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One-Step Purification of Murine IgM and Human α_2-Macroglobulin by Affinity Chromatography on Immobilized Snowdrop Bulb Lectin[1]

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A new mannose-specific plant lectin (GNA) isolated from the snowdrop bulb was immobilized on Sepharose 4B and employed for the purification of certain glycoproteins with high-mannose type glycan chains. Murine IgM bound tightly to this column and was eluted with 0.1 M methyl α-D-mannoside whereas bovine and murine IgG were not bound. When a murine hybridoma serum containing IgM monoclonal antibody was applied to this column, highly purified IgM antibody was obtained after elution with methyl α-D-mannoside. On the contrary, human IgM was not bound by this column despite reports that it contains high-mannose type glycan chains. α_2-Macroglobulin was the sole glycoprotein present in human serum which was bound by the immobilized snowdrop lectin column. It appears that only glycoproteins containing multiple Man(α1,3)Man units are bound to the immobilized lectin.

Snowdrop (*Galanthus nivalis*) bulb lectin (GNA) is a new plant lectin which is highly specific for D-mannose (1, 2). GNA requires the presence of equatorial hydroxyl groups at C-3 and C-4, and an axial hydroxyl at C-2 of the D-hexopyranose ring for binding; thus, it can distinguish D-mannose from D-glucose. This binding specificity makes it quite different from the previously known mannose/glucose-specific plant lectins such as concanavalin A (Con A), pea, or lentil lectin. Only one plant lectin which was recently isolated from tulip bulb (3) appears to belong to the same group of D-mannose-specific plant lectins. The combining site of GNA is most complementary to the disaccharide unit, Man(α1,3)Man, showing 10-30 times higher affinity compared to D-mannose. Glycoproteins with high-mannose type glycan chains behaved differently on an immobilized GNA column depending on the structure, density, and probably accessibility of their glycan chains (1). These results suggested the possible use of the snowdrop bulb lectin for the purification of certain glycoproteins with high-mannose type glycan chains. We report here the one-step purification of murine IgM and human α_2-macroglobulin from the corresponding sera of these species.

MATERIALS AND METHODS

GNA-Sepharose. GNA was purified as previously described (1) and coupled to Sepharose 4B using CNBr[4] (4). Methyl α-D-mannoside (final concentration, 0.2 M) was added to the GNA solution to protect the carbohydrate binding site during the immobilization procedure. The final product contained approximately 1.3 mg protein/ml of the gel.

Immunoglobulins and serum proteins. Murine hybridoma serum containing IgM-type monoclonal an-

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[4] Abbreviations used: CNBr, cyanogen bromide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin.

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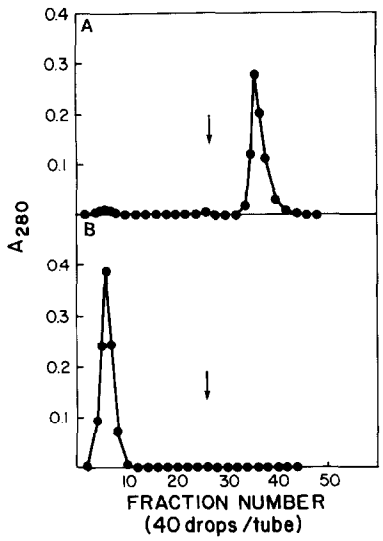

FIG. 1. Elution profile of immunoglobulins on GNA-Sepharose. (A) Purified murine IgM (1 mg in 0.5 ml PBS) was applied to the GNA-Sepharose column; (B) bovine IgG was applied to the same column. The arrow indicates the addition of 0.5 M methyl α-D-mannoside (same for all the following figures).

tibody was supplied by Dr. J. L. Claflin and Dr. G. W. Jourdian of the University of Michigan. Purified murine immunoglobulins of both IgG and IgM type were obtained from Dr. Claflin. Normal human serum was obtained from the University of Michigan Hospital.

Affinity chromatography. A 0.5-ml aliquot of glycoprotein solution (approx 2 mg/ml PBS) or serum was applied to a column of GNA-Sepharose (0.7 × 15 cm) and incubated for 1 h. The column was eluted with PBS, followed by 0.5 M methyl α-D-mannoside in PBS at a flow rate of 1 ml/h. Fractions were monitored by absorbance at 280 nm. All experiments were conducted in a cold room at 4°C.

