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# NON-SPECIFIC INTERACTION OF PROTEOGLYCANS WITH SURFACES AND MATRICES

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Summary: Evidence is presented that reversible non-specific adsorption of proteoglycans (PGs) to surfaces and matrices is an inherent property of the PGs. This adsorption is dependent on the intact PG structure as the glycosaminoglycans (GAGs), which are isolated after papain digestion of the PG show no such non-specific adsorption. The interaction of the PG with surfaces and matrices is also highly dependent on the internal milieu and can be both inhibited and enhanced by such factors as the ionic composition and concentration, pH, detergents and chaotropic reagents such as guanidine hydrochloride (Gdn-HCl). It is suggested that this inherent stickiness of the PGs allows them to function like a reversible fluid adhesant in the connective tissues. This weak binding force thus not only aids in maintaining the integrity of the connective tissues, but its reversible nature may provide for easy movement of other materials through the connective tissue matrix. 

Introduction: A large number of interactions of the GAGs and PGs with tissue macromolecules and cell membranes have been described and discussed (1-10), including the specific associations of hyaluronic acid and the globular domains of the core-proteins of PGs from cartilage and other tissues (6,7). This interaction is stabilized by link proteins, which have affinity for both the binding region of the PG and the hyaluronate (6,9,11). Another important interaction of PGs from various tissues is their highly regular association with collagens (8,12-15). This association probably occurs between the PG protein cores and the collagen fibrils (13,14) although there may also be interactions between the GAG side chains of the bound PGs (13). A large body of data also has accumulated on the interaction of arterial PGs with lipoproteins, suggesting that they play an important role in the development of atherosclerotic lesions (2). These associations appear to depend not only on the degree of sulfation of the GAGs, but also on their other chemical characteristics since GAGs and PGs from different sources showed variable LDL binding affinities (16). There is also considerable evidence for an association between elastin and PGs (17). In a recent report the binding of heparins to various proteins has been discussed and forty-nine regions in twenty-one proteins were identified as potential heparin-binding sites (18). The interaction of cartilage PGs

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with a variety of matrix proteins via thiol-disulphide interchange has also been demonstrated (19). The cell membrane associated PGs (3,4,20) have been shown to interact with a large number of extracellular substrates including lipoprotein lipase (21), thrombospondin (22), the matrix glycoprotein fibronectin (23,24) and the basal lamina separating the nerve terminal and the post synaptic cell of the electric organ (25). It has also been demonstrated that the PGs bind to the matrix proteins, cytotactin (26), chondronectin (27) and laminin (28). Other interactions, which have been observed include the association of heparan sulfate PGs with unidentified protein(s) to modulate their affinity binding to fibronectin (29) and inter-associations between PG molecules extracted from pig arterial smooth muscle cells (30). Finally, specific binding of PGs to concanavalin A-Sepharose 4B (31) as well as a lectin-like activity in a segment of the protein core (32) have also been shown.

In the various interactions described above the binding involves the protein core, the carbohydrate chains or both. In addition, virtually all of these associations and interactions are between the PGs and other tissue macromolecules or cell membranes. There has been very little discussion of the non-specific binding properties of the PGs although these properties are encountered during the isolation and analysis of these macromolecules. The present study addresses the non-specific adhesive properties of the PGs and demonstrates that these non-specific interactions between matrices and surfaces are highly dependent on the internal milieu.

Materials and Methods: Controlled pore glass, PG-3000-200 (CPG), glyceryl controlled pore glass, GG-3000-400 (G-CPG) and Bis-Tris Propane (1,3-bis[tris(hydroxymethyl)methylamino]propane) were purchased from Sigma Chemical Co. (St. Louis, MO). Glass beads (diameter 250-149 m) were obtained from Johns Scientific (Toronto, Ont.). Silica gel 60 (70-230 mesh) was obtained from BDH Chemicals (Toronto, Ont.). Cellulose powder (Cellex-410) was purchased from Bio-Rad Laboratories (Mississauga, Ont.). Sepharose CL-2B, Sephacryl S-1000, Sephadex G-50 (fine), G-25 (superfine) and G-15 were obtained from Pharmacia Fine Chemicals (Dorval, Quebec). Test tubes: polystyrene, Falcon 2057 (17x100 mm), polystyrene and polypropylene Fisherbrand (12x75 mm) and borosilicate glass Fisherbrand (10-75 mm) were purchased from Fisher Scientific Co. (Pittsburgh, PA). All other chemicals and reagents were obtained from sources described previously (33,34).

