of labeled precursors reflected real increases in proteoglycan synthesis, as chemical analyses showed a 4-fold increase in the accumulation of macromolecules containing hexuronic acid in concanavalin A-maintained cultures. Furthermore, the effect of concanavalin A on [35S]sulfate incorporation into proteoglycans was greater than that of various growth factors or hormones. However, concanavalin A had smaller effects on [35S]sulfate incorporation into small proteoglycans and [3H]glucosamine incorporation into hyaluronic acid and chondroitinase AC-resistant glycosaminoglycans. Since other lectins tested, such as wheat germ agglutinin, lentil lectin, and phytohemagglutinin, had little effect on [35S]sulfate incorporation into proteoglycans, the concanavalin A action on chondrocytes seems specific. Although concanavalin A decreased [3H]thymidine incorporation in chondrocytes, the stimulation of proteoglycan synthesis could be observed in chondrocytes exposed to the inhibitor of DNA synthesis, cytosine arabinoside. These results indicate that concanavalin A is a potent modulator of proteoglycan synthesis by chondrocytes.

A group of plant lectins induces blastoid transformation of lymphocytes and has been used extensively as a tool for studying the mechanism of cell proliferation (1). However, no information about the effect of lectins on chondrocyte proliferation or differentiation or both is currently available. Since certain lectin(s) may mimic chondrotrrophic actions of growth factor or hormones, in the present study we tested various lectins with a broad spectrum of sugar-binding specificity for their ability to stimulate DNA or proteoglycan synthesis by rabbit chondrocytes. Results show that none of lectins increase [3H]thymidine incorporation into DNA in chondrocytes. On the contrary, several lectins such as concanavalin A (ConA) and phytohemagglutinin (PHA-P) suppressed [3H]thymidine incorporation in chondrocytes. Nevertheless, ConA, but not other lectins, stimulated the incorporation of [35S]sulfate and [3H]radioactivity with glucosamine into cartilage-matrix proteoglycan in chondrocyte cultures. These observations suggest that ConA is a unique lectin that stimulates proteoglycan synthesis by chondrocytes.

EXPERIMENTAL PROCEDURES

Materials—Lectins, methyl-a-D-mannopyranoside, dibutyryl cyclic AMP, insulin, isoproterenol, and phytohemagglutinin (PHA-L) were purchased from Sigma. Transforming growth factor-d1 (bovine) and insulin-like growth factor-I (human) were generously supplied by Dr. Seyedin, Collagen Corporation, Palo Alto, CA and Dr. Horii, Fujisawa Pharmaceutical Co., Osaka, respectively. Bovine parathyroid hormone fragment(1-34) was purchased from Peninsula, Belmont, CA. Dulbecco’s modified Eagle’s medium (DME), Ham’s F-12 medium, and Eagle’s minimum essential medium were obtained from Nissui Pharmaceutical Co., Tokyo, Japan. Fetal bovine serum was from Gibco. Chondroitinase AC was obtained from Seikagaku Kogyo Co. (Tokyo). [35S]Sulfate (carrier-free) was obtained from Japanese Atomic Energy Research Institute (Tokyo). L-[3H]Methionine (800 Ci/mmole) and D-3H]glucosamine (27 Ci/mMole) were obtained from Du Pont-New England Nuclear and Amersham Corp. (United Kingdom), respectively. Monomeric proteoglycans were isolated from rabbit costal cartilages, as described previously (2).

Chondrocyte Cultures—Chondrocytes were isolated from articular cartilage of femur knee joints of 4-week-old New Zealand rabbits as described previously (2, 3), except that cartilage fragments were incubated for 2.5 h with 0.1% crude collagenase (Sigma, Type IA) were purchased from Sigma. Transforming growth factor-d1 (bovine) and insulin-like growth factor-I (human) were generously supplied by Dr. Seyedin, Collagen Corporation, Palo Alto, CA and Dr. Horii, Fujisawa Pharmaceutical Co., Osaka, respectively. Bovine parathyroid hormone fragment(1-34) was purchased from Peninsula, Belmont, CA. Dulbecco’s modified Eagle’s medium (DME), Ham’s F-12 medium, and Eagle’s minimum essential medium were obtained from Nissui Pharmaceutical Co., Tokyo, Japan. Fetal bovine serum was from Gibco. Chondroitinase AC was obtained from Seikagaku Kogyo Co. (Tokyo). [35S]Sulfate (carrier-free) was obtained from Japanese Atomic Energy Research Institute (Tokyo). L-[3H]Methionine (800 Ci/mmole) and D-3H]glucosamine (27 Ci/mMole) were obtained from Du Pont-New England Nuclear and Amersham Corp. (United Kingdom), respectively. Monomeric proteoglycans were isolated from rabbit costal cartilages, as described previously (2).

