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Jacalin, the human IgA1 and IgD precipitating lectin, also binds IgA2 of both allotypes

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The lectin jacalin from jackfruit seeds shows a human IgA-subclass specificity by gel precipitation and Western blotting. However, its reactivity with IgA2 is a matter of controversy. We further studied the immunoglobulin isotype specificity of jacalin by affinity chromatography with myeloma sera and by inhibition of jacalin binding to solid-phase IgA1 by purified monoclonal immunoglobulins. The lectin proved to bind IgA2 of both allotypes with a lower apparent affinity than for IgA1 and IgD.

Key words: Jacalin; Lectin; Chromatography, affinity; IgA subclass; IgA2 allotype

Introduction

The lectin jacalin from *Artocarpus heterophyllus* (or *A. integrifolia* L.) seeds first came to the attention of immunologists because of its mitogenic properties (Bunn-Moreno and Campos-Neto, 1981; Saxon et al., 1987). It was subsequently reported that jacalin could selectively bind human IgA (Roque-Barreira and Campos-Neto, 1985). This lectin thus seemed to be potentially useful for the purification of IgA from biological fluids. It soon became apparent that jacalin predominantly reacts with IgA1 and IgD (Kondoh et al., 1986; Aucouturier et al., 1987; Zehr and Litwin, 1987) but the IgA2 reactivity of jacalin has been a

matter of controversy (Table I). We therefore undertook a re-evaluation of jacalin reactivity with purified monoclonal IgA2 of both allotypes. This study confirms the ability of jacalin to bind IgA2 weakly but significantly.

Material and methods

Jacalin

Jacalin crude extract (JCE) was prepared from jackfruit seeds collected in the island La Réunion. Jacalin was purified by diethylaminoethyl (DEAE) chromatography as previously described (Aucouturier et al., 1987). Purification was confirmed by the presence of two single bands (the 15 kDa (glycosylated) and 12 kDa (unglycosylated) subunits) after sodium dodecyl sulfate 12% polyacrylamide gel electrophoresis and by thin layer agarose electrophoresis (Paragon, Beckman, Brea, CA). All the following experiments were performed with jacalin fractions with a 3:1 ratio of unglycosylated and glycosylated subunits (Aucou-

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Abbreviations: JCE, jackfruit seed crude extract; DEAE, diethylaminoethyl; JBP, jacalin-binding protein; PBS, phosphate-buffered saline; OD, optical density; IEL, immunoelectrophoresis; HRP, horseradish peroxidase.

TABLE I

IgA2 REACTIVITY OF JACALIN

Data from the literature and from the present study.

Reference	Origin of jacalin	IgA2 Allotypes	Number studied	Assay		Western blot with jacalin-peroxidase	Competitive ELISA on IgA1 coat	Radiometric assay on IgA2 coat	Inhibition of latex agglutination	Affinity chromatography purified material	Comments
				Gel precipitation							
Kondoh et al., 1986, 1987	Japan	A2m(1)	?	-							
		A2m(2)	?	+							
		A2m(1)	?	-							
		A2m(2)	?	-							
Aucouturier et al., 1987	Brazil	?	2-5	-		-	+			+	Reactivity much weaker than that of IgA1
	La Réunion	?	2-5	-		-	+			+	
Gregory et al., 1987	?	?	Polyclonal secretory IgA							+	8% IgA2 in isolates from secretory IgA
Zehr and Litwin, 1987	?	?	1							-	
Skea et al., 1988	?	?	2					-			
Hagiwara et al., 1988		?	Polyclonal secretory IgA								Subclasses evaluated by protease resistance
		A2m(1)	5	-							
		A2m(2)	1	-					-		Weak inhibition of latex agglutination assumed to reflect IgA1 contamination
		?	Polyclonal serum and secretory IgA	-					-		
Present study	La Réunion	A2m(1)	2	-		-	+			+	Weak but significant reactivity in non-precipitating assays
		A2m(2)	2	-		-	+			+	

turier et al., 1987). More anodic fractions containing a higher proportion of unglycosylated subunit were not used in this work.

