Natural human antibodies to dietary lectins

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Abstract Natural antibodies to self and non-self proteins, including dietary proteins, are a significant part of the immune repertoire of humans. Antibodies to three structurally related legume lectins (*Erythrina corallodendron* lectin (ECorL), peanut agglutinin (PNA), and soybean agglutinin (SBA)) and to one cereal lectin (wheat germ agglutinin (WGA)) were purified by affinity chromatography from human sera and their binding specificity examined. The anti-SBA, anti-ECorL and anti-WGA antibodies exhibited high specificity, whereas the anti-PNA antibodies were polyreactive. Although the anti-WGA antibodies were highly specific for WGA, they also crossreacted slightly toward some other proteins. The anti-ECorL antibodies bound to native SBA, but the anti-SBA antibodies failed to bind to the native ECorL. Although the anti-SBA and anti-ECorL antibodies both exhibited specificity when interacting with native lectins, they bound to a wider range of denatured lectins, indicating a common or universal epitope which is recognized by many natural antibodies. Interestingly, the natural antibodies did not interfere with the agglutination properties of the lectins. These findings may provide a basis for studying the in vivo biological effects of anti-dietary protein antibodies, including those against carbohydrate-binding proteins.

Key words: Food antigen; Legume lectin; Cereal lectin; Natural autoantibody; Human serum

1. Introduction

Recently, we demonstrated that human serum contains natural antibodies to dietary proteins from eggs and garlic [1,2]. The observed natural antibodies to the garlic proteins can be divided into specific and polyreactive categories. The specific antibodies interact with the major protein component of garlic, the enzyme alliinase, and the polyreactive antibodies, which are against the mannose specific lectin (ASA), interact with many other proteins. Whether this poly(cross)-reactive property is specific for anti-ASA natural antibodies or is a general property of other anti-dietary lectin antibodies is intriguing.

Lectins, which are carbohydrate-binding proteins, are important biological tools, particularly in the isolation, localization and determination of glycoconjugates [3]. Lectins are also used for blood typing [4] and for fractionation of bone marrow cells for transplantation [5]. The presence of natural antibodies in human serum can interfere with some of these applications.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; ECorL, *Erythrina corallodendron* lectin; PNA, peanut agglutinin; RBP, riboflavin-binding protein; SBA, soybean agglutinin; WGA, wheat germ agglutinin

The human diet typically includes many substances of plant origin, several of which contain different and functionally active lectins, which are main components of various seeds and vegetables. Some lectins present in common dietary vegetables are resistant to degradation by digestive enzymes [6], and thus remain active during passage through the gut and can mediate a variety of biological effects [7–9]. In rodents, a diet containing lectins can provoke intestinal and systemic immune responses to these carbohydrate-binding proteins [10,11].

Whether human serum contains natural antibodies to dietary and non-dietary lectins and the properties of such affinity-purified antibodies were examined. Of the lectins chosen for this study, three (peanut agglutinin (PNA), soybean agglutinin (SBA), and *Erythrina corallodendron* lectin (ECorL)) have similar carbohydrate specificities and are derived from related legumes, whereas the fourth (wheat germ agglutinin (WGA)) is derived from a cereal and is specific for *N*-acetylglucosamine and sialic acid.

2. Materials and methods

2.1. Materials

Alkaline phosphatase-conjugated goat anti-human F(ab)', *N*-acetyl-D-glucosamine, avidin, bovine serum albumin (BSA), conalbumin, D-galactose anhydrous, lysozyme, *p*-nitrophenyl phosphate, ovalbumin, ovomucoid, PNA, riboflavin-binding protein (RBP), SBA, Tween 20 and WGA were from Sigma Chemical Co. (St. Louis, MO); cross-linked Sepharose 4B and protein-A Sepharose were from Pharmacia (Uppsala, Sweden): and 96-welled, flat-bottomed and conical-shaped microtiter plates were obtained from Nunc Co. (Roskilde, Denmark). Purified ECorL and recombinant ECorL (rECorL) were kind gifts from Prof. Nathan Sharon (The Weizmann Institute of Science) [12]. Alliinase and ASA were purified as previously described [2]. Human sera were obtained from the Tel Aviv Blood Bank of the Israel Blood Center/Magen David Adom.