Determination of the Rate of Proteoglycan Synthesis—When chondrocytes in 6-mm microwells became confluent, they were preincubated for 24 h in 0.1 ml of DME with 0.3% fetal bovine serum. The cells were then incubated for 24 h with various lectins (0.01–20 µg/ml) in 0.1 ml of fresh DME supplemented with 0.3–10% fetal bovine serum in the presence of 0.3 µCi of [35S]sulfate. Alternatively, cells were incubated for 6–36 h with 3 µg/ml of ConA in 0.1 ml of DME with 0.3% fetal bovine serum. Five µl of DME supplemented with 1 µCi of [35S]sulfate was added 6 h before the end of incubation. In some experiments, chondrocytes were seeded in 1 ml of DME with 2% fetal bovine serum in 15-ml plastic centrifuge tubes (Corning, 25019).

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

§ To whom correspondence should be addressed.
of \[^{35}S\]sulfate into material precipitated with cetylpyridinium chloride after treatment with Pronase E (5).

**Relative Hydrodynamic Sizes of Proteoglycans**—Chondrocytes were seeded at a density of \(10^6\) cells/35-mm plastic culture dish and grown in 2 ml of Medium A. They were then incubated for 24 h in the presence or absence of 3 \(\mu\)g/ml of ConA in 2 ml of a 1:1 mixture of DME and Ham's F-12 medium with 0.3% fetal bovine serum. Twenty \(\mu\)l of DME supplemented with 100 \(\mu\)Ci of \[^{35}S\]sulfate was added 6 h before the end of incubation. Alternatively, 20 \(\mu\)l of DME supplemented with 60 \(\mu\)Ci of \[^{3}H\]glucosamine was added 12 h before the end of incubation. The medium was kept frozen at \(-70^\circ C\) until analyzed. Chondrocytes-Rabbit articular chondrocytes were seeded and grown to confluency in Medium A in 35-mm plastic culture dishes and exposed to \[^{3}H\]glucosamine for 12 h in the presence or absence of 3 \(\mu\)g/ml of ConA. The \(^{3}H\)-labeled glycosaminoglycans were extracted from the cell layer and medium fractions and digested with chondroitinase AC, as described previously (2, 6-8). The resulting disaccharides were analyzed by thin-layer chromatography (2, 6, 8).

**Determination of the Rate of DNA Synthesis**—DNA synthesis was determined by measuring incorporation of \[^{3}H\]thymidine into 5% trichloroacetic acid-insoluble cell precipitate (9). DNA content was determined by a fluorometric procedure (11). Total protein was determined by a dye-binding procedure (12).

**RESULTS**

**ConA-induced Morphologic Differentiation of Chondrocytes**—Rabbit chondrocytes were seeded in 35-mm plastic culture dishes in the presence of DME supplemented with 0.3% fetal bovine serum and 3 \(\mu\)g/ml of ConA (0). The cells were exposed to \[^{35}S\]sulfate for 6 h before the end of incubation. Values are averages \(\pm\) S.D. for four cultures.

**Effect of ConA on \[^{35}S\]sulfate Incorporation into Proteoglycans by Chondrocytes**—ConA, at 0.3 (B), 2 (C), or 5 \(\mu\)g/ml (D), was added 24 h before pictures were taken with a phase-contrast photomicroscope.

**Fig. 1. Morphologic appearance of rabbit chondrocytes treated with increasing concentrations of ConA.** Rabbit chondrocytes were grown to 90-95% confluency in Medium A. They were then exposed to DME supplemented with 0.3% fetal bovine serum for 24 h. ConA, at 0.3 (B), 2 (C), or 5 \(\mu\)g/ml (D), was added 24 h before pictures were taken with a phase-contrast photomicroscope.

**Fig. 2. Effect of ConA on \[^{35}S\]sulfate incorporation into proteoglycans by chondrocytes.** A, confluent cultures were exposed for 24 h to DME supplemented with 0.3% fetal bovine serum and increasing concentrations of ConA in the presence of \[^{35}S\]sulfate. Values are averages \(\pm\) S.D. for four cultures. B, confluent cultures were incubated for 6-36 h in DME supplemented with 0.3% fetal bovine serum alone (0) or DME supplemented with 0.3% fetal bovine serum and 3 \(\mu\)g/ml of ConA (O). The cells were exposed to \[^{35}S\]sulfate for 6 h before the end of incubation. Values are averages \(\pm\) S.D. for four cultures.