Affinity chromatography purification of serum jacalin binding proteins (JBP)

Sephacrose 4B (Pharmacia, Uppsala, Sweden) was activated with cyanogen bromide according to March et al. (1974) and coupled to JCE or purified jacalin (12 mg protein/ml of sedimented beads). In five separate experiments, the yields of coupling were 0.63, 0.53 and 0.54 with JCE and 0.87 on two occasions with purified jacalin. Sepharose-coupled JCE or purified jacalin yielded identical results and hence were used interchangeably. Ammonium sulfate precipitates from myeloma sera containing the proteins Mia (IgA2m(1) λ), Gir (IgA2m(1) κ), Felg (IgA2m(2) λ) or Bel (IgA2m(2) κ) (the allotypes were kindly determined by Dr. G. De Lange, Amsterdam), in addition to the controls, Ga (IgM κ) and Ja (IgG2 κ), were adjusted to a concentration of 6.5 mg of protein/ml in 0.01 M phosphate-buffered saline pH 7.4 (PBS) and incubated for 20 min at 4°C with jacalin-Sepharose (5 ml of settled beads for 10 ml of protein solution). After washing with cold PBS (at least 2 liters with numerous 2 or 3 min incubations) until the optical density (OD) of the effluent at 280 nm was below 0.002, JBP were eluted from the adsorbent by overnight incubation with 20 ml of 0.5 M D-galactose (Merck, Darmstadt, F.R.G.) at 4°C, concentrated by vacuum dialysis against 0.15 M NaCl and analyzed by standard immunoelectrophoresis (IEL) with polyvalent and anti-immunoglobulin light and heavy chain-specific antisera (Dako, Copenhagen, Denmark) and with JCE (1.8 mg/ml). With the anti- α antiserum, IgA1 spurred over IgA2.

Purified monoclonal human Ig

Six monoclonal Ig were purified for this study; all steps were performed at 4°C and the purified concentrated solutions were stored in the presence of 50% glycerol at -30°C. The IgA1 κ Min was isolated from serum by ammonium sulfate precipitation, gel filtration on Sepharose 6B (Pharmacia) followed by jacalin-Sepharose affinity chromatography of the 7-8 S fraction. It was pure by IEL at

27 mg/ml. The IgA2m(1) κ Gir and IgA2m(2) κ Bel were purified by ammonium sulfate precipitation, gel filtration on Ultrogel ACA34 (IBF, Villerueve-la-Garenne, France) and DEAE-Trisacryl (IBF) chromatography of the 7-8 S fractions in 0.01 M Tris/HCl buffer pH 7.5 with a linear 0-0.3 M NaCl gradient. Residual IgG of fast mobility and traces of polyclonal IgA1 were removed by adsorption on protein A-Sepharose (Pharmacia) and on a limited quantity of jacalin-Sepharose. After concentration by vacuum dialysis, the two IgA2 preparations were pure by IEL at 72 and 23 mg/ml respectively. The IgG3 κ Met has a very low electrophoretic mobility and was easily purified by DEAE-Trisacryl chromatography. The IgM κ Mil was purified by ammonium sulfate precipitation, gel filtration on Sepharose 6B and euglobulin precipitation. It was then adsorbed on jacalin-Sepharose to remove small amounts of residual polyclonal IgA. The pure IgD λ Car was prepared as previously described (Aucouturier et al., 1987) and stored at -30°C with 5 mM ϵ -aminocaproic acid and 1 mM phenylmethylsulfonyl fluoride in 50 % glycerol. Concentrations of the purified Ig solutions were calculated from their absorbance at 280 nm using $A_{1\%}^{1\text{cm}}$ values of 10.6 for IgA, 17.0 for IgD, 13.5 for IgG and 11.85 for IgM (Hudson and Hay, 1980; Johnstone and Thorpe, 1982). The monoclonal IgA2m(2) λ Felg and IgA2m(1) λ Mia were purified as previously described (Aucouturier et al., 1987). Purity was controlled by IEL and by aminoterminal aminoacid sequencing.

Determination of isotype specificity of jacalin by ELISA

Polystyrene microtitration plates (Nunc, Roskilde, Denmark) were coated with the IgA1 Min at 2 μ g/ml in 0.1 M carbonate buffer pH 9.6 (200 μ l/well) overnight at 4°C, and saturated with PBS 0.05% Tween 20 (v/v) for 1 h at 37°C. After washing with PBS-Tween, 100 μ l/well of purified Ig at various dilutions were added in triplicate to 100 μ l of purified jacalin conjugated to horseradish peroxidase (HRP, Grade I, Boehringer, Mannheim, F.R.G.) by the two-step glutaraldehyde method (Avrameas and Ternynck, 1971) at a final dilution of 1.04 μ g of jacalin/ml. The plates were incubated for 2 h at room temperature and washed

six times with PBS-Tween; enzyme activity was revealed with 0.4 mg/ml *o*-phenylenediamine 0.012% H₂O₂ in 0.05 M sodium citrate pH 5. Absorbance values were read in a Titertek (Flow, McLean, VA) plate reader at a wavelength of 492 nm. As a control the same purified Ig were similarly tested on identical plates with a monoclonal anti-human IgA1 antibody (clone M4D8, kindly provided by Professor I.C.M. MacLennan, Birmingham, U.K.) diluted 1/1000 instead of HRP-jacalin. Binding of the antibody to the immobi-