Electrophoresis. SDS-PAGE was carried out with 7.5% acrylamide gel at pH 8.8. Protein bands were visualized using a silver stain procedure (5). Immunoelectrophoresis was carried out using goat anti-human IgM and anti-whole human serum. Immunofixation of human serum protein on an electrophoretic gel using antibodies to human serum components was carried out by the method of Ritchie and Smith (6, 7).

ELISA assay of immunoglobulins. The amount of IgM and IgG in the eluate of affinity chromatography was determined by ELISA as follows. Aliquots of the eluate were coated directly on an ELISA plate and incubated overnight at room temperature. After blocking unoccupied binding sites on the plastic surface with BSA, appropriate alkaline phosphatase-labeled antibodies were applied and incubated for 4–5 h. The plates were developed with Sigma 104 phosphatase substrate.

RESULTS

Purification of murine IgM from hybridoma serum. IgM immunoglobulin has been reported to contain high-mannose-type glycan chains in its molecular structure (8–10) and is considered to be a good candidate to test the applicability of the immobilized GNA for the isolation of specific glycoproteins. Purified murine IgM bound tightly to the GNA column and was eluted with 0.5 M methyl α-D-mannoside, whereas bovine γ-globulin (IgG) did not bind to this column (Fig. 1). When a murine hybridoma serum which contained IgM-type monoclonal antibody was applied to the GNA column, a significant amount of protein was bound to the column and was eluted with 0.5 M methyl α-D-mannoside (Fig. 2). SDS-PAGE of this fraction revealed that almost pure IgM was obtained by this simple procedure (Fig. 3). Similar results were obtained in three separate experiments using murine serum obtained from several different hybridoma cell lines. To confirm the specificity of the lectin for the IgM-type antibody, various IgG subclass molecules were added to the starting serum and applied to the GNA column. Analysis of the bound fraction by ELISA using antibodies specific to IgG subclasses or IgM, and also by isoelectric focusing (data not shown), showed that most of the IgM molecules were recovered in the bound fraction, whereas the IgG subclasses did not bind to

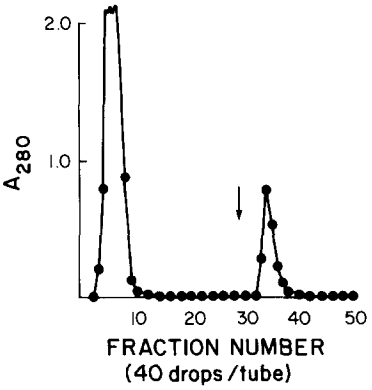

FIG. 2. Elution profile of IgM-producing murine hybridoma serum on GNA-Sepharose. A murine hybridoma serum containing IgM-type monoclonal antibody (0.4 ml) was applied to the GNA column.

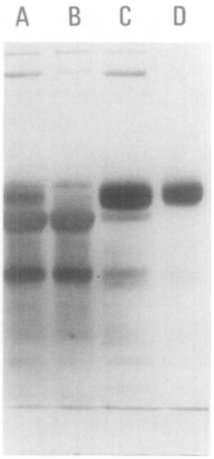

FIG. 3. SDS-polyacrylamide gel electrophoresis of the affinity purified murine IgM. (A) Original murine hybridoma serum; (B) flowthrough fraction; (C) IgM fraction eluted from the GNA column by 0.5 M methyl α-D-mannoside; (D) murine IgM standard.