**Effect of ConA on \[^{35}S\]sulfate Incorporation into Proteoglycans**—Because ConA increased the number of spherical chondrocytes that were surrounded by a refractile matrix (Fig. 1), the effect of ConA on proteoglycan synthesis by chondrocytes was examined. Proteoglycan synthesis was estimated by measuring incorporation of \[^{35}S\]sulfate into macromolecules (glycosaminoglycans) precipitated with cetylpyridinium chloride after protease digestion (5). In cartilage, the majority of sulfated glycosaminoglycans are associated with proteoglycans. When rabbit chondrocytes were incubated with ConA, the incorporation of \[^{35}S\]sulfate into glycosaminoglycans increased in a dose-dependent manner (Fig. 2A). This effect of ConA was detected at a concentration of 0.5 \(\mu\)g/ml and maximal at 3 \(\mu\)g/ml. The level of \[^{35}S\]sulfate incorporation into glycosaminoglycans in the presence of 3 \(\mu\)g/ml of ConA was 2.5 times that in its absence. At a high concentration (20 \(\mu\)g/ml), ConA slightly suppressed \[^{35}S\]sulfate incorporation into glycosaminoglycans (Fig. 2A).

**Effect of ConA on \[^{35}S\]sulfate Incorporation into Proteoglycans**—Because ConA increased the number of spherical chondrocytes that were surrounded by a refractile matrix (Fig. 1),
sulfate incorporation into glycosaminoglycans in the cell layer and medium fractions in 0.3% serum, but not in 10-15% serum (Fig. 3). The lack of the ConA effect on proteoglycan synthesis in the presence of high concentrations of serum may be due to its binding to serum glycoproteins.

Table I shows that the ConA-induced stimulation of $^{35}$S sulfate incorporation into glycosaminoglycans is abolished by 10 mM methyl-$\alpha$-D-mannopyranoside. Methyl-$\alpha$-D-mannopyranoside alone did not have any effect on $^{35}$S sulfate incorporation into glycosaminoglycans. Methyl-$\alpha$-D-mannopyranoside also suppressed the ConA-induced change in the chondrocyte morphology (not shown).

Effects of ConA on Syntheses of Cartilage Proteoglycan and Low Molecular Weight Proteoglycan—Next we analyzed the hydrodynamic sizes of $^{35}$S-labeled proteoglycans synthesized by chondrocytes exposed or not to ConA by gel exclusion chromatography on Sepharose CL-2B. The elution profiles are shown in Fig. 4A. Chondrocytes produce two proteoglycan species: a large proteoglycan characteristically found in the cartilage matrix and small proteoglycans found in both non-chondrogenic and chondrogenic cells (14-17). ConA increased $^{35}$S sulfate incorporation into the large proteoglycan synthesized by articular chondrocytes 2.4-fold (Fig. 4A). ConA also increased $^{35}$S sulfate incorporation into the large proteoglycan synthesized by growth plate chondrocytes 3-fold (data not shown). The effect of ConA on $^{35}$S sulfate incorporation into the large proteoglycan was greater than its effect on $^{35}$S sulfate incorporation into small proteoglycans. No changes in the monomer size of the large proteoglycan (Fig. 4) or the length of the glycosaminoglycan chains associated with the large proteoglycan were detected in ConA-exposed chondrocyte cultures (data not shown).

Effect of ConA on $^{3}H$ Glucosamine Incorporation into Glycosaminoglycans—To examine whether ConA might increase not only the incorporation of $^{35}$S sulfate but also that of $^{3}H$ glucosamine into proteoglycans, we incubated chondrocytes with or without 3 μg/ml of ConA. The proportions of $^{3}H$ radioactivity incorporated into hyaluronic acid (HA), chondroitin 6-sulfate (C-6S), chondroitin 4-sulfate (C-4S), chondroitin (C-OS), and chondroitinase AC (Ch-ase)-resistant material were shown. Similar results were obtained in two independent series of studies.

