Specific binding of bovine immunoglobulins to Sepharose and Sephadex

Hiroshi Shimada, Yuji Saito, Teruaki Imada and Yuji Inada

Laboratory of Biological Chemistry, Tokyo Institute of Technology, Ookayama, Meguroku, Tokyo 152, Japan

Received 30 March 1983

Abstract and keywords not received

1. INTRODUCTION

Commercially available beaded polysaccharides, Sephadex (polymer of D-glucose) and Sepharose (copolymer of D-galactose and 3,6-anhydro-L-galactose), are widely used for gel filtration and affinity chromatography to purify biological materials including plasma proteins [1]. They are usually regarded as ideal materials to serve for these purposes because they are thought to be inert and not to bind biological materials except for some lectins [2].

However, during our study on purification of fibronectin from plasma by gelatin–Sepharose [3], it became apparent that certain plasma proteins do bind to Sepharose rather than to a ligand attached to this polysaccharide [4]. Here, we show by electrophoresis and immunodiffusion analysis that those plasma proteins are very specific immunoglobulins, IgM and IgG. Those which bound to Sepharose did not bind to Sephadex, and vice versa. This result would warn us of the necessity to remove those immunoglobulins by plain (non-ligand-attached) Sepharose or Sephadex before applying plasma directly to ligand-attached Sepharose or Sephadex. This is true especially when the bound materials are to be eluted from a ligand by denaturing solvents.

2. MATERIALS AND METHODS

Sepharose 4B and Sephadex G-200 were purchased from Pharmacia. Centriflo membrane cones type CF25 were from Amicon. Antibovine IgM was purchased from Miles Labs. All other chemicals were of analytical grade. Polyacrylamide gel electrophoresis (3.5%) in the presence of 0.1% sodium dodecyl sulfate (SDS) with or without reduction of the sample with 1% 2-mercaptoethanol were performed as in [5]. Immunodiffusion analysis was carried out in 0.85% agar dissolved in 150 mM NaCl containing 0.1% NaN_3 [6].

3. RESULTS AND DISCUSSION

Bovine plasma (800 ml) was passed through a column of Sepharose 4B (4.4 × 27.5 cm), and the column was washed thoroughly with phosphate-buffered saline, PBS (pH 7.2) containing 10 mM sodium citrate. Bound proteins were eluted with 4 M urea in 50 mM Tris–HCl buffer (pH 7.5) and were dialyzed against PBS (pH 7.0). To see whether those proteins bound specifically to Sepharose or whether they were merely trapped by the matrix, we applied them again to the same Sepharose 4B column. Almost all of the proteins bound to Sepharose again (fig.1a). The results strongly indicate that the proteins have specific affinity to Sepharose. The eluted proteins were dialyzed against PBS (pH 7.0) and concentrated by Centriflo. To investigate what kinds of proteins bound to Sepharose, we performed gel electrophoresis of those proteins. The results shown in the inset of fig.1a revealed that they consisted of two kinds with very high and relatively low M_r-values.