The preparation of labeled PG from bovine ankle cartilage (PG-BAC), unlabeled PG from bovine nasal septum (PG-BNS), labeled PG from human mononuclear cells (PG-HMC) has been performed as described previously (33). Both of the labeled PGs were extracted and purified in the presence of protease inhibitors. The labeled GAGs, GAG-BAC and GAG-HMC, were prepared from PG-BAC and PG-HMC respectively by papain digestion of the PG as described previously (34). Quantitation of the GAGs and PGs was performed by the Alcian blue-DMSO method (33,35). The amounts of the PGs and GAGs are expressed in terms of their hexuronic acid content.

The chromatography media used in all of the experiments, except for those shown in Table 2, were equilibrated with the buffer in which they were to be studied for at least 4h. They were suction filtered on a sintered glass (coarse) Buchner funnel and rinsed with the same buffer (10 ml). The

media were then suspended in fresh buffer to give a final volume of 1.5 or 3.0 ml. After the mixtures were cooled to 4°C the PG or GAG was added and the contents were gently mixed in a 4-8°C cold room. The media were allowed to settle to the bottom of the tubes by gravity and aliquots of the supernatant were removed for analysis as indicated in each experiment.

Results: The non-specific adsorption of labeled PG from bovine ankle cartilage to a variety of chromatography media and test tube surfaces in four different buffers is illustrated in Table 1. The magnitude of this adsorption is inversely related to the PG remaining in solution (Table 1). In addition, it can be calculated for controlled pore glass (CPG) that there is negligible adsorption to CPG and test tube surfaces when the mixtures are incubated in buffer C (0.05 M Tris and 0.5%, W/V Chaps at pH 8.0). Since the excluded volume (the volume not accessible to the PG) for the different media varies widely because of different pore sizes, the solution volume available to the PGs also varies. Thus, the percent of added PG remaining in solution, in the other three buffers, was calculated relative to that observed in buffer C, where it is assumed to be 100%. As seen in Table 1,

Table 1: Comparison of non-specific adsorption of tritium labeled PG to various chromatography media and surfaces in four different buffers [a]

| Chromatography media | % Of added DPM remaining in solution [b] | | | |
|---|---|---|---|---|
| | Buffer A | Buffer B | Buffer C | Buffer D |
| CPG[c] | 103(440) | 13(54) | 100(429) | 15(65) |
| G-CPG[d] | 56(327) | 9(52) | 100(579) | 12(70) |
| Glass beads | 87(583) | 63(418) | 100(668) | 69(458) |
| Silica gel | 86(520) | 27(164) | 100(606) | 29(177) |
| Cellulose | 50(275) | 7(38) | 100(550) | 7(40) |
| Sepharose CL-2B | 74(454) | 39(241) | 100(617) | 47(288) |
| Sephacryl S-1000 | 89(561) | 24(154) | 100(633) | 38(243) |
| Sephadex G-50 | 73(532) | 30(221) | 100(731) | 28(203) |
| Sephadex G-25 | 84(571) | 12(83) | 100(682) | 28(188) |
| Sephadex G-15 | 90(672) | 22(167) | 100(748) | 51(379) |