![Fig. 4. Sepharose CL-2B chromatography of proteoglycans from cultures grown in the presence (solid line) or absence (broken line) of ConA.](http://example.com/fig4.png)

**TABLE I**

| Addition | $^{35}$S Sulfate incorporation (dpm x 10$^3$/well) |
|----------|-----------------------------------------------|
| None     | 94 ± 15                                       |
| ConA     | 236 ± 13                                      |
| ConA + MeMan | 105 ± 10                                   |
| MeMan    | 108 ± 7                                       |

**TABLE II**

| Fraction | $^{3}H$ Glucosamine uptake (% of total incorporation) |
|----------|-----------------------------------------------------|
| Cell layer | HA C-6S C-4S C-OS Ch-ase-resistant 6/4 | 18.1 2.9 1.6 1.5 1.7 |
| Medium     | HA C-6S C-4S C-OS Ch-ase-resistant 6/4 | 38.1 18.1 17.6 17.6 17.6 |

*Ratio of C-6S to C-4S.*

The $^{3}H$-labeled glycosaminoglycans were obtained from the cell layer and medium fractions of confluent rabbit chondrocytes with or without 3 μg/ml of ConA. The proportions of $^{3}H$ radioactivity incorporated into hyaluronic acid (HA), chondroitin 6-sulfate (C-6S), chondroitin 4-sulfate (C-4S), chondroitin (C-OS), and chondroitinase AC (Ch-ase)-resistant material were shown. Similar results were obtained in two independent series of studies.
Differentiated Chondrocytes—Rabbit chondrocytes exposed to retinoic acid became fibroblastic and lost their ability to synthesize the large proteoglycan (Fig. 5), as expected from previous studies (19). ConA did not increase $^{[35]S}$sulfate incorporation into proteoglycans synthesized by the dedifferentiated cartilage cells (Fig. 5). The dedifferentiated cartilage cells did not recover their proper cell shape even in the presence of ConA. Retinoic acid may cause chondrocytes to lose cell surface glycoproteins which are required for the ConA stimulation of proteoglycan synthesis. In any case, ConA seems to act selectively on overtly differentiated chondrocytes.

Comparison of the Effect of ConA on Proteoglycan Synthesis with Growth Factor and Hormone Stimulation of Proteoglycan Synthesis—Insulin (14, 20), insulin-like growth factor-I (21), transforming growth factor-β-1 (22), parathyroid hormone (23–25), and dibutyryl cyclic AMP (24, 25), at their optimal concentrations (14, 20–25), increased the incorporation of $^{[35]S}$sulfate into glycosaminoglycans synthesized by chondrocytes 1.4- to 2.2-fold (Table III). On the other hand, ConA increased the incorporation of $^{[35]S}$sulfate into glycosaminoglycans 3.3-fold. The effect of ConA on $^{[35]S}$sulfate incorporation into glycosaminoglycans was consistently greater than that of growth factors or hormones in several series of experiments.

Lectin Specificity of the ConA Stimulation of Proteoglycan Synthesis—The lectin specificity of the ConA effect on proteoglycan synthesis was examined by exposing rabbit chondrocytes to various lectins that were shown previously to stimulate proliferation of lymphocytes. Lentil lectin, WGA, Phaseolus vulgaris agglutinin (PHA-L), PHA-P, garden pea lectin, lima bean lectin, UEA, or Scotch broom lectin did not have any substantial effect on $^{[35]S}$sulfate incorporation into glycosaminoglycans at concentrations ranging from 0.1 to 20 μg/ml (Table IV). Lotus lectin and Maclura pomifera agglutinin (MPA, 1 and 10 μg/ml) also had little effect on $^{[35]S}$sulfate incorporation into glycosaminoglycans (data not shown).

Chondrocytes exposed to WGA or garden pea lectin became spherical or polygonal within 2 days, although they had a marginal effect on proteoglycan synthesis (Table IV). Abrin lectin (Abrus precatorius), at 1 μg/ml, caused cell death (not shown).

Effects of Various Lectins on $^{[3]H}$Thymidine Incorporation—Although ConA is a potent mitogen for lymphocytes (1), in chondrocyte cultures it decreased $^{[3]H}$thymidine incorporation into DNA in a dose-dependent manner with an ED₅₀ of 0.4–1 μg/ml in the presence of various concentrations of serum ranging from 0.3 to 20% (Fig. 6). Note that ConA stimulated proteoglycan synthesis by chondrocytes in 0.3–3% serum, but not in 10–15% serum (see Fig. 3). This discrepancy suggests that ConA affects the syntheses of DNA and proteoglycans in chondrocytes by different mechanisms. ConA, at 1–3 μg/ml, also suppressed the proliferation of chondrocytes, dedifferentiated cartilage cells, and fibroblasts in monolayer cultures (data not shown). The inhibition of cell division was not explained by binding of ConA to serum mitogens, because ConA, at 1–5 μg/ml, suppressed DNA synthesis in chondrocytes in the absence of serum or in the presence of serum fraction which eluted from a ConA-Sepharose column.²

² Y. Kato and W. Yan, unpublished data.