2.2. Affinity purification of human anti-lectin antibodies

Lectins were coupled to nitrophenylchloroformate-activated Sepharose [13] for 48 h in PBS, pH 8. Unreacted nitrophenyl groups were blocked with 100 mM NH_4OH. Using this procedure about 2 mg of protein were bound per ml of resin.

Immunoglobulins were initially enriched from pooled normal human plasma from 7 healthy adult donors by precipitation with 45% ammonium sulfate. The precipitate was dialyzed against PBS, and the resulting dialysate was applied to an antigen-affinity column (overnight, 4°C, recycling mode at 6 ml/min). The column was then washed extensively with 0.05 M Tris-HCl, pH 7.5, followed the same buffer containing 0.5 M NaCl. Bound antibodies were eluted using either 0.1 M glycine (pH 3) or 0.1 M triethylamine (pH 11) buffer. The pH of the eluted fractions was immediately neutralized with 1 M Tris-HCl buffer, pH 8.

Since antibodies are glycoproteins, it was important, during the immunopurification procedure, to prevent unwanted binding of extraneous antibodies, due to the lectin-sugar interaction. Free saccharides were thus included in the appropriate solutions (i.e. the dialysates applied to the columns and subsequent washing buffers) – 0.2 M galactose for SBA-, PNA- or ECorL-containing affinity columns and 0.2 M *N*-acetylglucosamine for WGA-containing columns. The antibodies, eluted either with acidic or basic buffers, were then rechromatographed on a protein A column to enrich the IgG fraction.

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2.3. Detection of antibodies

The presence of antibodies was detected by ELISA. Briefly, microtiter plates were coated (100 μl/well) with an antigen solution (10 μg/ml) in 50 mM carbonate buffer, pH 9.8, and allowed to stand overnight at 4°C. The plates were then washed three times with phosphate-buffered saline (PBS) and blocked for 2 h at 37°C with PBS (100 μl/well) containing 3% BSA and 0.05% Tween 20 (BSA-Tween buffer). The plates were washed with PBS, and solutions containing purified antibodies were added to the antigen-coated wells (100 μl/well). Following an additional incubation for 2 h at 37°C, the plates were washed 3 times with PBS, and a solution (100 μl/well) containing alkaline phosphatase-conjugated goat anti-human F(ab)′ (diluted 1:2000) was added. After 2 h at 37°C, the plates were washed extensively with PBS, and substrate solution (10 mg of *p*-nitrophenyl phosphate in 10 ml of 100 mM diethanolamine buffer, pH 9.5) was added (100 μl/well). 30 min later, the OD_{405} was measured using an ELISA reader.

2.4. Electrophoresis and immunoblotting

SDS-polyacrylamide gel electrophoresis of lectins was performed under reducing conditions with 15% acrylamide slab gels. Some gels were stained with 0.05% Coomassie Brilliant Blue R 250 (BioRad, Richmond, CA), while others were used for immunoblotting. For immunoblotting, proteins were transferred from unstained gels to nitrocellulose sheets (150 mA for 2 h; transfer buffer consisted of 25 mM Tris-HCl and 192 mM glycine in methanol/water (1:5)), and the blots were blocked with BSA-Tween buffer and then incubated with primary antibodies (20 μg per ml of BSA-Tween buffer), affinity-purified on a lectin column. Blots were washed 3 times with PBS and incubated for 2 h with alkaline phosphatase-conjugated goat anti-human F(ab), diluted 1:2000 in BSA-Tween buffer. The blots were washed thoroughly and developed using 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT) (Sigma Chemical Co., St. Louis, MO).

2.5. Agglutination assay

The presence of anti-lectin antibodies was monitored by examining the inhibition of agglutination of sialidase-treated human red blood cells. Lectin (ECorL, SBA, PNA or WGA) solutions (5 μl of 1 mg/ml) were incubated overnight with affinity-purified human anti-lectin antibodies (40 μl, 1 mg/ml) or with total human IgG (50 μl, 10 mg/ml). Aliquots (50 μl of serial dilutions) of the resulting mixtures were mixed with sialidase-treated red blood cells, prepared as previously described [14], and incubated (1 h, room temperature) in conically shaped microtiter plates (4% suspension of sialidase-treated red blood cells in a final volume of 100 μl/well). Agglutination was assessed visually.