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

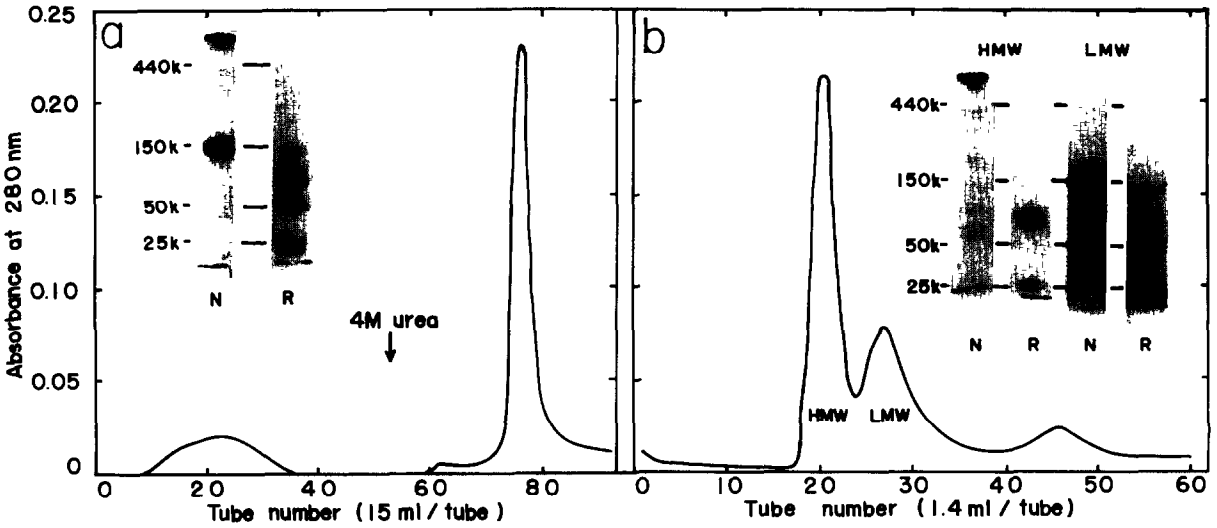

Fig.1. Binding specificity and separation of Sepharose-bound proteins. (a) Sepharose-bound proteins were applied again to the same Sepharose 4B column, after dialysis against PBS. The bound fraction was eluted with 4 M urea as indicated by an arrow. (b) Sepharose-bound proteins (A_{280} = 0.83, 3 ml) were separated into two components, high-M_r (HMW) and low-M_r (LMW) by gel filtration on Sephadex G-200 (1.5 × 45 cm). The insets are the electrophoregrams obtained in the absence (N) and presence (R) of 1% 2-mercaptoethanol. M_r-standards used are bovine fibronectin (440000), bovine IgG (150000), heavy (50000) and light (25000) chains of bovine IgG.

They will be designated high-M_r and low-M_r, respectively, hereafter. These two proteins yielded 3 major polypeptides upon reduction with 2-mercaptoethanol. In order to clarify from which proteins each of these 3 polypeptides were derived, we tried to separate high-M_r from low-M_r. Preliminary experiments suggested that these proteins did not bind to Sephadex. Taking advantage of this observation, we performed gel filtration of Sepharose-bound proteins on Sephadex G-200 (fig.1b). First and second peaks of gel filtration fractions (high-M_r and low-M_r) were pooled separately and were dialyzed against distilled water. They were then concentrated by lyophilization and were dissolved in PBS (pH 7.0) prior to electrophoresis shown in the inset. High-M_r has a value larger than that of fibronectin (440000) and consists of two kinds of subunits with M_r 80000 and 25000. Low-M_r has a value of 150000 and is made up of two kinds of subunits with M_r 50000 and 25000.

We have obtained proteins which bound to Sepharose but did not bind to Sephadex. To see if there are also proteins in plasma which have specific affinity to Sephadex we performed experiments as follows. Bovine plasma (600 ml) was added to 300 ml Sephadex G-200 in a beaker which was pre-equilibrated with PBS (pH 7.2) containing 10 mM sodium citrate. The mixture was washed with 10 mM sodium citrate–PBS on a piece of filter paper. It was then packed into a column with 4.4 cm diam. and was washed sufficiently with 10 mM sodium citrate–PBS. Bound proteins were eluted with 8 M urea in 50 mM Tris–HCl buffer (pH 7.5). These Sephadex-bound proteins were then applied to columns of Sephadex G-200 and Sepharose 4B. These proteins bound to Sephadex G-200 again, but they failed to bind to Sepharose 4B, as shown in fig.2a and 2b, respectively. The results of gel electrophoresis of Sephadex-bound proteins and Sepharose-bound proteins revealed that these proteins are identical in terms of molecular mass and subunit composition (fig.3). The Sephadex-bound proteins were eluted from the column with 0.5 M glucose. The Sepharose-bound proteins, on the other hand, could never be eluted from the matrix with glucose.

Values of low-M_r and its subunits are the same as those of IgG (150000, and its subunits: heavy chain, 50000; light chain, 25000). Subunits of high-M_r seem to be similar to those of IgM (heavy chain, 72000; light chain, 25000). To prove this