![Cell Layer ConA Control](image)

**Fig. 5.** Effect of ConA on syntheses of large and small proteoglycans by chondrocytes whose phenotypic expression is suppressed by treatment with retinoic acid. Before chondrocytes became confluent, they were preincubated with or without 0.02 μM of retinoic acid in Medium A for 4 days. They were then incubated for 24 h in Medium A with 0.3% fetal bovine serum with or without 0.02 μM of retinoic acid in the presence or absence of 3 μg/ml of ConA. $^{[35]S}$Sulfate (100 μCi/culture) was added to the medium 6 h before the end of incubation. Samples were applied onto a Sepharose CL-2B column as described in Fig. 4.

| Addition     | $^{[35]S}$Sulfate incorporation (μg/ml) | % of control |
|--------------|----------------------------------------|-------------|
| None         | 104 ± 17                                | 100         |
| ConA 3 μg/ml | 114 ± 17                                | 118         |
| Insulin 1 μg/ml | 93 ± 17                             | 114         |
| IGF-1 0.2 μg/ml | 93 ± 17                           | 114         |
| PTHb 10−7 M | 93 ± 17                                 | 114         |
| Dibutyryl cyclic AMP 0.5 mM | 93 ± 17                       | 114         |
| TGF-β 3 ng/ml | 93 ± 17                                 | 114         |

*Insulin-like growth factor-1.
Parathyroid hormone active fragment (1–34).
Transforming growth factor-β-1.

| TABLE III Comparison of the effect of various growth factor and hormones on $^{[35]S}$sulfate incorporation into proteoglycans with that of ConA |
|----------------------------------|-----------------|-----------------|
| Addition                        | $^{[35]S}$Sulfate incorporation (dpm × 10⁻⁶/well) | % of control |
| None                            | 184 ± 15                    | 100         |
| ConA                            | 604 ± 23                   | 328         |
| Insulin                         | 365 ± 27                   | 144         |
| IGF-1                           | 287 ± 21                   | 116         |
| PTHb                            | 307 ± 54                   | 167         |
| Dibutyryl cyclic AMP             | 345 ± 34                   | 188         |
| TGF-β                           | 409 ± 35                   | 222         |

**TABLE IV**

Effect of various lectins on the incorporation of $^{[35]S}$sulfate into proteoglycans in rabbit chondrocyte cultures

Rabbit chondrocytes in confluent cultures were exposed for 24 h to DME supplemented with 0.3% fetal bovine serum and increasing concentrations of various lectins. Values are averages for six to eight cultures in two independent series of experiments.

| Addition | Morphologic change | $^{[35]S}$Sulfate incorporation (μg/ml) | % of control |
|----------|--------------------|----------------------------------------|-------------|
| ConA     | +++                | 100 ± 15                               | 100         |
| Lentin   | –                  | 93 ± 17                                | 114         |
| WGA      | ++                 | 93 ± 17                                | 114         |
| PHA-L    | –                  | 93 ± 17                                | 114         |
| PHA-P    | +                  | 93 ± 17                                | 114         |
| Garden pea | +                  | 93 ± 17                                | 114         |
| Lima bean| –                  | 93 ± 17                                | 114         |
| UEA (I + II) | –                | 93 ± 17                               | 114         |
| Scotch broom | –                  | 93 ± 17                               | 114         |

* Morphologic changes were observed 48 h after the addition of 10 μg/ml of lectins.
* Not determined.
ConA Actions in Chondrocytes

Fig. 6. Effect of ConA on [3H]thymidine incorporation in rabbit chondrocytes in culture. Quiescent cultures maintained in 6-mm wells were exposed to DME supplemented with 0.3 (○), 2 (●), 10 (▲), or 20% (△) fetal bovine serum in the presence of increasing concentrations of ConA. After 24 h, they were labeled with [3H]thymidine for 3 h. Values are averages for three cultures. The level of [3H] radioactivity incorporated into DNA in ConA-free chondrocytes was 1051, 4365, 39972, and 89764 dpm/well at 0.3, 2, 10, and 20% serum, respectively. The standard deviation in the different determinations did not exceed 10% of the average.

