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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

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)		

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