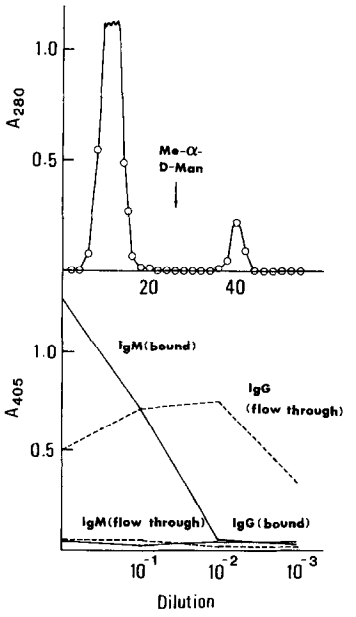

FIG. 4. Effect of murine IgG on the purification of IgM from hybridoma serum. An IgM-producing murine hybridoma serum was mixed with murine IgG of known subclass and applied to the GNA-Sepharose column. The amount of IgG and IgM in the bound and flowthrough fractions were determined by ELISA using antibodies to each immunoglobulin.

this column nor did they interfere with the adsorption of IgM to the column (Fig. 4).

Purification of α_2-macroglobulin from human serum. Contrary to the murine IgM, human IgM which is also known to have high-mannose-type glycan chains (9, 10) did not bind to the GNA-Sepharose column (Fig. 5). A protein fraction was obtained when normal human serum was applied to this column and eluted with 0.5 M methyl α-D-mannoside after the elution of the unbound fraction (Fig. 6). SDS-PAGE of this fraction showed a protein band which corresponded to a component of much higher molecular weight than the IgM subunit (Fig. 7). This glycoprotein component reacted with goat antisera against whole human serum on immunoelectrophoresis but did not react with antisera against human IgM (Fig. 8). The main component of this fraction was finally identified as α_2-macroglobulin by high-resolution immunofixation using antibodies specific to various human serum components (Fig. 9).

DISCUSSION

The results described herein demonstrate the usefulness of immobilized GNA for the purification of IgM-type antibodies from murine sera. This finding is of practical importance because most monoclonal antibodies presently available are produced using murine hybridoma cell lines and the production of IgM-type monoclonal antibodies is often encountered in

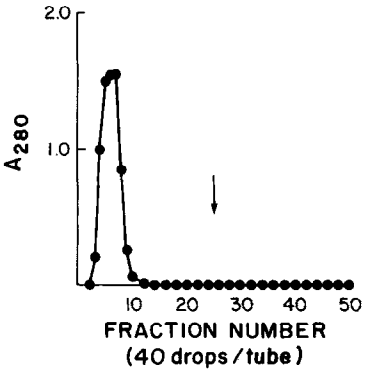

FIG. 5. Elution profile of human IgM on GNA-Sepharose. Purified human IgM (1.4 mg in 0.2 ml PBS) was applied to the GNA column.

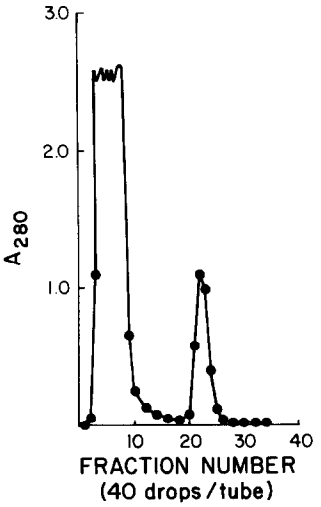

FIG. 6. Elution profile of whole human serum on GNA-Sepharose. Normal human serum (0.5 ml) was applied to the GNA column.

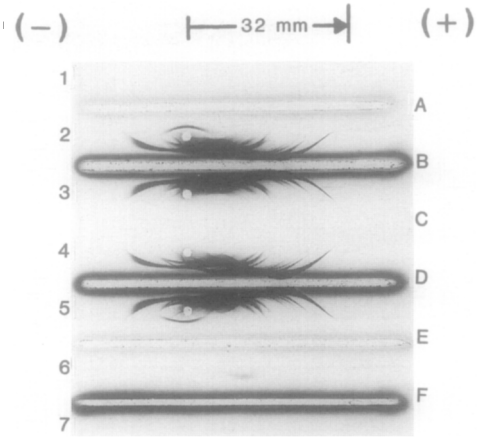

FIG. 8. Immunoelectrophoresis of human serum glycoprotein bound to GNA-Sepharose. Wells 2 and 3, normal human serum 1; wells 4 and 5, normal human serum 2; well 6, serum glycoprotein bound to GNA-Sepharose. Troughs A and E, anti-human IgM; troughs B, D, and F, anti-whole human serum; trough C, GNA. Protein bands were stained using Coomassie brilliant blue.

