ANTIGENIC DETERMINANTS OF TWO COMPONENTS OF PROTEOGLYCAN COMPLEX FROM BOVINE CARTILAGE

Nicola Di FERRANTE and Patricia V.DONNELLY
Laboratories of Connective Tissue Research of the Department of Biochemistry and the Division of Orthopedic Surgery, Department of Surgery, Baylor College of Medicine, Houston, Texas 77025, USA

and

John D.GREGORY and Stanley W.SAJDERA
The Rockefeller University, New York, N. Y., USA

Received 15 June 1970

The immunological properties of a glycoprotein fraction and of proteoglycan subunits obtained from bovine nasal cartilage by nondisruptive methods of isolation have been studied. Using the techniques of hemagglutination and hemagglutination inhibition, we found that the glycoprotein contains most of the species-specific determinants, whereas the proteoglycan subunits contain most of the cross-reacting ones.

1. Introduction

A major part of cartilage matrix consists of proteoglycan complex (PGC) which contains chondroitin sulfate covalently linked to noncollagenous protein. The slowly sedimenting fraction of this complex has been called proteinpolysaccharide-light (PPL) [1]. Various laboratories have demonstrated that antisera to PPL contain at least two types of antibodies: one directed against species-specific determinants and the other directed against determinants common to different species [2–5]. It has been reported that both antigenic sites are destroyed by treatment with various proteolytic enzymes, but remain intact after treatment with testicular hyaluronidase [4–7]. Thus, the latter enzyme has been widely employed to obtain antigens more suitable for immunodiffusion or hemagglutination techniques [5–7]. Recent studies, however, have indicated that treatment of PPL with testicular hayluronidase causes a loss of species-specific and cross-reacting determinants [8], possibly becase of conformational changes of the protein residue as a result of the removal of part of the chondroitin sulfate chains. This finding emphasizes the importance of performing immunochemical work with proteoglycan preparations which have not been subjected to disruptive methods of isolation [9] or to the action of proteolytic enzymes present in cartilage [10, 11].

For these reasons the PGC extracted from bovine nasal cartilage by dissociative, nondisruptive, methods [9] is particularly suitable for the continuation of our studies. The complex has a low protein content (8%). Moreover, this complex, in which the proteoglycan is partially aggregated may be separated by equilibrium centrifugation in a density gradient into two different fractions [12]: a glycoprotein link (GPL) and proteoglycan subunits (PGS). The latter preparation contains only 5% protein, and accounts for 95% of the hexuronic acid present in the complex, but it cannot reaggregate unless the glycoprotein link is present.

This present report describes the immunological properties of the glycoprotein link and the proteoglycan subunits obtained from bovine proteoglycan complex.

2. Methods

The techniques used for isolation of PGC, GPL and PGS from bovine nasal septa have been published [9, 12]. Antisera to bovine or human PPL were prepared by injection of white rabbits (5 kg) with 1 ml of Freund's adjuvant containing 1 mg of either human or bovine PPL. Injections were given weekly for four weeks. One month after the last injection, the rabbits received a booster injection of 1 mg antigen, and after another month they were bled.

The immune sera were inactivated, absorbed with lyophilized [5] or polymerized [13] bovine or human plasma and then absorbed with fresh group O human erythrocytes.

The details of the hemagglutination and hemagglutination inhibition tests have been described previously [3]. Tanned human erythrocytes could not be coated with PGS by these techniques, perhaps because of the low protein content. Instead, glutaraldehyde-fixed, untanned human erythrocytes [14] were coated as follows. One ml of a 2.5% suspension of glutaraldehyde-fixed erythrocytes in saline was added to 4 ml of phosphate-buffered saline at pH 6.4 containing 3 mg of PGS. The cells were incubated for 18 hr at room temperature, collected by centrifugation, washed twice with 1:200 normal rabbit serum, and suspended in 1 ml of the same. Aliquots of 0.10 ml were added to 0.90 ml of antiserum dilutions. Glutaraldehyde-fixed erythrocytes were coated with GPL by the same technique, but the GPL concentration was 0.5 mg in 4 ml, and the incubation was for 2 hr. Higher concentrations of GPL caused spontaneous hemagglutination.

Erythrocytes coated with GPL or PGS were added to increasing dilutions of the two immune sera. The results of hemagglutination are presented in table 1. GPL and PGS were used in progressively decreasing amounts as inhibitors of the systems: anti-*bovine PPL* immune serum, or anti-*human PPL* immune serum, versus red cells coated respectively with PGS or GPL. The results are presented in table 2.

Table 1
Hemagglutination of erythrocytes coated with components of proteoglycan complex.