3. Results

The yield of human anti-lectin antibodies purified from 100 ml of human serum by affinity chromatography on lectin (SBA, ECorL, PNA or WGA)-Sepharose columns was about 140 ± 20 μg for each. The specificity and polyreactivity of the purified anti-lectin antibodies were determined both by an ELISA procedure and by their binding to immobilized lectins. The antibodies to SBA and ECorL were highly selective and demonstrated little crossreaction with the other proteins (Table 1). In contrast, the antibodies to PNA were polyreactive and bound to most of the other proteins examined. Although the anti-WGA antibodies exhibited the strongest binding with WGA, they were slightly crossreactive, since they also exhibited binding to alliinase, ASA, and lysozyme. Whether antigen-antibody or sugar-ligand interactions were involved in the binding the anti-lectin antibodies to their respective lectins was examined. This was achieved by (i) preincubating (1 h, 37°C) SBA, ECorL and PNA with galactose and WGA with *N*-acetylglucosamine or (ii) including the sugars (200 mM) in the first and second antibody solutions during the ELISA procedure. These manipulations did not cause significant changes in the adsorption of anti-lectin antibody to the immobilized lectins (data not shown), thus indicating that the antibody binding was due to the antigen-antibody interaction. Moreover, the binding of the antibodies to the immobilized lectins could be inhibited by the corresponding lectin in solution, thereby strengthening this conclusion.

Certain lectins, such as, ECorL and SBA, are immunologically related, since rabbit serum to one lectin can crossreact with another (D. Belenky, personal communication). Therefore, the ability of the lectin-affinity purified human antibodies to bind to other lectins was also examined in non-competitive binding assays. Serial dilutions of purified anti-SBA and anti-ECorL antibodies were added to wells on which either ECorL, rECorL or SBA had been immobilized. The binding of anti-ECorL antibodies to ECorL, rECorL, and SBA was similar (Fig. 1A). In contrast, the anti-SBA antibodies, which bound avidly to SBA, did not bind with the immobilized ECorL, even at concentrations of 20 μg/ml (Fig. 1B).

ECorL is a glycoprotein containing two fucose residues/subunit, whereas its recombinant form is non-glycosylated [12]. The purified anti-ECorL antibodies bound similarly to both the intact glycosylated and recombinant forms of immobilized rECorL (Fig. 1A), thus indicating that the protein part of the lectin, not the sugar residue, is the portion recognized.

It was next assessed whether the affinity-purified antibodies could bind to denatured (Western blotted) lectins. Both the anti-SBA and anti-ECorL antibodies bound to all three denatured legume lectins (Fig. 2A,B). Similarly, the affinity-purified anti-WGA antibodies bound to denatured WGA (Fig. 2C). Thus, the purified anti-lectin antibodies recognized both intact (native; Table 1) and denatured forms of the lectins, and the specificity depends on the state of the lectin.

Whether interaction with human anti-lectin antibodies can affect the carbohydrate-binding capacity of lectins was examined in hemagglutination assays. The presence of non-fractionated human IgG or affinity purified anti-SBA, -ECorL or -WGA antibodies (40 μg/ml) did not affect the lectin-mediated agglutination of sialidase-treated human erythrocytes (data not shown).

Table 1
Specificity of human antibodies purified by lectin affinity chromatography

Protein	Anti-lectin antibodies[a] (OD_{405})[b,c,d]			
	Anti-SBA	Anti-PNA	Anti-ECorL	Anti-WGA
Ovalbumin	0.131	0.172	0.129	0.216
Ovomucoid	0.118	0.126	0.106	0.137
RBP[b]	0.127	0.190	0.147	0.199
Lysozyme	0.185	0.389	0.229	0.643
Conalbumin	0.129	0.202	0.146	0.235
Avidin	0.212	0.289	0.234	0.210
ASA	0.147	0.234	0.174	0.276
Alliinase	0.169	0.237	0.348	0.279
SBA	**1.114**	0.344	0.785	–
PNA	0.169	**0.333**	0.236	–
ECorL	0.110	0.329	**1.161**	–
WGA	–	–	–	**1.038**

[a]20 μg/ml.
[b]OD_{405} observed after 30 min incubation with the substrate.
[c]Averages obtained from three preparations of purified antibodies.
[d]$OD_{405} > 0.060$ was considered to be above background and therefore indicative of binding.

