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Bacterial Proteins and Their Proposed Interactions with Fc or Fab Fragments of Immunoglobulins

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Abstract

The reactivity of Immunoglobulin Binding Proteins (IBP) to Fc and/or Fab fragments of immunoglobulins was summarized in this review. Staphylococcal protein A (SpA), Streptococcal protein G and Peptostreptococcal protein L (SpL) were the IBP reported. SpA reacted with IgG from skunk, coyote, raccoon, mule and donkey. SpG reacted almost with the entire panel of immunoglobulins and SpL binding was restricted to some immunoglobulins including raccoon, ostrich and duck. The various immunological techniques that have been used to test the binding capacity of IBP to Igs were double immunodiffusion, Enzyme-Linked Immunosorbent Assay (ELISA), SpA-affinity chromatography and immunoblot analysis. These protein-protein interactions are important because they can be used in the immunodiagnosis and in the purification of intact Igs or their fragments.

Keywords: Immunoglobulin; Streptococcal protein; Bacterial proteins; ELISA; Immunoblot analysis

Introduction

The binding of Immunoglobulin-Binding Protein (IBP) such as Staphylococcal protein A (SpA) [1], Streptococcal protein G [2] and Peptostreptococcal protein L (SpL) [3,4] to immunoglobulins (Igs) from distinct animal species is known [1-9]. However, the information about their binding capacity to Fc or Fab fragments is scanty. The aim of this review is to report on the reactivity of IBP to immunoglobulin regions from a number of mammalian and avian Ig molecules.

The following is what is well-known about IBP: SpA has a molecular weight of 42 KDa. It binds to the Fc fragment of IgG produced by several animal species. The native protein consists of five domains. Of these, four show high structural homology of about 58 aminoacids and they have binding capacity to immunoglobulins [1]. Streptococcal protein G, Type III bacterial Fc receptor, is a small globular protein produced by several streptococcal species and is composed of two or three nearly identical domains, each of 55 amminoacids. SpG is well-known for binding to many species including human, mouse, rat and hamster [2]. SpL comprised of an alpha-helix packed against a 4 stranded beta-sheet. Utilizing immunoblot assays showed that the isolated protein binds to immunoglobulins through L chain interaction [3-5].

Materials and Methods

All materials used in the following experiments were obtained from Sigma-Aldrich Co, St. Louis, Missouri, USA. The methods used to study the reactivity or binding capacity of IBP to Igs: Fc, Fab or both regions were double immunodiffusion [6,10], ELISA [6,8,9,11], immunoblot analysis [2,3,6,12] and SpA-affinity chromatography [5-7]. In addition the IgY purification from avian egg yolks was carried out by the method of Polson [5-8,12,13].

Immunoglobulin Y isolation

The IgY fraction was isolated from the egg yolks of a variety of birds including chicken, bantam hen, duck, and ostrich. The IgY fraction was isolated by the chloroform-polyethylene glycol (PEG) method [12]. The eggs were washed with warm water and the egg yolk was separated from the egg white. The membrane was broken and the egg yolk collected and diluted 1:3 in Phosphate Buffered Saline (PBS), pH 7.4. To one third (1/3) of the egg yolk mixture an equal volume of chloroform was added, the mixture was then shaken and centrifuged for 30 min, 1000 x g, Room Temperature (RT). The supernatant was decanted and mixed with PEG 6000 (12%, w/v), stirred and incubated for 30 min at RT. The mixture was then centrifuged as described above. The precipitate containing IgY was dissolved in PBS (pH 7.4) at a volume equivalent to one sixth (1/6) of the original volume of the egg yolk and dialyzed against 1L of PBS (pH: 7.4 for 24 h at 4°C). The IgY was removed from the dialysis tubing. IgY concentration was determined by the Bradford method [13]. IgY samples were stored at –20°C.

Purification of immunoglobulins from animal sera and avian eggs

A commercially prepared protein-A antibody purification kit, PURE-1A (Sigma-Aldrich Co, St. Louis Missouri) based on SpA-affinity chromatography was used to purify IgG from the sera of skunk, coyote, raccoon, mule, horse, donkey; IgY from ostrich, bantam hen and duck egg yolks and ostrich IgM from the ostrich egg white. The procedure was performed according to the manufacturer's instructions [7,14].

Binding properties of bacterial immunoglobulin receptors by double immunodiffusion (Ouchterlony) technique

The binding of SpA, SpL, and SpG with animal sera, avian IgY, avian egg whites and purified IgG were investigated by double immunodiffusion as previously described [8].