Erythrocyte-coating preparation	Titer of antiserum	
	Anti-bovine PPL	Anti-human PPL
Bovine GPL	1:20,480	1:1,280
Bovine PGS	1:5,120	1:10,240

3. Results and discussion

The species-specific determinants are mostly located on the glycoprotein, while the cross-reacting ones are present on the proteoglycan (table 1). It is clear, however, that PGS also has some species-specific determinants, unless its reactivity with anti-bovine PPL immune serum can be attributed to a contamination with GPL. The possibility that PGS is grossly contaminated with GPL is eliminated by the hemagglutination inhibition experiments (table 2), which indicate that 2 μg/ml of bovine GPL are sufficient to inhibit the agglutination of PGS-coated erythrocytes by anti-bovine PPL immune serum but that 250 μg/ml of bovine PGS are required to inhibit the agglutination of GPL-coated erythrocytes by the same immune serum. The results indicate rather that PGS and GPL have a very low level of similar species-specific determinants and that GPL possesses a high level of species-specific determinants different from those present on PGS.

When the hemagglutination inhibition experiments were performed with anti-human PPL immune serum at 1:400 dilution, 8 μg/ml of bovine GPL were required to inhibit the agglutination of PGS-coated erythrocytes; with anti-human PPL immune serum at 1:80 dilution, 2 μg/ml of PGS were sufficient to inhibit the agglutination of GPL-coated erythrocytes. These results indicate that both fractions have cross-reacting antigenic determinants and that these are most abundant on PGS.

The chondroitin sulfate chains present in PGS are known to be structurally similar in various species; our results indicate that even the small protein moiety of these subunits seems to be immunologically similar in various species. The immunological determinants which are unique to GPL suggest additional considerations and dictate the direction for further experiments. It is conceivable that, in the native arrangement of PGC, the GPL molecules are shielded by the dense cloud of negative charges of the PGS. If this were the case, the immunocompetent cells of the organism could not detect GPL under normal conditions, but

Table 2
Inhibition of hemagglutination by components of proteoglycan complex.

Erythrocyte-coating preparation	Antiserum and titer	Inhibitor (μg/ml)	Results of hemagglutination
Bovine GPL	Anti-bovine PPL, 1:400	Bovine PGS 250	–
		125	+
	Anti-human PPL, 1:80	4	–
		2	+
Bovine PGS	Anti-bovine PPL, 1:400	Bovine GPL 2	–
		1	+
	Anti-human PPL, 1:400	8	–
		4	+

would immediately recognize it as "foreign" if, under pathological conditions, it became exposed.

The high titer of antibodies against GPL present in our antisera is not contrary to the latter hypothesis, because the antisera were produced against PPL, a disrupted and desorganized preparation in which GPL might have become mechanically exposed during the process of isolation. The necessity of using PGC rather than PPL as immunizing antigens and the opportunity of investigating the possible antigenicity of homologous GPL are evident.

Acknowledgements

This investigation was supported by grants from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service (AM 10811 and AM 04973) and the Robert A. Welch Foundation (Q-236).

References

[1] B.R.Gerber, E.C.Franklin and M.Schubert, J. Biol. Chem. 235 (1960) 2870.
[2] N.Di Ferrante, Science 143 (1964) 250.
[3] N.Di Ferrante and M.Pauling, J. Lab. Clin. Med. 63 (1964) 945.
[4] G.Loewi and H.Muir, Immunology 9 (1965) 119.
[5] J.Sandson, L.Rosenberg and D.White, J. Exptl. Med. 123 (1966) 817.
[6] A.Hirschman and D.D.Dziewiatkowski, Science 154 (1966) 393.
[7] A.M.Pankovich and L.Korngold, J. Immunol. 99 (1967) 431.
[8] P.V.Donnelly, N.Di Ferrante and R.Hrgovcic, Immunochemistry 6 (1969) 353.
[9] S.W.Sajdera and V.C.Hascall, J. Biol. Chem. 244 (1969) 77.
[10] S.Y.Ali, Biochem. J. 93 (1964) 611.
[11] D.D.Dziewiatkowski, C.D.Tourtellotte and R.D.Campo, in: The Chemical Physiology of Mucopolysaccharides, ed. G.Quintarelli (Little, Brown, Boston, 1968) p. 63.
[12] V.C.Hascall and S.W.Sajdera, J. Biol. Chem. 244 (1969) 2384.
[13] N.Di Ferrante, in: Chemistry and Molecular Biology of the Intercellular Matrix, Vol. III, ed. E.Balazs (Academic Press, London, 1970) p. 1551.
[14] D.H.Bing, J.G.M.Weyand and A.B.Stavitsky, Proc. Soc. Exptl. Biol. Med. 124 (1967) 1166.