(a) The four buffers used here are: A, 0.05 M Tris-HCl pH 9.0; B, 0.05 M NaOAc + 0.5 M Gdn-HCl pH 5.8; C, 0.05 M Tris-HCl + 0.5% (w/v) Chaps pH 8.0; D, 0.05 M NaOAc + 4.0 M Gdn-HCl pH 5.8.
(b) A constant amount of each of the chromatography media (0.60-0.80 ml, wet volume) was equilibrated and rinsed with one of the four buffers and then suspended in the same buffer to give a final volume of 1.5 ml. To each mixture, labeled PG (PG-BAC, 6.68 x $10^3$ DPM, 1.1 $\mu$g of hexuronic acid equivalent, in 25 1 of ddd water) was added and the mixtures were gently shaken for 10 hrs at 8°C. The chromatography media was allowed to settle to the bottom of the tube and 100 $\mu$l of the supernatant was removed for radioactivity measurement. The percentages are expressed relative to the values obtained in buffer C, where it is assumed that 100% of the labeled PG is in solution. The actual radioactivities measured are shown in brackets.
(c) CPG is controlled pore glass.
(d) G-CPG is glyceryl controlled pore glass.

in buffer A (0.05 M Tris at pH 9.0) there is some adsorption of the PG in the presence of all media except for CPG and it is highest in the presence of cellulose and G-CPG. The highest degree of adsorption occurred when the mixtures were incubated in buffer B (0.05 M sodium acetate and 0.5 M Gdn-HCl at pH 5.8). In this buffer, almost all of the PG becomes adsorbed in tubes containing CPG, G-CPG, cellulose and Sephadex G-25 and an amount greater than 50% is adsorbed in the presence of all the other materials except for the glass beads. As shown in Table 1, somewhat less PG is adsorbed in buffer D (0.05 M sodium acetate and 4.0 M Gdn-HCl at pH 5.8), however, there is still almost complete adsorption in the presence of CPG, G-CPG or cellulose and in the presence of most media there is more than 50% adsorption.

Using CPG and the same four buffers the interactions of PGs from bovine ankle cartilage (PG-BAC) and human mononuclear cells (PG-HMC) and their GAGs, which were obtained by papain digestion of the PGs and isolated by the cetyl pyridinium chloride precipitation procedure (34), are compared in Table 2. In these experiments the PGs or the GAGs were first incubated in

Table 2: Non-specific adsorption of PGs and GAGs to polystyrene tubes and controlled pore glass (CPG) (a)

| Sample | Buffer | % Of DPM in solution after incubation in buffer alone | % Of DPM in solution after incubation in the presence of CPG |
|---|---|---|---|
| PG-BAC | A | 89 | 92 |
| PG-BAC | B | 33 | 21 |
| PG-BAC | C | 101 | 86 |
| PG-BAC | D | 52 | 15 |
| GAG-BAC | A | 102 | 102 |
| GAG-BAC | B | 102 | 94 |
| GAG-BAC | C | 102 | 101 |
| GAG-BAC | D | 99 | 101 |
| PG-HMC | A | 99 | 105 |
| PG-HMC | B | 79 | 87 |
| PG-HMC | C | 104 | 107 |
| PG-HMC | D | 99 | 97 |
| GAG-HMC | A | 99 | 100 |
| GAG-HMC | B | 101 | 103 |
| GAG-HMC | C | 100 | 101 |
| GAG-HMC | D | 99 | 99 |

(a) A constant amount of labeled PG (PG-BAC, $7.07 \times 10^3$ $^3$H DPM, 1.2 $\mu$g of hexuronic acid equivalent; PG-HMC, $37.8 \times 10^3$ $^3$H DPM, 0.44 $\mu$g of hexuronic acid equivalent) or GAG (GAG-BAC, $4.99 \times 10^3$ $^3$H DPM, 1.0 $\mu$g of hexuronic acid equivalent; GAG-HMC, $5.16 \times 10^3$ $^3$H DPM, 6.0 $\mu$g of hexuronic acid equivalent) in 25 $\mu$l of ddd water was added to 1.5 ml of each buffer in polystyrene tubes. The tubes were gently shaken at 4°C for 1 hr and 50 $\mu$l was removed for radioactivity measurement. To each tube was added 0.25 g of CPG and after gentle mixing at 4°C for 1 hr, 50 $\mu$l of the supernatant was removed for radioactivity measurement.

the presence of the buffer only for 1 hour. The CPG was then added and the incubation continued. As shown in Table 2, the adsorption of PG-BAC, which occurs both on the test tube walls and CPG, follows similar patterns in the four buffers as was observed in Table 1. There is, however, only a minor amount of adsorption of the small PG (36) from HMC in buffer B and negligible adsorption in the other three buffers. The GAGs obtained from these PGs showed no non-specific adsorption in all four buffers. Similar effects were obtained when PG-BAC was incubated in borosilicate or polypropylene tubes.

