ISOLATION OF ANTIBODIES SPECIFIC FOR THE CARBOHYDRATE BINDING SITE OF CONCANAVALIN A

Raquel A CALDERON and Felix CORDOBA*

Departamento de Biologia Experimental Facultad de Medicina, UNAM, Mexico, DF and **Departamento de Biotecnologia, Centro de Investigacions Biologicas de Baja California, AC, 5 de Mayo no 9, Apdo Postal no 128, La Paz BCS, Baja California, Mexico*

Received 4 April 1979

1. Introduction

Concanavalin A (Con A), one of the most extensively studied plant lectins, has proved a useful tool for studying the mammalian cell membrane It binds specifically to membrane glycoproteins containing α-D-Man or α-DGlc residues [1], and is a powerful mitogen for T-lymphocytes [2] The binding of Con A to membrane oligosaccharides may be reversed by α-D-mannopyranosides [3]

Con A is antigenic, and antibodies raised in rabbits have been reported [4–6] However, in most instances these are heterogeneous and include specificities towards a variety of antigenic determinants on the Con A molecule

Here we describe an affinity chromatography procedure to obtain antibodies specific for the 'active site' of Con A Distinct differences in biological activity were found between Con A 'active site' antibodies and antibodies directed to other antigenic determinants on the Con A molecule

2. Materials and methods

2 1 *Preparation of antibodies*

Antisera were raised in rabbits (locally bred) by giving 4 weekly injections of 1 mg Con A (Sigma Chemical Co , St Louis, MO) emulsified with complete Freund's adjuvant Venous blood was obtained 1 month after the last injection The isolation and purified of serum immunoglobulins were performed by ammonium sulphate precipitation and DEAE (DE52, Whatman Ltd) column chromatography

Address correspondence to Dr Felix Cordoba

2 2 *Fractionation of anti-Con A antibodies by affinity chromatography*

Sepharose 4B–Con A (Pharmacia Fine Chemicals), kindly donated by Dr R M E Parkhouse, was packed in a plastic column (2 × 15 cm) and equilibrated with 0 15 M phosphate buffer (pH 7 2) containing 0 9% (w/v) NaCl (PBS) A sample (246 mg) of the anti-Con A IgG fraction (prep A) was applied to the column and washed with PBS until all unbound protein (fraction I) was removed A linear 0–0 5 M α-methyl-D-mannoside (αMM) gradient in PBS was passed through the column and the eluate collected (fraction II) Residual antibody was eluted with 0 1 M glycine–HCl buffer at pH 2 8 (fraction III) The eluates were dialysed against PBS

2 3 *Radioimmunoassay*

The technique in [7] was used with slight modifications The reagents were pipetted in a haemagglutination plate (Falcon Plastic no 3040) as follows 25 μl ^{125}I-labeled Con A (2 5 μg, 10 000 cpm) prepared according to [8] 25 μl of the IgG fractions obtained by affinity chromatography (4 mg/ml) and 25 μl PBS in the presence or absence of 1 M αMM The mixture was incubated for 30 min at room temperature, then 25 μl of sheep anti-rabbit IgG serum was added and incubated for a further 30 min at 37°C Finally, the suspension was adjusted to 400 μl with 2% (w/v) bovine serum albumin solution and centrifuged

(16 000 rev./min) for 4 min. The pellets were counted in a gamma counter.

2.4. *Murine spleen cell culture*

Mouse spleen cell suspensions were prepared as in [9]. Briefly, 1.5×10^6 Babl/c (Charles River Breeding Lab., Wilkington, MA) spleen cells were cultured for 72 h at 37°C in RPMI-1640 containing 5% FCS, and a 10% CO_2 atmosphere. At initiation, Con A (1 μg) and the antibodies under study (1 μg) were added. A set of replicate cultures were treated with 1 M αMM solution (25 μl) in PBS. [^{3}H]Thymidine (1 μCi, New England Nuclear, Boston, MA) was added 18 h before harvesting the cells.

2.5. *Immunodiffusion*

Double diffusion tests were performed according to the Ouchterlony procedure; with Con A (50 μg) and various antibody fractions (whole rabbit anti-Con A serum, preparation A, fractions I–III). After development of immunoprecipitin lines, the plates were washed extensively with PBS and then with a 1 M αMM solution.

3. Results

Figure 1 shows the elution profile of rabbit antibodies to Con A from an affinity chromatography column of Sepharose 4B–Con A. A purified IgG fraction (246 mg) was applied to the column, and 170 mg was not retained (peak I). A gradient of αMM eluted a second fraction (33.1 mg) later shown to contain antibodies directed towards the 'active site' of the Con A molecule (peak II). A further 13.4 mg protein was recovered by acid elution (peak III).

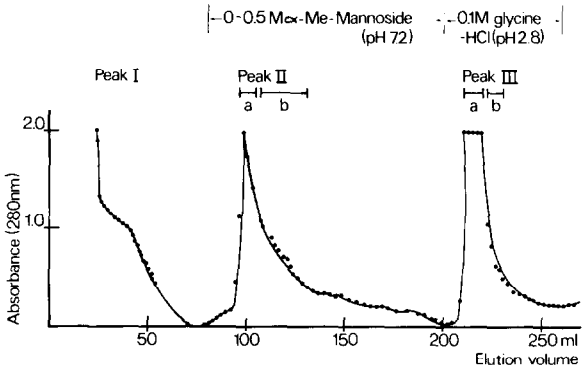

Fig.1. Fractionation of anti-Con A rabbit IgG on Sepharose 4B–Con A column (2 × 15 cm). Arrows indicate the change of buffer. The effluent was monitored at 280 nm.