Ig Samples	Positive double immunodiffusion	Positive ELISA	SpA affinity Chromatography	Positive Immunoblot analysis
Pig IgG*	SpA, SpG, SpL	SpA, SpG, SpL	Positive	SpA, SpG, SpL
Rabbit IgG*	SpA, SpG, SpL	SpA, SpG, SpL	Positive	SpA, SpG, SpL
Goat IgG*	SpA, SpG,	SpA, SpG	Positive	SpA, SpG
Sheep IgG*	SpA, SpG,	SpA, SpG	Positive	SpA, SpG
Human IgG*	SpA, SpG, SpL	SpA, SpG, SpL	Positive	SpA, SpG, SpL
Mouse IgG*	SpA, SpG, SpL	SpA, SpG, SpL	Positive	SpA, SpG, SpL
Cat IgG	SpA	None	Positive	SpA
Skunk IgG	SpA, SpG	SpA, SpG	Positive	SpA, SpG
Coyote IgG	SpA, SpG	SpA, SpG	Positive	SpA, SpG
Raccoon IgG	SpA	SpA	Positive	SpA
Mule IgG	SpG	SpA, SpG	Positive	SpA, SpG
Donkey IgG	SpA, SpG	SpA, SpG	Positive	SpA, SpG
Ostrich IgY	None	SpA, SpL	Positive	SpA, SpL
Duck IgY	None	SpA, SpG. SpL	Positive	SpA, SpG, SpL
Duck serum	None	SpA, SpL	Positive	SpA, SpG, SpL
Bantam hen IgY	None	SpA, SpL	Positive	SpA, SpL
Chicken IgY*	None	None	Negative	None
Ostrich E.W. Ig	None	SpL, SpA	Positive	SpA, SpL

Table 1: Comparison of the reactivities of SpA, SpG and SpL using various immunological techniques. Keys: NB: No binding, EW: Egg white. *: Previously reported for proteins A, L and G

Briefly, 1% agarose gels were prepared and wells cut into the gel using a template. Initially, aliquots of 25 µl each of SpA, SpL or SpG at 1 µg /µl were applied to the centre well. The peripheral wells were filled with 25 µl each of IgY (30 µg/µl), avian egg white diluted 1:2 in PBS pH 7.4, purified IgG or animal serum. The gels were incubated at RT for 48-72 hours and then examined for precipitin lines. Human serum and human IgG were included as positive controls. The positive results were taken as the presence of precipitin lines and negative results, the absence of precipitin lines. The experiments were repeated using concentrations of each bacterial protein and animal serum or purified immunoglobulin ranging from (1-51 µg /µl).

ELISAs

The microplates were coated with the samples (20 to 50 µl of sera, various concentration of IgG, IgY or egg whites) diluted in 50 µl of carbonate coating buffer (pH 9.6), and incubated overnight at 4°C. Washing was conducted 4 times with PBS (phosphate buffered saline, pH 7.4) containing 0.05% Tween 20 buffer. Binding of the IBP was using the IBP-HRP (Horseradish Peroxidase) conjugate (SpA-HRP, SpG-HRP or SpL-HRP), and 50 µl of 3 mg/ml o-phenylene diamine solution in PBS-Tween 20 was used as substrate. The reaction of ELISA was stopped with 25 µl of 3M H_2SO_4 solution. The plates were read in a microplate reader at 492 nm.

Immunoblot analysis

Aliquots of 3-5 µl [1 µg/µl] of the Ig samples applied to the gel and run on a protein electrophoresis (SDS-PAGE). Gels were transferred to nitrocellulose membranes (Immobilon-Nc, pore size 0.45 µm, during 75 min at 40 mAmps using a semi-dry electro blotter, HEP-1 Model, Owl Scientific Inc). The running buffer contained 25mM Tris, 192mM glycine pH 8.3 and 20% methanol. The nitrocellulose membranes were blocked overnight in 10% nonfat skim milk in PBS with 0.05% Tween-20 pH 7.4 and then washed 4 times for 10 minutes with PBS-Tween 20. Peroxidase-labeled SpA, SpG or SpL (Sigma-Aldrich Co, USA) was added and incubated at 4°C overnight. Membranes were washed as above and then tetra-methyl-benzidine was added and the reaction was stopped with deionised water. A positive test displays colored bands. Positive and negative controls were included in the test. This test was used for confirmation of the results obtained using other techniques. The test was repeated three times and similar results were gotten.