The rates of the adsorptive and desorptive processes are illustrated in Figure 1 using labeled PG-BAC and unlabeled PG from bovine nasal septum cartilage (PG-BNS). The labeled PG-BAC reached an adsorptive equilibrium within about eight min and the amount of adsorbed material did not change over the next four hours. The unlabeled PG-BNS, which was measured by the Alcian Blue-DMSO method (33), took about twice as long to reach an adsorption steady state and the proportion of the material that became adsorbed was significantly less than that of the PG-BAC. Addition of 7.2 mg of Chaps to the reaction mixture to give a 0.5% (w/v) Chaps concentration, produced rapid desorption of the PG and a new steady state was achieved in less than two minutes. In the case of the BNS cartilage PG there was negligible non-specific adsorption in the presence of 0.5% (w/v) Chaps in this buffer.

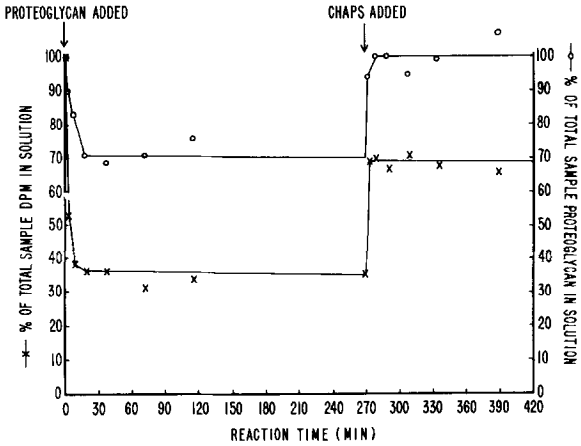


Fig. 1: Kinetics of PG adsorption to surfaces in 0.05 M NaOAc pH 5.8 buffer. To a polystyrene tube containing 0.25 g of CPG and the above buffer in a total volume of 1.5 ml was added 40 $\mu$l of PG-BAC ($1.46 \times 10^4$ $^3$H DPM, 2.4 $\mu$g of hexuronic acid equivalent) and 300 $\mu$l of PG-BNS (49.4 $\mu$g of hexuronic acid equivalent). The contents were gently mixed at 8$^{o}$C and aliquots of the supernatant were removed after various reaction times for radioactivity (25 $\mu$l samples) and PG (10 $\mu$l samples) measurement. After 270 min. 7.2 mg of Chaps was added as shown in the figure and the gentle mixing and sampling was continued.

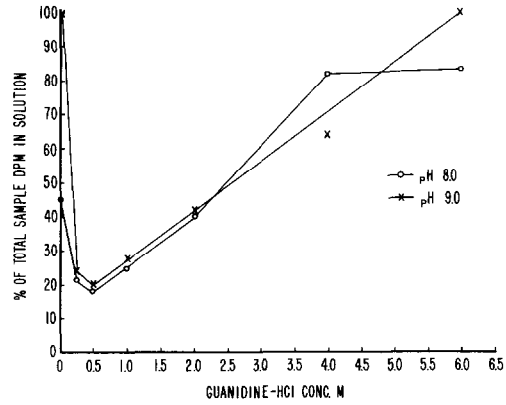


Fig. 2: Effect of guanidine hydrochloride concentration, in the 0.05 M Tris-HCl pH 8.0 and pH 9.0 buffers, on the non-specific adsorption of PG to surfaces. Into polystyrene tubes, containing 0.25 g of CPG in 0.05 M Tris-HCl pH 8.0 or pH 9.0 buffer and various concentrations of Gdn-HCl in a total volume of 1.5 ml, was added 25 $\mu$l of PG-BAC ($1.43 \times 10^4$ $^3$H DPM, 2.4 $\mu$g of hexuronic acid equivalent). After 10 hours of gentle mixing at 4°C, 50 $\mu$l aliquots of the supernatant were removed for radioactivity measurement.