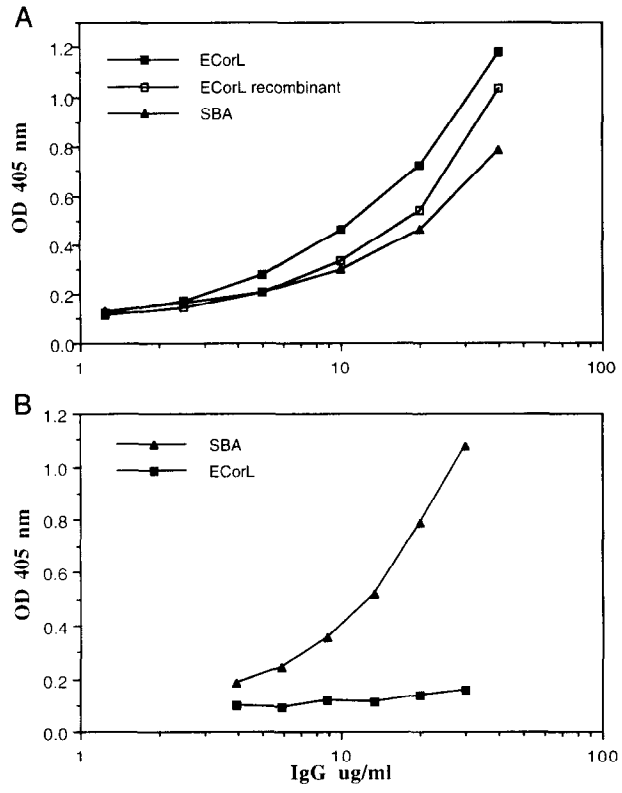

Fig. 1. Dose dependence of the binding of affinity-purified human anti-lectin antibodies to lectins. (A) Binding of anti-ECorL antibodies with ECorL (■), recombinant rECorL (□) and SBA (▲). (B) Binding of anti-SBA antibodies with SBA (▲) and ECorL (■).

4. Discussion

Antibodies (specific and polyreactive) to dietary proteins exist in the sera of healthy humans [2]. Since common vegetarian foods contain many different plant lectins, the presence in human sera of natural antibodies to dietary lectins and their characteristics were examined. From the sera of healthy subjects, we purified, using lectin-affinity chromatography, antibodies that recognized and bound to three legume lectins (ECorL, PNA, and SBA) and one cereal agglutinin (WGA). The purified anti-legume lectin antibodies exhibited a range of specificities. The anti-SBA antibodies were specific and did not react with other proteins in ELISA assays. Similarly, the anti-ECorL antibodies reacted with SBA, but not with PNA. In contrast, the anti-PNA antibodies were polyreactive and crossreacted with all proteins tested. In this respect, they were similar to the anti-ASA antibodies previously isolated in our laboratory [2]. The anti-WGA antibodies reacted with WGA and lysozyme, but not with the other proteins tested.

Although ECorL is not a dietary protein, and has never been injected into humans, significant amounts of antibodies that demonstrate specificity for this lectin are present in human sera. Legume lectins share considerable structural homology [15] and can be antigenically related [16]. Thus, antibodies that specifically interact with ECorL and other lectins (dietary and non-dietary) may actually be elicited by antigenically related dietary lectins.