these systems. In addition, the purification of IgM molecules is usually very difficult compared to that of IgG because of the lack of suitable adsorbants such as protein A which has a very low affinity for IgM. The unique binding specificity of GNA, i.e., a strict requirement for nonreducing terminal D-mannose residues (1), gives rise to a high selectivity for murine IgM which carries high-mannose-type glycan chains (8). On the contrary, traditional mannose/glucose-specific lectins such as Con A, pea, and lentil lectins bind many glycoproteins present in human serum and do not lead to such high selectivity.

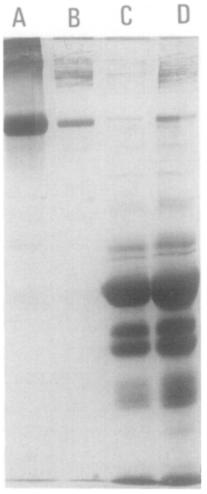

FIG. 7. SDS-polyacrylamide gel electrophoresis of the human serum fraction bound to GNA-Sepharose. (A, B) Human serum glycoprotein bound to GNA-Sepharose; (C) flowthrough fraction; (D) original human serum.

The very low affinity of human IgM for the GNA column was quite unexpected because it has also been reported that human IgM contains high-mannose-type glycan chains (9, 10). There are at least two possibilities for the low reactivity of human IgM. One possibility is that the glycan chains of human IgM are structurally different compared to those of murine IgM and do not accommodate well to the combining site of GNA which requires nonreducing terminal Man(α1-3)Man disaccharide units for high affinity binding. In fact, glycoproteins with high-mannose-type glycan chains of different structure displayed different affinity for the immobilized GNA column (1). Or, the glycan chains of human IgM are sterically hindered and not accessible to the lectin. Day *et al.* (11) found that rat IgM was bound by Con A only when complexed with antigen

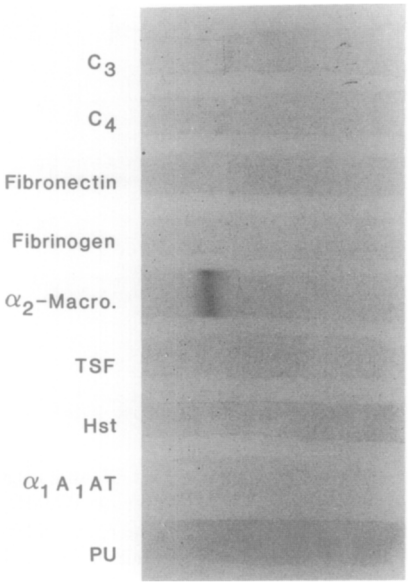

FIG. 9. High-resolution immunofixation of the glycoprotein bound to GNA-Sepharose after gel electrophoresis. Aliquots of the human serum glycoprotein which was bound to GNA-Sepharose were electrophoresed on agarose gel and then overlayed with strips containing antisera against each human serum component. Immunocomplex was visualized using Coomassie brilliant blue after rinsing out the free serum protein and antisera. The glycoprotein was stained only with the anti-human α_2-macroglobulin.

and suggested that the glycan chains might be buried within the quarternary structure of the polypeptide and that a conformational change was necessary to make it accessible by Con A.

The finding that human α_2-macroglobulin can also be purified by the GNA column is not only methodologically useful but also affords information regarding the structure of the glycan chains of this glycoprotein. Although α_2-macroglobulin was reported to have eight glycan chains in its subunit polypeptide of M_r 180,000 (12), the detailed structure of these carbohydrate units has not yet been fully clarified. Dunn and Shapiro (13) suggested the presence of high-mannose-type carbohydrate units and Pan *et al.* (14) isolated a glycopeptide which showed a sugar composition corresponding to a hybrid-type structure. The presence of high-mannose-type glycan chains in the human α_2-macroglobulin is suggested from the results described here and also previous results (1) in which only glycoproteins with high-mannose-type glycan chains of a specific type could be bound by this column. We have not yet determined whether hybrid-type glycopeptides are bound to the GNA column.

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