Fig. 1. Immunoelectrophoretic analysis of jacalin-binding proteins (JBP1) and effluent (E1) obtained by affinity chromatography of an ammonium sulfate precipitate from the IgA2m(1)κ myeloma serum Gir (S1). Anti-NHS: polyvalent anti-normal human serum antiserum. Note (1) that although the bulk of the IgA2 is in the effluent, it is also present in JBP, and (2) the spur of polyclonal IgA1 over IgA2 (arrow).

Fig. 2. Immunoelectrophoretic analysis of jacalin-binding proteins (JBP2) and effluent (E2) obtained by affinity chromatography of an ammonium sulfate precipitate from the IgA2m(2)κ myeloma serum Bel (S2).

lized IgA1 was revealed by incubation for 1 h at room temperature with 200 µl/well of HRP-conjugated rabbit anti-mouse IgG (2 µg/ml, prepared in our laboratory).

Western blotting

Purified IgA1 Min, IgA2m(1) Gir and IgA2m(2) Bel at 2.8 mg/ml were analyzed by thin layer agarose electrophoresis (Paragon, Beckman) followed by pressure blotting on nitrocellulose (HAHY, Millipore, Molsheim, France) and detection with either HRP-anti-α antiserum (Institut Pasteur Production, Paris, France, 1/2000) or

HRP-jacalin (0.52 μg jacalin/ml) for 90 min at room temperature. The enzymatic activity was developed with benzidine 0.13% H_2O_2 0.03% in 0.1 M Tris/HCl buffer pH 7.6.

Results

Study of proteins isolated by affinity chromatography on jacalin-Sepharose

IEL analysis of JBP from the four IgA2 myeloma sera (Figs. 1 and 2) revealed the presence of significant amounts of monoclonal IgA as shown in each case by a typical curvature of the IgA line with the anti- α and relevant anti-light chain antisera, and by a spur of polyclonal IgA1 with the anti- α serum. Indeed, as in our previous experiments (Aucouturier et al., 1987), residual polyclonal IgA1 was considerably enriched in jacalin affinity chromatography-purified material.

Fig. 3. Competition ELISA. Inhibition of HRP-jacalin binding to coated IgA1 by increasing concentration of purified human monoclonal IgA1 (●), IgA2m(1) (□), IgA2m(2) (○), IgD (▼), IgG3 (☆) and IgM (★).

Fig. 4. Inhibition of binding of the anti- $\alpha 1$ antibody M4D8 to coated IgA1 by increasing concentrations of purified human monoclonal IgA1 (●), IgA2m(1) (□), IgA2m(2) (○), IgD (▼), IgG3 (☆) and IgM (★).

Jacalin precipitated neither the IgA2m(1) nor the IgA2m(2) present in these JBP, but yielded a faint precipitating line (barely visible on the photographs) corresponding to polyclonal IgA1. In contrast, no traces of monoclonal IgG2 κ or IgM κ could be found in JBP from the serum Ja and Ga respectively.

Competition ELISA

As previously observed, monoclonal IgA1 and IgD strongly inhibited the binding of HRP-jacalin to IgA1 coated plates while IgA2 of both allotypes yielding a weaker but significant inhibition (Fig. 3). The absence of contamination by IgA1 was confirmed by the control assay with the monoclonal anti- $\alpha 1$ antibody instead of jacalin (Fig. 4), where only IgA1 was found to inhibit the binding of the antibody.

Gel precipitation and western blotting

As previously described and in striking contrast to IgA1, all IgA2 preparations tested failed to react with jacalin in Ouchterlony and IEL analysis or by Western blotting following agarose electro-

phoresis and detection with HRP-jacalin (not shown).

