THE INTERACTION OF WHEAT GERM AGGLUTININ WITH KERATAN FROM CORNEA AND NASAL CARTILAGE

Hans Erik CARLSSON,* Jörgen LÖNNGREN,** Irwin J. GOLDSTEIN, James E. CHRISTNER*** and George W. JOURDIAN

Department of Biological Chemistry and the Rackham Arthritis Research Unit, The University of Michigan, Ann Arbor, Michigan 48104 USA

Received 24 November 1975

1. Introduction

Wheat-germ agglutinin (WGA), a lectin isolated from *Triticum vulgaris*, is widely used as a probe for studying carbohydrates found on the surfaces of malignant cells [1,2]. The agglutinin consists of two identical polypeptide chains. Each chain is reported to contain two identical binding sites, which can accomodate a *N,N',N''*-triacetyl chitotriosyl residue [1–7]; internal (1→4)-*N*-acetyl-β-D-glucosaminyl residues as well as terminal non-reducing *N*-acetyl-β-D-glucosaminyl groups also interact with WGA [5,8,9].

Keratan sulfate, a connective tissue glycosaminoglycan, is composed of six to nine repeating disaccharide units of 4-*O*-β-D-galactopyranosyl-*N*-acetyl-D-glucosamine [10,11]; the disaccharide units are glycosidically linked β, 1→3 [12,13]. Each of the carbohydrate residues contains varying amounts of *O*-sulfate ester groups at carbon 6.

The carbohydrate-binding specificity of WGA suggested that desulfated keratan sulfate should interact with WGA. The present communication documents the specific precipitation of desulfated keratan sulfate with WGA.

* On leave from National Defense Research Institute, Department 4, Division of Applied Microbiology, S-172 04 Sundbyberg, Sweden.

** On leave from the University of Stockholm, Arrhenius Laboratory, Department of Organic Chemistry, S-104 05 Stockholm, Sweden.

*** Postdoctoral Fellow, National Arthritis Foundation.

2. Materials and methods

Highly purified WGA was prepared by specific adsorption to chitin by the procedure of Bloch and Burger [14]. *N,N'*-Diacetyl chitobiose was isolated from chitin [15]. Keratan sulfate was prepared from bovine cornea (KS-I) and from bovine nasal cartilage (KS-II) [16]. The sulfate content of each preparation (18%) was determined with a barium chloroanilate reagent [17]. Each preparation was desulfated by the method of Bhavanandan and Meyer [13]. Highly purified preparations of chondroitin 4- and 6-sulfates, chondroitin, dermatan sulfate, heparin and heparan sulfate were the generous gift of Dr J. A. Cifonelli (University of Chicago, Chicago, Ill.). Polysaccharide concentrations were quantitated either by determination of hexose content by an anthrone–sulfuric acid method [18] or a phenol–sulfuric acid method [19]. Quantitative precipitation and inhibition studies were performed by a microprecipitin technique [20]. Reaction mixtures contained WGA, glycosaminoglycan, carbohydrate inhibitor (where appropriate), and 0.15 *M* saline buffered at pH 7.2 with 0.01 *M* sodium phosphate, in a final volume of 200 μl. The reaction mixtures were maintained at room temperature for two days. The nitrogen content of the precipitates was determined by a modification [21] of the method of Rosen [22]. Capillary tube tests of various glycosaminoglycans were performed in Micro Hematocrit tubes at concentrations of 2 mg of polysaccharide per ml and 1 mg of WGA per ml.

3. Results

The quantitative precipitation curves obtained when WGA was incubated with desulfated bovine corneal (KS-I) and nasal keratan sulfate (KS-II) preparations are presented in fig.1. Desulfated KS-I contained 3.2% residual sulfate and two preparations of desulfated KS-II contained 0.9% and 3.4% residual sulfate, respectively. The bovine nasal keratan sulfate preparation with the lowest sulfate content (0.9%) precipitated the largest amount of WGA. This result suggests that the residual sulfate groups may block loci in the *N*-acetyl-D-glucosaminyl residues required for binding to the lectin. The precipitation curves obtained when WGA was incubated with desulfated KS-I and KS-II preparations (containing equivalent residual sulfate content) were distinct. This result may reflect a difference in the carbohydrate structure in the linkage region between the amino acid residue and the carbohydrate residue that initiates the KS-I and KS-II chains [16], and/or a difference in the number of branches in each type of keratan [12].