Results and Discussion

The various immunological techniques reported [6] were double immunodiffusion (Ouchterlony technique) that tested positive high protein reactivities. ELISAs tested positive moderate and low protein-binding capacities. SpA-affinity chromatography and immunoblot analysis were sensitive tests and helpful in the screening of IBP-Igs

interactions as confirmative tests. The comparison of the reactivities of SpA, SpG and SpL using various immunological techniques are shown in Table 1.

Table 2 addresses the known and newly proposed interaction between Immunoglobulin specimens and IBP. From all these interactions, SpA is the better known studied and reported, followed by protein G and protein L. SpA binds to IgG from pigs, rabbits, goats, sheep, human, mouse and cat and it is a new finding the SpA interaction with the IgGs of skunk, coyote, raccoon, mule, donkey, ostrich and duck. These interactions of SpA with immunoglobulins may involve the Fc, Fab or both fragments.

Ig Samples	SpG	SpL	SpA	Comments
Pig IgG*	Fc	L chain (kappa)	Fc	Reported [1-3]
Rabbit IgG*	Fc	L chain (kappa)	Fc	Reported [1-3]
Goat IgG*	Fc	NB	Fc	Reported [1-3]
Sheep IgG*	Fc	NB	Fc	Reported [1-3]
Human IgG*	Fc	L chain (kappa)	Fc	Reported [1-3]
Mouse IgG*	Fc	L chain (kappa)	Fc	Reported [1-3]
Cat IgG	Fc	L chain (kappa)	Fc	Reported only for SpA [1,6]
Skunk IgG	IgG (Fc)	NB	Fc or Fab	Newly reported [6]
Coyote IgG	IgG (Fc)	NB	Fc or Fab	Newly reported [6]
Raccoon IgG	NB	L chain (kappa)	Fc or Fab	Newly reported [6]
Mule IgG	IgG (Fc)	NB	Fc or Fab	Newly reported [6]
Donkey IgG	IgG (Fc)	NB	Fc or Fab	Reported only for SpA [6]
Ostrich IgY	Fc	Lchain (kappa)	Fc	Newly reported [6]
Duck IgY	Fc	Lchain (kappa)	Fc	Newly reported (SpL) [6,15]
Duck serum	NB	Lchain (kappa)	IgY (Fc)	Newly reported [6]
Bantam hen IgY	NB	Lchain (kappa)	Fc	Newly reported [6]
Chicken IgY*	NB	NB	NB	Reported [3]
Ostrich EW Igs	NB	IgM-kappa chain	IgM (Fab)	Newly reported [6,7]
Pheasant EW Igs	NB	NB	IgM (Fab)	Newly reported [6]

Table 2: Newly proposed Fc and /or Fab regions of immunoglobulins involved in the reactivity with Ig-binding proteins (proteins A, G or L). Keys: NB: No binding, EW: Egg white *: Previously reported for proteins A, L and G

SpL binds to kappa chains of some Igs from pigs, rabbits, mouse and human. We newly reports that SpL binds to IgG from cat and raccoon, to IgY from ostrich, duck, bantam hen and egg white immunoglobulins (IgM, IgA or both). SpG binds mainly to the entire panel of mammalian immunoglobulins by their Fc fraction. It is newly reported that SpG also binds to IgG from skunk, coyote, raccoon, mule and donkey. These interactions mainly involved the Fc region of Igs.

The IBP-Igs reactivity is important since these bacterial proteins can be used as immunological tools in immunoassays including Enzyme-Linked Immunosorbent Assays (ELISA) [5-6,8,9,11,12], dot blotting for the immunodiagnosis of infectious diseases [12] and also purification of IgG molecules and their fragments [5-7,15].

Protein A (SpA) binds mainly to the Fc portion of IgGs and some IgM and IgA molecules by its Fab region. Protein L (SpL) binds to IgG Kappa chains from raccoon and does not bind to IgGs from the other species tested. SpG binds to a broader spectrum of immunoglobulins by their Fc region.

Conclusion

This review suggests newly reported interactions of IBP with the Fc or Fab regions of immunoglobulins, for example mule, donkey, coyote, skunk and raccoon. It demonstrates once more the importance of IBP for immunodiagnosis and purification of immunoglobulins.

Competing Interests

The authors declared that no competing interests exist.

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