Antibodies to lectins contained in popular food items can display completely different properties, as demonstrated by the various specificities to native lectins of the anti-lectin antibodies purified in this study. Since many of these dietary lectins have similar molecular weight and structures, the specificity of the same antibodies for denatured forms of the lectins was examined. Anti-SBA antibodies, which did not react with native ECorL or PNA (Table 1), reacted with denatured ECorL, PNA, and SBA legume lectins (Fig. 2A). Similarly, anti-ECorL, which did not react with native PNA, reacted with denatured ECorL, PNA, and SBA (Fig. 2B). In the primary sequence of all legume lectins, > 10% of their amino acids are invariant [15]. Some of these residues, which are involved in metal binding and are grouped in extended regions, may serve as common epitopes. Upon denaturation, some common epitopes may become available for binding with lectin-specific antibodies. Similarly, human anti-avidin antibodies, previously purified in our laboratory, bind to denatured, but not native streptavidin (Tchernychev, unpublished results). The anti-WGA, which is a non-legume lectin, antibodies reacted with both native and denatured WGA (Fig. 2C) and exhibited crossreactivity with several of the proteins and lectins tested (Table 1). Furthermore, the

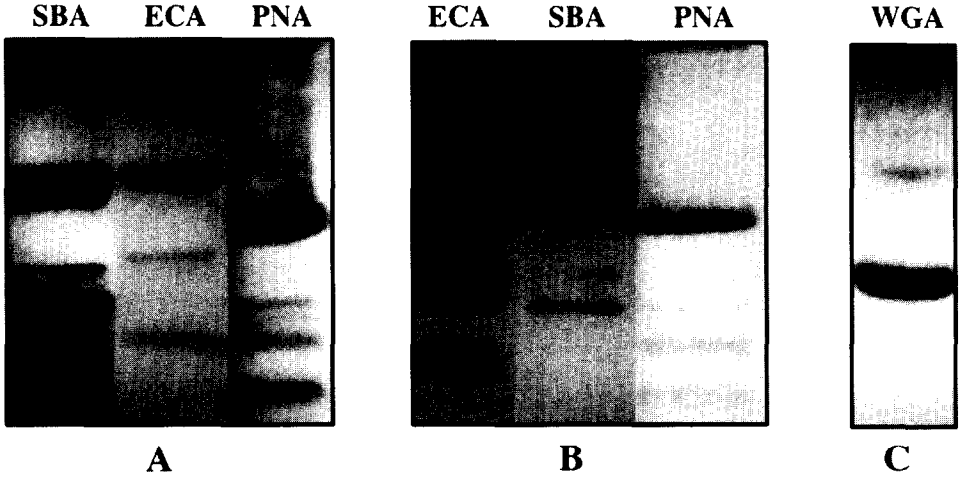

Fig. 2. Binding of human anti-SBA (A), anti-ECorL (B), and anti-WGA (C) antibodies to denatured ECorL, PNA, SBA, and WGA. Bound antibodies were visualized by incubation with alkaline phosphatase conjugated goat anti-human F(ab)′ followed by BCIP and NBT.

interaction of the purified antibodies with the lectins involved protein-protein interactions, not protein-carbohydrate interactions.

The sera of some rabbits immunized against legume lectins inhibit the carbohydrate-binding activity of the immunizing and immunologically crossreactive legume lectins, as assessed by agglutination [16]. In contrast, the natural human anti-ECorL, -SBA, -PNA, and -WGA antibodies purified in this study did not affect the agglutinating capacity of their specific lectins. This difference between anti-lectin antibodies elicited in different species may be due to variations in the avidity of the antibodies or, possibly, to a masking by glycoconjugates of the carbohydrate-binding site of ingested lectins as they pass through the gut. The latter of which would mask antigenic epitopes associated with the carbohydrate-binding site.

In summary, human serum contains natural antibodies to lectins commonly present in human diets. These antibodies, which were purified by affinity chromatography using lectins, can be either specific or polyreactive, as was previously seen with other natural antibodies to other dietary proteins. Since diagnosis of food allergies involves determining the titer of IgE type antibodies, it may be necessary to also consider the presence of IgG type antibodies that interact with food antigens. Furthermore, natural anti-lectin antibodies may affect in situ and in vitro procedures in which lectins participate.

Elucidation of the mechanisms by which human anti-dietary antibodies, including those against carbohydrate-binding proteins, are invoked and the intestinal and systemic immune responses in which such antibodies participate may be important in understanding of immunity in general.

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