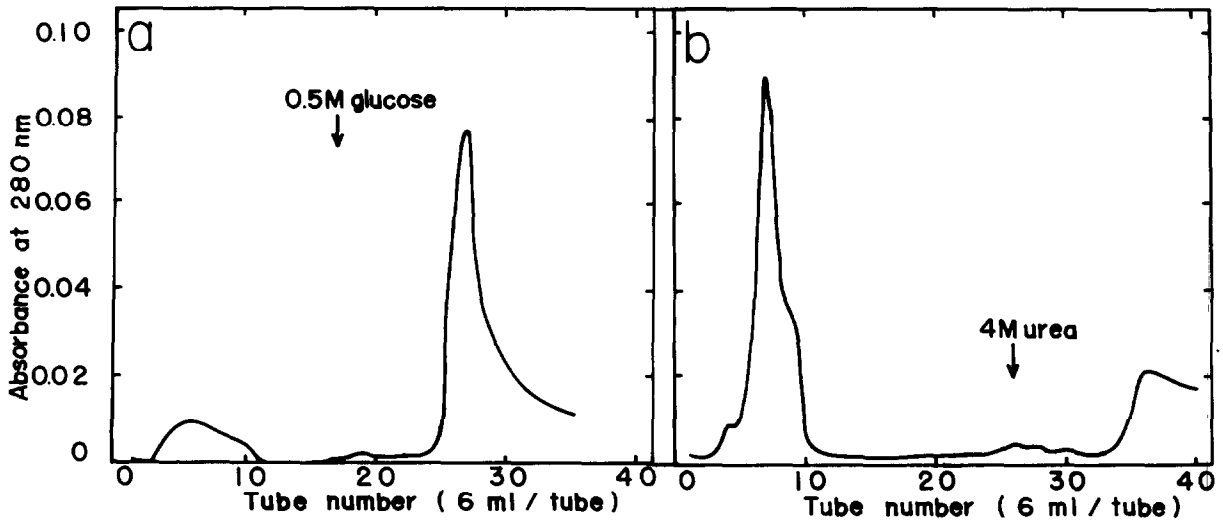

Fig.2. Binding specificity of Sephadex-bound proteins. Sephadex-bound proteins (A_{280} = 1.3, 1.5 ml) were applied to columns of (a) Sephadex G-200 (2.4 × 13.5 cm) and (b) Sepharose 4B (2.4 × 10.5 cm) after dialysis against PBS. The bound fractions were eluted with 0.5 M glucose and 4 M urea, respectively, as indicated by the arrows.

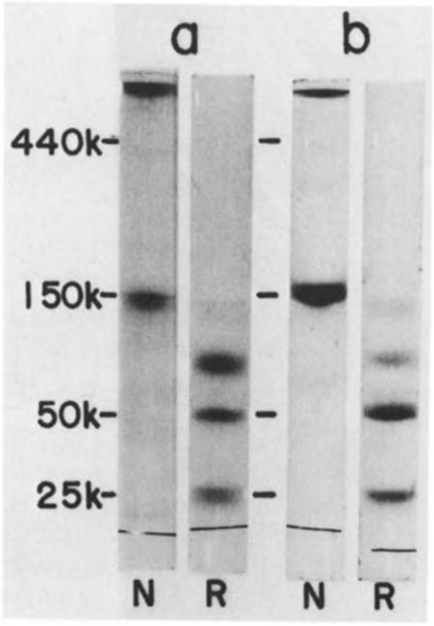

Fig.3. Comparison of Sepharose- and Sephadex-bound proteins by polyacrylamide gel electrophoresis. The electrophoresis was performed in the absence (N) and presence (R) of 1% 2-mercaptoethanol: (a) Sepharose-; (b) Sephadex-bound protein. The M_r-standards used were the same as in fig.1.

immunodiffusion analysis was conducted (fig.4). As we expected, anti-IgM antibodies reacted specifically with high-M_r. These results suggested that high-M_r is truly IgM. These results also reveal that antibodies specific against Sepharose and Sephadex occur naturally in plasma. They would amount to 2–3 mg in 100 ml bovine plasma. High-M_r and low-M_r were also obtained from human

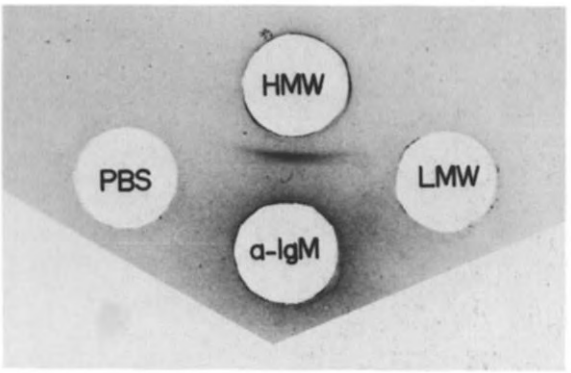

Fig.4. Immunological characterization of Sepharose-bound proteins. Sepharose-bound proteins were separated into low-M_r and high-M_r by gel filtration as in fig.1. They were subjected to immunodiffusion analysis against anti-bovine IgM antiserum from rabbits.

plasma with the same method as used for bovine plasma (not shown).

These experiments indicate that if we use Sepharose or Sephadex to purify proteins from plasma, we must carefully use these matrices because small amounts of immunoglobulins strongly bind to them. Especially in the case of affinity chromatography, it is advisable to pass plasma through a non-ligand-attached column beforehand.

REFERENCES

[1] Cuatrecasas, P. and Anfinsen, C.B. (1971) in: Methods in Enzymology (Jakoby, W.B. ed) vol.22, pp.345–378, Academic Press, New York.

[2] Agrawl, B.B.L. and Goldstein, I.J. (1967) Biochim. Biophys. Acta 147, 262–271.

[3] Engvall, E. and Ruoslahti, E. (1977) Int. J. Cancer 20, 1–5.

[4] Ruoslahti, E., Hayman, E.G., Kuusela, P., Shively, J.E. and Engvall, E. (1979) J. Biol. Chem. 254, 6054–6059.

[5] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.

[6] Kabat, E.A. (1961) in: Experimental Immunochemistry, 2nd edn (Kabat, E.A. and Mayer, M.M. eds) pp.85–88, Charles C. Thomas, Springfield IL.