BRIEF NOTE

Purification and Identification of Horse Serum IgA

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(Received: May 11, 1981)

The horse T-globulin [8] has been considered to be a homologue of human IgA on the basis of its β_2 electrophoretic mobility and high carbohydrate contents [2, 6]. T-globulin was, however, considered to be an IgG subclass designated as IgG(T) [9]. Vaerman et al. [7] first identified an immunoglobulin in horse serum and milk which exhibited an immunological cross-reaction with anti-human-IgA antiserum. Secretory IgA (SIgA) of the horse has been purified from colostrum [5] and tears [4]. Purification of horse serum IgA, however, has not been successful [4, 5, 7]. The present study was therefore designed to establish the procedures for purification of horse serum IgA.

In the present study, purification of horse serum IgA was performed by the immunoabsorbent method. For preparation of immunoabsorbents, several antisera against horse serum proteins were prepared. All of them were obtained from the rabbits immunized with antigens in Freund's complete adjuvant and utilized for preparation of immunoabsorbent columns of Sepharose 4B activated with BrCN [1]. The properties and the efficiency of the immunoabsorbent columns are tabulated (Table 1). Procedures for preparation of the immunoabsorbent columns are as follows:

Anti-IgG immunoabsorbent: IgG isolated according to Vaerman et al. [7] was injected into a rabbit to make anti-IgG serum. The antiserum was absorbed with fixed IgM to remove the anti-light-chain (anti-L-chain) activity. Antibodies against IgG in the absorbed antiserum were purified specifically with fixed IgG. The purified antibodies were bound to Sepharose to form an anti-IgG immunoabsorbent column.

Anti-IgG(T) immunoabsorbent: IgG(T) isolated according to McGuire et al. [4] was injected to make anti-IgG(T) serum. Absorption of the antiserum was carried out with fixed IgG. After absorption, antibodies against IgG(T) were purified specifically with fixed IgG(T) and then bound to Sepharose to form an anti-IgG(T) immunoabsorbent column.

Anti-IgM immunoabsorbent: IgM isolated according to Mach and Pahud [3] was injected to make anti-IgM serum. Absorption of the antiserum was carried out with fixed IgG. After absorption, antibodies against IgM were purified specifically with fixed IgM and then bound to Sepharose to form an anti-IgM immunoabsorbent column.

Anti-L-chain immunoabsorbent: Specifically purified antibodies containing anti-L-

Table 1. Properties and efficiency of the immunoabsorbent column for purification of horse serum IgA

Immunoabsorbent column	Composition of column		Maximum capacity of column ²⁾
	Antibodies ¹⁾ /Sepharose		
Anti-L-chain	340 mg	50 g	40 mg
Anti-IgM	70 mg	15 g	25 mg
Anti-IgG	33 mg	10 g	6 mg
Anti-IgG (T)	84 mg	15 g	10 mg
Anti-non-Ig	— ³⁾	15 g	NT ⁴⁾

¹⁾ Protein concentration were determined from optical density at 280 nm and a 1% extinction coefficient of 13.5, determined with the Folin phenol reagent.

²⁾ Protein concentrations were determined by the micro-kjeldahl method and from optical density at 280 nm.

³⁾ The gammaglobulin fraction was bound to Sepharose.

⁴⁾ NT=Not tested.

chain activity were recovered from the unabsorbed anti-IgM antiserum with fixed IgG and then bound to Sepharose to form an anti-L-chain immunoabsorbent column.

Anti-non-Ig immunoabsorbent: Immunoglobulins in 2 ml of horse sera were removed with the anti-L-chain immunoabsorbent column. The break through fraction from this column was concentrated and injected to make anti-non-IgG serum. The antiserum obtained, designated as anti-non-immunoglobulin-serum-components (anti-non-Ig), developed no precipitin arc of immunoglobulins against whole horse serum in immunoelectrophoresis. The antiserum (10 ml) was precipitated with ammonium sulfate at 50% saturation. The sediment, dialyzed against sodium bicarbonate buffer (0.1 M; pH 9.0), was bound to Sepharose to form an anti-non-Ig immunoabsorbent column.

The procedures for purification of horse serum IgA are illustrated in Fig. 1. The serum taken from 11 healthy horses were dialyzed against 0.01 M sodium phosphate buffer (PB; pH 6.2). It was centrifuged to separate pseudoglobulin from euglobulin. The pseudoglobulin fraction was precipi-

tated with ammonium sulfate at 50% saturation. After dialysis against PB (0.01 M; pH 7.4), the sediment was chromatographed on a DEAE-cellulose column equilibrated with the same buffer. The fractions eluted with 0.01–0.04 M PB, pH 7.4 were discarded. The fraction eluted with 0.04 M PB, pH 7.4 containing 0.1 M NaCl was concentrated and applied to the anti-L-chain immunoabsorbent column to purify immunoglobulins. The column was well washed with 0.01 M phosphate buffered saline (PBS), pH 7.4 and then treated with 3 M NaSCN to dissociate immunoglobulins from this column. Immunoglobulins obtained were concentrated with polyethylene glycol after dialysis against PBS and centrifuged at 3,000 rpm for 30 min to remove insoluble materials. Pure IgA was recovered from the supernatant after removal of IgG, IgG(T), IgM and a traces of nonimmunoglobulin serum components (non Ig). Non Ig were traces of contaminants based on nonspecific interaction on the anti-L-chain immunoabsorbent column. Such removal was performed by use of the immunoabsorbent columns of anti-IgG, anti-IgG(T), anti-IgM and anti-non-Ig. By these pro-