The specificity of the 2 Con A antibody fractions (peaks II, III) was assessed by testing their capacity to inhibit Con A binding in the standard systems of radioimmunoassay, mouse spleen lymphocyte transformation and immunoprecipitation. For the assay, peaks II and III were divided into 2 subfractions (fig.1).

The results of the radioimmunoassay (RIA) are shown in table 1. The peak IIa bound 69.8% of the total ^{125}I-labeled Con A and in the presence of αMM the binding was reduced to 22.3%, giving an inhibition of 68%; the peak IIb showed a similar profile of inhibition (67%). Peak III also bound Con A (47%), however, this binding was not inhibited (6%) by the glycoside.

Table 1
Inhibition of ^{125}I-labeled Con A binding to antibody fractions by α-methyl-D-mannoside

Additions	^{125}I-Labeled Con A binding (cpm)[a]		% Binding inhibition
	−αMM	+αMM	
^{125}I-Labeled Con A (2.5 μg)	10 000		
^{125}I-Labeled Con A + peak I	1665	1730	
^{125}I-Labeled Con A + peak IIa	6986	2236	68.0
^{125}I-Labeled Con A + peak IIb	6186	2070	67.0
^{125}I-Labeled Con A + peak IIIa	4703	4446	5.5
^{125}I-Labeled Con A + peak IIIb	2606	2796	
^{125}I-Labeled Con A + sheep anti rabbit IgG	1270	1530	

[a] Average of triplicate runs

Table 2
Effect of various antibody fractions on lymphocyte activation by Con A

Addition	[^{3}H]Thymidine incorp (cpm)[a]	% Inhib
None	629	–
Con A	17 025	–
Con A + αMM	845	95
Con A + peak II antibodies	3325	80
Con A + peak III antibodies	15 093	11
Con A + peak I	24 005	–

[a] Average of triplicate runs

Con A is a potent mitogen for mouse spleen lymphocytes as measured by increased [^{3}H]thymidine incorporation (table 2) and addition of αMM blocked lymphocyte transformation Similarly, addition of peak II antibodies reduced the thymidine incorporation by 80%, whereas the peak III had no appreciable effect

Immunodiffusion revealed a further difference between peaks II and III Agarose plates were set up with Con A and various antibody fractions (whole rabbit anti-Con A serum, prep A, fractions I–III) Immunoprecipitin lines were developed between Con A and each of the preparations (except with fraction I), and then the plates were washed with a solution of αMM This treatment only caused solubilisation of the precipitin line for fraction II Thus, two functionally different classes of antibodies have been resolved by affinity chromatography

4. Discussion

A simple procedure for the isolation, from immune rabbit sera, of antibodies specific for the 'active site' of concanavalin A is described Two antibody fractions were resolved from a Sepharose 4B–Con A column, the first (peak II, fig 1) by elution with αMM, known to interact with the 'active site' of Con A [10] and the second, by low pH (peak III)

Distinct differences were observed in the biological activity of these antibody fractions

1. The binding of peak II antibodies to Con A (RIA) was inhibited by αMM, whereas no inhibition was shown for peak III
2. The antigen–antibody complex (immunodiffusion) between Con A and peak III was not dissociated by the glycoside
3. Lymphocyte activation by Con A involves binding of the lectin-'carbohydrate site' to oligosaccharides in the cell membrane [11], inhibition only by peak II antibodies supports the view that these antibodies are indeed directed to the combining site

Inhibition of lymphocyte transformation by antibody to whole Con A is considered to be due to changes in the tertiary structure of the molecule [4], as reported for antibodies to other biologically-active molecules [12–14] However, we have found that even though peak III antibodies bind to Con A, there is no effect on mitogenic activity

In summary, the method reported here, is applicable to almost any system in which a specific ligand of low molecular weight is known For example, in the production of antibodies to the active site of lectins, enzymes, hormones or even other antibodies

References

[1] Goldstein, I J , Hollerman, C E and Smith, E E (1965) Biochemistry 4, 876–883
[2] Anderson, J , Moller, G and Sjoberg, O (1972) Cell Immunol 4, 381–393
[3] Greaves, M F , Bauminger, S and Janossy, G (1972) Clin Exp Imm 10, 537–554
[4] Sell, S and Sheppard, H W (1974) Exp Cell Res 84, 153–158
[5] Leon, M A (1975) Adv Exp Med Biol 55, 261–271
[6] Northoff, H , Jungfer, H and Resch, K (1978) Exp Cell Res 115, 151–158
[7] Yallow, R S , Glick, S M , Roth, J and Berson, S A (1964) J Clin Endocrin 24, 1219–1225
[8] Hunter, W M and Greenwood, F C (1962) Nature 194, 495–496
[9] Calderon, R A and Cordoba, F (1976) Eur J Immunol 6, 522–525
[10] So, L L and Goldstein, I J (1968) J Biol Chem 243, 2003–2007
[11] Powell, J T and Leon, M A (1970) Exp Cell Res 62, 315–325
[12] Bucci, E and Bowman, D E (1970) Immunochemistry 7, 289–303
[13] Omenn, G S , Ontjes, D A and Anfinsen, C B (1970) Biochemistry 9, 313–321
[14] Shapira, E and Arnon, R (1967) Biochemistry 6, 3951–3956