Inhibition studies were performed to establish that precipitin reactions with desulfated KS preparations were the result of specific interaction at the carbohydrate binding sites of WGA. *N,N'*-Diacetyl chitobiose at concentrations of 50, 8 and 10 nmol per 200 μl resulted in 50% inhibition of the precipitation reaction between WGA and desulfated KS-II (0.9% sulfate), desulfated KS-II (3.4% sulfate) and desulfated KS-I (3.2% sulfate), respectively. These concentrations of *N,N'*-diacetyl chitobiose were of the same order of magnitude required for 50% inhibition of the precipitation reactions between WGA and *p*-azophenyl β-*N*-acetyl-D-glucosamine–bovine serum albumin [8]. The precipitation reaction was completely abolished at higher *N,N'*-diacetyl chitobiose concentrations. When sodium chloride (final concentration 2 M) was added to reaction mixtures that contained WGA and desulfated KS-I or KS-II, no inhibition of the precipitation reaction occurred. Charge-charge interactions, therefore, do not play a major role in these precipitation reactions. On the contrary, these results demonstrate that the interactions are governed primarily by the carbohydrate-binding capacity of WGA.

The following glycosaminoglycans did not give a precipitation reaction with WGA under the experimental conditions described in Materials and methods: chondroitin 4- and 6-sulfates, chondroitin, dermatan sulfate, heparan sulfate, heparin, hyaluronic acid and keratan sulfates (KS-I and KS-II).

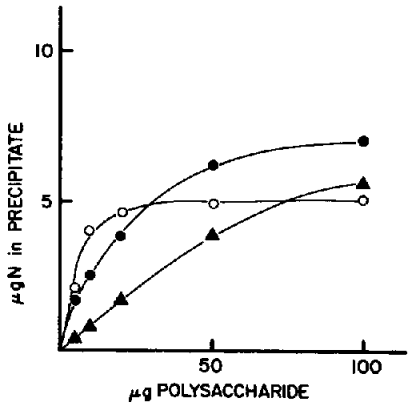

Fig.1. Precipitation of desulfated keratan sulfate preparations by WGA. (●) Nasal cartilage keratan (0.9% sulfate) and WGA (4.9 μg N); (○) nasal cartilage keratan (3.4% sulfate) and WGA (8.4 μg N); (▲) corneal keratan (3.2% sulfate) and WGA (8.4 μg N).

4. Discussion

The results reported herein support the view that non-consecutive (1→4)-*N*-acetyl-β-glucosaminyl residues in polysaccharides form sufficiently strong interactions with WGA to cause precipitation. These results are in agreement with the studies by Allen et al. [5] and Lotan et al. [9], who investigated the interactions of WGA with *Micrococcus luteus* and *Staphylococcus aureus H* cell-wall oligomers and polymers, and with studies by Goldstein and coworkers [8], who studied the interaction of WGA with a synthetic glycoprotein.

To the best of our knowledge the present study constitutes the first report which documents the specific precipitation reaction of a glycosaminoglycan mediated by a lectin's carbohydrate binding site. Previously reported precipitation reactions of highly anionic glycosaminoglycans with lectins (e.g., interaction of concanavalin A with heparin) were demonstrated to result from non-specific charge–charge interactions

[23–25]. Similar non-specific interactions between teichoic acids and concanavalin A were observed at low pH values [26].

The 4- and 6-mono-*O*-methyl derivatives of *N*-acetyl-D-glucosamine inhibit the WGA hemagglutination reaction, whereas 3-*O*-methyl-*N*-acetyl-D-glucosamine does not [5]. The interaction of WGA with desulfated KS-I and KS-II preparations reported in this communication are consistent with these findings, e.g., 4-*O*-substitution of *N*-acetyl-D-glucosaminyl residues as they occur in desulfated KS preparations provide binding loci for interactions with WGA. Hyaluronic acid, a glycosaminoglycan that contains alternating units of 3-*O*-substituted *N*-acetyl-β-D-glucosaminyl residues, does not interact with WGA.