Fig. 1. Procedures for purification of horse serum IgA.

cedures, 4.6 mg of horse serum IgA was obtained from 150 ml of pooled serum. Purified serum IgA showed only one precipitin arc against anti-whole-horse serum by immunoelectrophoresis (Fig. 2) and no reaction with the antisera against IgG, IgG(T), IgM or non Ig by the double diffusion method.

When purified horse serum IgA was analyzed with anti-dog IgA (Miles Laboratories, Inc., Elkhart, U.S.A.), an immunological cross-reaction was observed by the double diffusion method (Fig. 3).

Antiserum against horse serum IgA was obtained in the rabbit immunized with 1 mg of purified horse serum IgA in Freund's complete adjuvant. After absorption with fixed IgG, the antiserum developed a single precipitin arc with whole horse serum in

immunoelectrophoresis (Fig. 2). The antiserum was therefore regarded as monospecific antiserum against horse serum IgA.

Antiserum against horse SIgA was obtained from the rabbit immunized with well-washed immunoprecipitate formed between the anti-horse-serum-IgA and colostrum whey. Absorption of the antiserum was performed with fixed IgG. After absorption, the anti-horse-SIgA showed a single precipitin arc against horse serum as well as colostrum in immunoelectrophoresis (Fig. 4). The anti-horse-SIgA antiserum reacted with them by the double diffusion method, it formed spur against horse serum (Fig. 5). The formation of spur indicates the presence of additional antigenic determinants on colostrum IgA compared to serum IgA. Similar observations have been

Fig. 6. Elution profiles of horse serum on a Sephacryl S-300 column; Distribution of serum proteins was determined by single radial immunodiffusion.

reported by Pahud and Mach [5] and McGuire et al. [4], who stated that the formation of spur corresponded to the secretory component (SC).

When horse serum was chromatographed on a Sephacryl S-300 column, serum IgA was eluted well behind IgM, and slightly

behind α_2 -macroglobulin and slightly before IgG and IgG(T). Albumin was separated well from the foregoing. Characteristic two peaks of serum IgA were also observed (Fig. 6).

It was proved that the immunoglobulin isolated in the present study was horse serum IgA on the basis of: (a) a single entity in immunoelectrophoresis and double diffusion; (b) its $\beta_2\text{--}\gamma_1$ electrophoretic mobility; (c) its immunological cross-reactivity with anti-dog-IgA; (d) its characteristic elution profiles from Sephacryl S-300; and (e) the demonstration of additional antigenic determinants on colostral secretory IgA.

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要 約

ウマ血清 IgA の分離精製および同定 (短報): 栗本 司・池田 敦・田中享一 (麻布大学獣医学部家畜衛生学教室)——ウマ分泌型 IgA (初乳, 涙) の分離精製については, すでに報告されているが, ウマ血清 IgA の分離精製に関する明確な手法を示した報告は見あたらない. われわれは, ウマ血清を脱塩, 硫酸塩析し, ついで DEAE セルロース, 免疫吸着体のカラム操作により, 抗原性および分子サイズにおいて, IgG, IgG(T), IgM とは明らかに区別される免疫グロブリンを分離精製した. この免疫グロブリンは抗イヌ IgA との交差反応性により, IgA と同定された. さらに作製した抗分泌型 IgA により, 初乳 IgA に secretory component の存在が想定された.

Explanation of Figures

Fig. 2. Evaluation of purity of isolated horse serum IgA and demonstration of monospecificity of prepared anti-horse-serum-IgA by immunoelectrophoresis. The anode is on the left side. The top and bottom wells were filled with horse serum (S).

Fig. 3. Double diffusion analysis demonstrating cross reactivity between purified horse serum IgA and anti-dog-IgA.

Fig. 4. Immunoelectrophoretic analysis of pre-

pared anti-horse-SIgA demonstrating development of a single precipitin arc when reacted with horse serum and colostrum. The anode is on the left side. Wells were filled with serum (S) and colostrum (C).

Fig. 5. Double diffusion analysis of horse colostrum and serum demonstrating the presence of additional antigenic determinants on colostrum IgA compared to serum IgA when reacted with the monospecific anti-horse-SIgA.