Fig. 7. Effect of various lectins on [3H]thymidine incorporation in rabbit articular chondrocytes in culture. Quiescent cultures maintained in 6-mm wells were exposed to DME supplemented with 0.3% fetal bovine serum in the presence of increasing concentrations of ConA (▲), WGA (●), UEA (○), PHA-L (□), lentil lectin (▲), or garden pea lectin (●) (upper panel). Alternatively, cultures were exposed to increasing concentrations of MPA (○), lima bean (□), Lotus (□), or Scotch broom lectin (▲) (lower panel). After 24 h, they were labeled with [3H]thymidine for 3 h. The level of [3H] radioactivity incorporated into DNA in lectin-free chondrocytes was 1115 dpm/well. Values are averages for three cultures. The standard deviation in the different determinations did not exceed 10% of the average.

The inhibition of chondrocyte proliferation does not seem to be specific to ConA. WGA, lentil lectin, PHA-P, garden pea, and UEA (I + II) also decreased, dose-dependently, [3H]thymidine incorporation into DNA in chondrocytes (Fig. 7). Other lectins had little effect on [3H]thymidine incorporation at 0.01-20 μg/ml (Fig. 7).

Table V shows that ConA, at 3 μg/ml, increases [35S]sulfate incorporation into glycosaminoglycans (Figs. 2-4). However, this might reflect changes in pool sizes rather than real increase in the synthetic activity. Therefore, chemical analyses were carried out to confirm the ConA stimulation of proteoglycan synthesis (Fig. 8). In subconfluent cultures, chondrocytes were incubated in the presence of 2% serum with ConA (0.05-10 μg/ml) for 48 h. The addition of ConA, at 3-10 μg/ml, resulted in a 4-fold increase in the accumulation of macromolecules containing hexuronic acid (proteoglycans) (Fig. 8A), whereas it sup-
pressed the increase of DNA during a 48-h period (Fig. 8C). Accordingly, the uronic acid content/µg DNA of the ConA exposed cultures was 10 times that of ConA-free cultures. ConA also increased the protein content to a lesser degree (Fig. 8B). In this situation, ConA promoted the conversion of proliferating chondrocytes into maturing chondrocytes that produced matrix proteoglycans.

Note that ConA did not increase the uronic acid content of chondrocyte cultures at 1 µg/ml (Fig. 8A) that abolished DNA synthesis (Fig. 8C). Cytosine arabinoside also had little effect on the uronic acid content (Fig. 8A), when it abolished DNA synthesis (Fig. 8C). Thus, the inhibition of DNA synthesis is not sufficient for the induction of proteoglycan synthesis.

**Effect of ConA on Proteoglycan Synthesis by Chondrocytes in Suspension Culture**—Freshly isolated chondrocytes were exposed to ConA in suspension cultures. The addition of ConA resulted in 1.3- to 1.5-fold increase in [35S]sulfate incorporation into glycosaminoglycans in two independent series of experiments (Table VI).

**Effect of ConA on the Cyclic AMP Level**—Previous studies have shown that parathyroid hormone increases the intracellular cyclic AMP concentration when it stimulates proteoglycan synthesis by chondrocytes (24, 25). However, the addition of ConA (3 µg/ml) resulted in only 1.5- to 2.5-fold increase in the cyclic AMP level after 10-120 min (data not shown), whereas parathyroid hormone increased the cyclic AMP level 40-fold in 2-5 min (24, 25). Thus, cyclic AMP does not seem to play a second messenger role in ConA-stimulated chondrocytes.

**DISCUSSION**

The present study showed that ConA has little effect on the monomer size of cartilage-matrix proteoglycan and the degree and position of sulfation of the glycosaminoglycan side chains. However, the lectin increased by 3- to 4-fold the incorporation of [35S]sulfate and [3H]glucosamine into cartilage proteoglycan synthesized by rabbit chondrocytes in early confluent cultures. The stimulation of incorporation of labeled precursors reflected real increases in proteoglycan synthesis, in that chemical analyses showed a marked increase in the accumulation of macromolecules that contained uronic acid in ConA-maintained cultures. However, ConA had less effects on the incorporation of [35S]sulfate or [3H]glucosamine into hyaluronic acid, chondroitinase AC-resistant glycosaminoglycans, and small proteoglycans. These results provide evidence that ConA selectively increases the synthesis of cartilage-matrix proteoglycan by chondrocytes.