In preliminary experiments we have observed that WGA interacts to form a precipitate with capsular polysaccharide from *Diplococcus pneumoniae*, Type XIV (S XIV). This finding is of great interest since the *N*-acetyl-D-glucosaminyl residues of this capsular polysaccharide are believed to be substituted at both oxygen atoms 4 and 6 [27,28]. We conclude, therefore, that 4,6-di-*O*-substituted *N*-acetyl-D-glucosaminyl residues are capable of interaction with WGA. This phenomenon is currently under investigation.

Acknowledgements

This work was supported by grants AM-10171 and AM-10531 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

Travel grants from the foundation Stiftelsen Blanceflor Boncompagni-Ludovisi, Född Bildt and the foundation Längmanska Kulturfonden to Dr Carlsson are gratefully acknowledged.

References

[1] Nicolson, G. L. (1974) Int. Rev. Cytol. 39, 89–190.
[2] Lis, H. and Sharon, N. (1973) Ann. Rev. Biochem. 42, 541–574.
[3] Nagata, Y. and Burger, M. M. (1974) J. Biol. Chem. 249, 3116–3122.
[4] Rice, R. H. and Etzler, M. E. (1974) Biochem. Biophys. Res. Commun. 59, 414–419.
[5] Allen, A. K., Neuberger, A. and Sharon, N. (1973) Biochem. J. 131, 155–162.
[6] Privat, J.-P., Delmotte, F., Mialonier, G., Bouchard, P. and Monsigny, M. (1974) Eur. J. Biochem. 47, 5–14.
[7] Privat, J.-P., Delmotte, F. and Monsigny, M. (1974) FEBS Lett. 46, 224–228.
[8] Goldstein, I. J., Hammarström, S. and Sundblad, G. (1975) Biochem. Biophys. Acta 405, 53–61.
[9] Lotan, R., Sharon, N. and Mirelman, D. (1975) Eur. J. Biochem. 55, 257–262.
[10] Greiling, H., Struhlsatz, H. W. and Kisters, R. (1970) in: Chemistry and Molecular Biology of the Intracellular Matrix (Balazs, E. A., ed.) 2, 873–877, Academic Press, New York.
[11] Hopwood, J. J. and Robinson, H. C. (1974) Biochem. J. 141, 517–526.
[12] Bhavanandan, V. P. and Meyer, K. (1967) J. Biol. Chem. 242, 4352–4359.
[13] Bhavanandan, V. P. and Meyer, K. (1968) J. Biol. Chem. 243, 1052–1059.
[14] Bloch, R. and Burger, M. M. (1974) Biochem. Biophys. Res. Commun. 58, 13–19.
[15] Rupley, J. A. (1964) Biochim. Biophys. Acta 83, 245–255.
[16] Rodén, L., Baker, J. R., Cifonelli, J. A. and Mathews, M. B. (1972) Methods Enzymol. 28B, 73–140.
[17] Spencer, B. (1960) Biochem. J. 75, 435–440.
[18] Scott, T. A., Jr. and Melvin, E. H. (1953) Anal. Chem. 25, 1656–1661.
[19] Hodge, J. E. and Hofreiter, B. T. (1962) Methods Carbohyd. Chem. 1, 380–394.
[20] So, L. L. and Goldstein, I. J. (1967) J. Biol. Chem. 242, 1617–1622.
[21] Hayes, C. E. and Goldstein, I. J. (1974) J. Biol. Chem. 249, 1904–1914.
[22] Rosen, H. (1957) Arch. Biochem. Biophys. 67, 10–15.
[23] Cifonelli, J. A., Montgomery, R. and Smith, F. (1956) J. Amer. Chem. Soc. 78, 2488–2489.
[24] Doyle, R. J. and Kan, T.-J. (1972) FEBS Lett. 20, 22–24.
[25] DiFerrante, N. and Hrgovcic, R. (1970) FEBS Lett. 9, 281–283.
[26] Doyle, R. J. and Birdsell, D. C. (1972) J. Bact. 109, 652–658.
[27] Baker, S. A., Pardoe, G. I., Stacey, M. and Hopton, J. W. (1964) Nature 204, 938–939.
[28] Erbing, S., Lindberg, B. and Svensson, S., unpublished results.