Discussion

We have previously reported that jacalin does not react with IgA2 by gel precipitation and western blotting (which makes it very useful for the subclass typing of monoclonal IgA proteins) (Aucouturier and Preud'homme, 1987) but weakly binds to IgA2 (Aucouturier et al., 1987). This is confirmed in the present study of further IgA2 of known allotypes. Indeed, affinity chromatography prepared JBP from IgA2 myeloma sera contained the monoclonal IgA (in contrast to JBP from IgG or IgM myeloma sera which were free of myeloma proteins). Residual polyclonal IgA1 bound jacalin (with a considerable enrichment of the IgA1 compared to the starting sera) which clearly shows the much higher affinity of jacalin for the IgA1 subclass. A large part of the myeloma IgA2 did not bind to jacalin-Sepharose and was recovered free of IgA1 in the effluent, thus permitting easy purification of IgA2, in agreement with the findings of Gregory et al. (1987). Interestingly, even the IgA2 isolated in the JBP fraction did not precipitate jacalin in gel diffusion experiments, which strongly suggests that partial binding of IgA2 to jacalin columns results from low affinity or functional monovalency rather than from the presence of different populations of IgA2 molecules.

All of the IgA2 preparations tested weakly inhibited the binding of jacalin (and not that of an anti-IgA1 monoclonal antibody) to insolubilized IgA1. In addition to the lack of reactivity with this monoclonal antibody, these IgA2 preparations were pure by electrophoretic, immunoelectrophoretic and (in two cases) sequence criteria.

The strong reactivity of jacalin with human IgA1 and IgD is well explained by the presence of several *o*-linked Gal β 1 $\rightarrow$ 3GalNAc in the hinge region of both heavy chains (Baenziger and Kornfeld 1974a,b; Mellis and Baenziger, 1983) since this disaccharide is the predominant reactive carbohydrate with jacalin (Sastry et al., 1986). Moreover, the location of the binding sites in the hinge region of IgA1 was confirmed by a study of proteolytic fragments (Skea et al., 1988). The

situation is not as clear for the other isotypes. Terminal galactose residues exist in all human Ig classes and subclasses, but their density and presentation differ. μ chains bear five kinds of asparagine-linked oligosaccharides, two of which are of the 'high mannose' type without galactose (Chapman and Kornfeld, 1979a,b), whilst the others have terminal NeuAc α 2 $\rightarrow$ 6Gal β 1 $\rightarrow$ 6GlcNAc or Gal β 1 $\rightarrow$ 6GlcNAc (Hickman et al., 1972). The glycoside present on gamma chains is often terminated with Gal β 1 $\rightarrow$ 4GlcNAc (Mizuochi et al., 1982; Takahashi et al., 1987), but its situation inside the pair of CH₂ domains in the entire molecule makes it almost inaccessible (Deisenhofer, 1981). The location of IgA2 oligosaccharides has been studied by Torano et al. (1977). However, their structures are not yet completely elucidated. Both allotypes seem to share two common *N*-glycosides with IgA1, one located in the CH3 domain and the other, in the CH2 region, having exposed terminal Gal β 1 $\rightarrow$ 4GlcNAc. IgA2m(1) and IgA2m(2) have two and three supplementary asparagine-linked oligosaccharides in contrast to IgA1, but their sequences have not been determined. Thus, the special feature of the carbohydrates found in IgA2 is the existence of at least one terminal Gal β 1 $\rightarrow$ 4GlcNAc exposed on the surface of the molecule. Sastry et al. (1986) showed that this disaccharide had some affinity for jacalin (although with a low association constant), and we previously showed that D-lactose (Gal β 1 $\rightarrow$ 4Glc) inhibits HRP-jacalin binding to IgA1 at higher concentration than GalNAc and Gal (Aucouturier et al., 1987). Although the determination of the structure of the other oligosaccharides on the α 2 chain may provide alternative explanations, the presence of Gal β 1 $\rightarrow$ 4GlcNAc in the CH2 domain is a possible explanation for IgA2 reactivity with jacalin.

The ability of jacalin to bind both IgA subclasses can be used to remove IgA from biological samples and Ig preparations. However, the complete elimination of IgA2 would require a large excess of jacalin because of its low affinity of interaction. Preliminary experiments with commercially available intravenous IgG preparations showed that the IgA level sharply decreased after a first adsorption on jacalin-Sepharose but that several steps of adsorption were required to re-

move all contaminating IgA. Nevertheless, a method based on this principle should be of great value for the preparation of human IgG for therapeutic use, in which the presence of IgA is an important and difficult problem.

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