Although ConA promoted conversion of proliferating chondrocytes into maturing chondrocytes in certain culture conditions, the stimulation of proteoglycan synthesis was not secondary to the inhibition of DNA synthesis. Even in the presence of cytosine arabinoside, ConA increased proteoglycan synthesis by chondrocytes. Furthermore, cytosine arabinoside alone had little effect on proteoglycan synthesis, when it inhibited DNA synthesis.

ConA, WGA, and several other lectins have been shown to mimic the effect of insulin in adipocytes (1, 26-28). However, their insulin-like effect is less than that of insulin itself. Although ConA may have direct effect on insulin receptor (26, 28), the extent of the stimulation of [35S]sulfate incorporation into glycosaminoglycans by ConA was greater than that of the stimulation induced by the optimal concentration of insulin. Furthermore, WGA had little effect on [35S]sulfate incorporation into glycosaminoglycans. These observations suggest that the ConA stimulation of proteoglycan synthesis is not simply due to activation of insulin receptor.

It is well known that there is a close correlation between rounded cell shape and chondrocyte phenotypic expression (29, 30). Zanetti and Solursh (30) have shown that cytochalasin D that induces cell shape changes from flat to spherical stimulates chondrogenic differentiation of limb bud mesenchymal cells. Thus, the ConA stimulation of proteoglycan synthesis by chondrocytes in monolayer cultures might be due to the change in morphology from flat to spherical. However, WGA and garden pea lectin induced similar cell shape changes without any increase in proteoglycan synthesis. Furthermore, ConA increased 1.3- to 1.5-fold [35S]sulfate incorporation into glycosaminoglycans in suspension cultures where chondrocytes maintained a spherical configuration. ConA (5-10 µg/ml) also increased [35S]sulfate incorporation into glycosaminoglycans by 1.6- to 2.2-fold in late confluent stage of chondrocyte cultures where 85-90% of the cells had already become spherical. Thus, the rounded cell shape alone

**TABLE VI**

**Effect of ConA on proteoglycans synthesis by chondrocytes in suspension cultures**

Rabbit chondrocytes in suspension cultures were exposed for 24 h to 5 µg/ml of ConA in the presence of DME supplemented with 2% fetal bovine serum. Values are averages ± S.D. for four cultures.

| Experiment | ConA | [35S]Sulfate incorporation | dpn × 10³/culture | % |
|------------|------|---------------------------|-----------------|---|
| I.         | -    | 128 ± 9                  | 100             |   |
|            | +    | 189 ± 12*                | 140             |   |
| II.        | -    | 101 ± 6                  | 100             |   |
|            | +    | 132 ± 8*                 | 131             |   |

*p < 0.01%.

*p < 0.05% versus control.

**TABLE VII**

**Lectin actions on lymphocytes and chondrocytes**

| Lectin | Sugar specificity | Lymphocyte activation | Inhibition of DNA synthesis | Morphologic change | Stimulation of proteoglycan synthesis |
|--------|------------------|-----------------------|----------------------------|-------------------|-------------------------------------|
| ConA   | D-Man, b-Glc     | +                     | +                          | +                 | +                                   |
| WGA    | (D-GlcNAc), NeuNAc | +                | +                          | +                 | +                                   |
| Garden pea | D-Man, b-Glc   | +                     | +                          | +                 | +                                   |
| PHA-L  | Oligosaccharide | +                     | +                          | -                 | +                                   |
| PHA-P  | Oligosaccharide | +                     | +                          | -                 | -                                   |
| Lentil | D-Man, b-Glc    | +                     | +                          | +                 | +                                   |
| UEA (1 → II) | n-PAc (n-PAc-NAc) | +                   | +                          | +                 | +                                   |
| Lima bean | D-GalNAc        | +                     | +                          | +                 | +                                   |
| Scotch broom | D-Gal, D-GalNAc | +                     | +                          | +                 | +                                   |
| Lotus  | L-Fuc            | -                     | -                          | -                 | -                                   |
| MPA    | D-Gal            | -                     | -                          | -                 | -                                   |
is insufficient to account for the stimulation of proteoglycan synthesis by ConA.