The extent of the non-specific association of the PG with surfaces and matrices is dependent in a complex way on a large number of variables, including the ionic strength, nature of the salts, pH and the types of surfaces and matrices. In the present communication only the effects of chaotropic reagents such as guanidine hydrochloride and pH will be illustrated. The effect of Gdn-HCl concentration in 0.05 M Tris buffer at pHs 8 and 9 is shown in Figure 2. As the Gdn-HCl concentration is increased the interaction of PG-BAC with CPG and the polystyrene tube surface

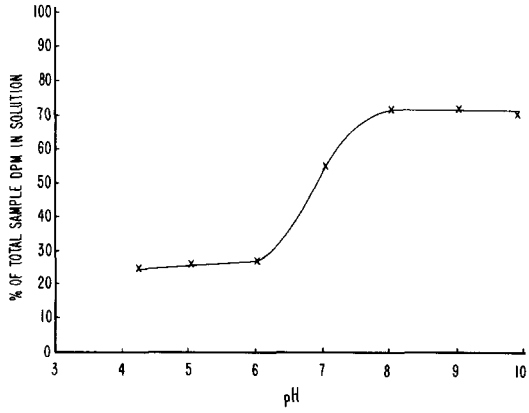


Fig. 3: Effect of pH on non-specific adsorption of PG to surfaces. Into polystyrene tubes, containing 0.5 g of CPG in 0.5 M Bis-Tris Propane + 0.5% (w/v) Chaps buffer at various pH in a total volume of 3.0 ml, was added 50 $\mu$l of PG-BAC ($2.93 \times 10^4$ $^3$H DPM, 4.8 $\mu$g of hexuronic acid equivalent). After 4 hours of gentle mixing at 4°C, 50 $\mu$l aliquots of the supernatant were removed for radioactivity measurement.

increases to reach maximum at approximately 0.5 M Gdn-HCl, and then with further increases in the Gdn-HCl concentration, there is a gradual decrease in the extent of adsorption so that in the pH 9.0 buffer it becomes negligible when the Gdn-HCl concentration reached 6.0 M. The effects of pH on non-specific adsorption are shown in Figure 3 using the Bis-Tris Propane buffer. There is, in general, less non-specific interaction (adsorption) as the pH is increased. Although the character and magnitudes varied widely, the same pattern of decreasing adsorption with increasing pH was observed for a variety of different buffers (data not shown).

Discussion: Nonspecific interaction with matrices and surfaces in a variety of buffers was observed for cartilage PGs and to a much lesser degree for human mononuclear cell PG, but not for the GAGs derived from them. The magnitude of this interaction depends on the precise buffer conditions and the largest effects were observed in the presence of 0.5 M Gdn-HCl in 0.05 M sodium acetate pH 5.8 buffer, which is also the buffer used to study aggregation of cartilage PG with hyaluronate (37). Similar buffer conditions (Gdn-HCl 0.24 M, 0.05 M sodium acetate pH 6.8) were found useful in the stabilization of the dimethylmethylene blue dye-PG interactions (38).

It is not clear at present which features of the PGs are responsible for these interactions except that digestion of the protein core abolishes these effects. One may speculate that the adsorption involves both the protein core and the GAG chains in a mechanism where the adhesion results from many weakly adsorbing GAG arms or may be due solely to the protein component. Additional work in order to further characterize these effects is in progress. The presence of such non-specific interactions, however, has a direct relevance to the analysis of the interactions of the PGs with other biomacromolecules and cell membranes both in the in vitro and in vivo situations. It has also relevance to studies which require their isolation and quantitation since as will be shown in a subsequent report, these effects affect the isolation efficiencies of the PGs.

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