It may not be surprising that plant lectins affect proliferation or differentiation of chondrocytes, because they can bind to many cell surface glycoproteins, some of which must be involved in receiving microenvironmental information. Interestingly enough, the ConA action on proteoglycan synthesis by chondrocytes is, however, specific (Table VII). The specificity of the ConA effect was demonstrated by the five observations. (a) While various plant lectins with distinct sugar-binding properties, as listed in Table VII, have potent mitogenic actions in lymphocytes (1), none of them, besides ConA, enhanced proteoglycan synthesis by chondrocytes. (b) Several lectins including ConA suppressed DNA synthesis in chondrocytes. (c) WGA, ConA, and garden pea lectin altered the morphology of chondrocytes, but only ConA enhanced their proteoglycan synthesis. (d) ConA increased proteoglycan synthesis by chondrocytes at low concentrations, whereas lentil lectin, whose sugar-binding specificity is similar to that of ConA (1) (Table VII), did not increase proteoglycan synthesis even at high concentrations. This could be explained by the fact that lentil lectin has lower affinity for mannose and glucose (1). Furthermore, lentil is a dimer, whereas ConA is a tetramer (1). (e) The stimulation of proteoglycan synthesis by ConA was greater than the stimulation by hormones or growth factors that were shown previously to increase proteoglycan synthesis. Furthermore, the ConA stimulation of proteoglycan synthesis was abolished by 10 mM methyl-α-D-mannopyranoside. These observations suggest that cell surface glycoprotein(s) that has N-linked sugar chains specific for ConA plays a special role in the control of proteoglycan synthesis by chondrocytes.

Recent studies showed that the COOH-terminal domain of cartilage proteoglycan core protein elicits lectin-like activity (31). However, it remains unknown whether animal lectins are involved in the control of proteoglycan synthesis in cartilage in vivo.

In conclusion, the present study showed that ConA is a specific modulator of proteoglycan synthesis by chondrocytes. Because the molecular structure of ConA and its mode of actions have been extensively characterized (1), chondrocytes exposed to this lectin will be useful as a novel model in studying the role of cell surface glycoproteins in the control of cellular differentiation.

Acknowledgments—We thank Dr. Dennis K. Fujii, Sterling Drug Inc. and Dr. Fujio Suzuki for critical comments on the manuscript and Dr. Tatsuya Koike, Osaka City University, who determined the cAMP level in chondrocytes.

REFERENCES

1. Lies, H., and Sharon, N. (1986) in The Lectins (Liener, I. E., Shafritz, N., and Goldstein, I. J., eds) pp. 265-291, Academic Press, Orlando, FL.
2. Kato, Y., and Gospodarowicz, D. (1985) J. Cell Biol. 100, 486-495.
3. Shimomura, Y., Yokoda, T., and Suzuki, F. (1975) J. Biol. Chem. 250, 179-183.
4. Iwamoto, M., Sato, K., Nakashima, K., Fujihata, H., Suzuki, F., and Kato, Y. (1980) Biochem. Biophys. Res. Commun. 159, 1006-1011.
5. Kato, Y., Nomura, Y., Tsuji, M., Ohmoe, H., Nakazawa, T., and Sato, K. (1981) J. Cell. Physiol. 109, 58-63.
6. Kato, Y., and Gospodarowicz, D. (1965) J. Biol. Chem. 260, 2364-2373.
7. Yamagata, T., Saito, H., Abuchi, O., and Suzuki, S. (1968) J. Biol. Chem. 243, 1523-1535.
8. Mason, R. M., Kimura, J. H., and Hascall, V. C. (1982) J. Biol. Chem. 257, 2236-2245.
9. Kato, Y., Hiraki, Y., Inoue, Y., Yutani, Y., and Suzuki, F. (1983) Eur. J. Biochem. 129, 685-690.
10. Homma, M., Satoh, T., Takevaza, J., and Ui, M. (1977) Biochem. Med. 16, 257-273.
11. Johnson-Wint, B., and Hollis, S. (1982) Anal. Biochem. 122, 338-344.
12. Bradford, M. M. (1976) Anal. Biochem. 72, 248-254.
13. Sibbiter, T., and Muir, H. M. (1982) Anal. Biochem. 4, 320-324.
14. Stevens, R. L., and Haswell, W. C. (1981) J. Biol. Chem. 256, 2053-2058.
15. Carrino, D. A., Lennnon, D. P., and Caplan, A. L. (1983) Dev. Biol. 99, 132-144.
16. Benya, P. D., and Shaffer, J. D. (1982) Cell 30, 215-224.
17. Zanetti, N. C., and Solursh, M. (1981) J. Cell. Physiol. 106, 259-268.
18. Osen, M., Kenyon, P., Mumford, R. A., and Green, B. G. (1981) Biochemistry 20, 5800-5809.
Stimulation by concanavalin A of cartilage-matrix proteoglycan synthesis in chondrocyte cultures.

W Q Yan, K Nakashima, M Iwamoto and Y Kato

J. Biol. Chem. 1990, 265:10125-10131.