

Partial characterization of an allergenic glycoprotein from peanut (*Arachis hypogaea* L.)

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A concanavalin A-reactive glycoprotein allergen has been isolated from peanut (*Arachis hypogaea*). The allergen was separated by affinity chromatography and purified by gel permeation and ion-exchange chromatography. The monomeric molecular weight is 65 000 and the *pI* is 4.6. The presence of one cysteine residue per molecule results in some dimer formation. Concanavalin A-reactive glycoprotein is a potent allergen for peanut-sensitive patients in both in vivo and in vitro tests. It is allergenically stable, on in vitro examination, at temperatures of up to 100°C and over the pH range 2.8-10. Removal of the carbohydrate moiety failed to eliminate the allergenicity. Concanavalin A-reactive glycoprotein is identified in the crossed immunoelectrophoretic pattern as a major antigen of peanut protein extract but its structural characteristics indicate that it is probably not a component of the major storage-protein complex, arachin.

Introduction

Peanuts are a commonly eaten food and in many countries contribute significantly to the total dietary protein. Despite the valuable nutritional properties of this legume, peanut can cause severe, IgE-mediated type I hypersensitivity reactions. The symptoms of such reactions may occur in a considerable number of allergic patients and include asthma, angioedema and occasionally anaphylaxis [1,2]. The substances responsible for these reactions are known to be proteins or glycoproteins, and various peanut globulins and fractions have been examined for IgE-binding in an earlier study [2]. Some 16 IgE-binding antigens in raw peanut extract were revealed by crossed radio-immunoelectrophoresis although none of these were unequivocally identified. In the same

study, arachin, conarachin, peanut lectin and phospholipase D were shown to bind specific IgE in the sera of hypersensitive patients, but of greatest significance was the concanavalin A-reactive glycoprotein from peanut. Gleeson and Jermyn [3] first described the isolation of this glycoprotein but did not recognise its allergenicity. In the work reported here we have carried out further purification and partial characterization of the glycoprotein. In our earlier study of the peanut allergens [2], crossed radio-immunoelectrophoresis patterns resulting from treatment with sera from peanut-sensitive patients all showed specific IgE binding to the component, giving rise to the major peak in the pattern. It was concluded that this peak corresponded to a major allergen of peanut and, on the basis of migration distance and immunodominance, was tentatively identified as arachin - the major storage protein of peanut. In the present study we have identified the position of con-

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canavalin A-reactive glycoprotein in the crossed radio-immunoelectrophoresis pattern. This paper thus extends the previous work, beginning the detailed examination of a clinically important and readily prepared allergen from peanut. With the notable exception of allergen M from codfish [4], no previous detailed studies of the allergenic or antigenic structure of a food allergen have been reported.

Materials and Methods

Materials

Raw peanut seeds (*Arachis hypogaea*), variety Virginia Bunch, were kindly supplied by the Peanut Marketing Board, Kingaroy, Queensland. The seeds were skinned and defatted as described previously [2]. The reagents for electrophoresis were obtained from Bio-Rad Laboratories and sodium dodecyl sulphate was from British Drug Houses. Molecular mass marker proteins and agarose for crossed immunoelectrophoresis were from Sigma Chemical Co. Sephacryl S-200, Con A-Sepharose 4B and agarose for isoelectric focusing were from Pharmacia Ltd. DEAE-trisacryl M was supplied by LKB Productor. Gelbond film was from FMC Corporation. All chemicals were analytical grade and the guanidinium hydrochloride (Fluka puriss grade) was recrystallised from ethanol before use.

Protein preparation

A crude protein extract was made from peanut seeds by a modification of the procedure of Gleeson and Jermyn [3]. Ground peanut meal was defatted with petroleum ether and then extracted (1:9, w/v) by stirring for 2 h in a buffer of Tris-HCl (0.05 M) (pH 8.0) in 0.5 M NaCl. The extract was centrifuged at $10000 \times g$ for 30 min to remove insoluble debris and the supernatant was then dialysed against several changes of distilled water. The retentate was lyophilised and stored at -20°C until required.

Isolation of the crude glycoprotein was effected using Con A-Sepharose 4B packed in a 1×10 cm column equilibrated with the extract buffer. The crude protein extract (250 mg) was dissolved in 10 ml buffer, the solution was shaken briefly and left standing for 5 min before filtering it through Whatman No. 1 paper. The filtrate was applied to

the column and eluted with the extract buffer at a flow rate of 10 ml/h. Unbound proteins were eluted until the effluent absorbance at 280 nm declined to zero. Bound material was then released by passage of 25 ml of a 0.5 M solution of methyl α -D-glucoside through the column. The concanavalin A-reactive fraction thus eluted was dialysed against 1% w/v NH_4HCO_3 and lyophilised.

The resulting crude allergen was partially purified by gel filtration on Sephacryl S-200 in the presence of 6 M guanidinium hydrochloride and under reducing conditions (Fig. 1a) [3]. The major earlier-eluting peak was dialysed against the Tris-HCl buffer without NaCl. A small amount of insoluble material was removed from the dialysate by centrifugation. The resulting supernatant was

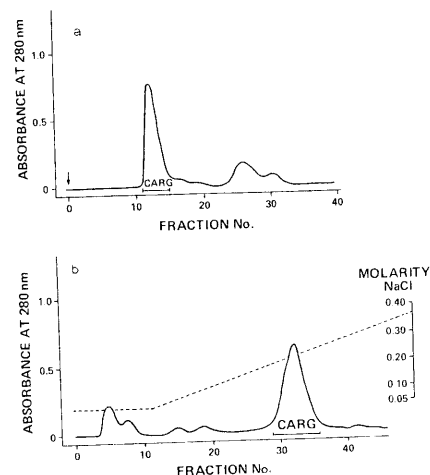


Fig. 1. (a) Elution profile of gel permeation chromatogram of crude concanavalin A-reactive glycoprotein (CARG) (50 mg, reduced in 5% v/v 2-mercaptoethanol) with a 1.6×75 cm column of Sephacryl S-200 equilibrated with 6 M guanidinium hydrochloride in 0.2 M phosphate buffer (pH 7.0). (b) Ion-exchange chromatogram of concanavalin A-reactive glycoprotein (40 mg) on DEAE-trisacryl M following gel permeation purification. The 1.6×15 cm column was eluted with a linear gradient of NaCl. The flow rate of both columns was 20 ml/h and fractions were 5 ml.

applied to a 1.6×15 cm column of DEAE-trisacryl M equilibrated with the dialysis buffer. The column was eluted with starting buffer (40 ml) followed by a linear gradient over 200 ml from 0 to 0.4 M NaCl in the Tris buffer. The material emerging from the column at the concentration of about 0.22 M NaCl was the major peak (Fig. 1b). This refined concanavalin A-reactive glycoprotein was re-run on the ion-exchange column and after dialysis was lyophilised and stored at -20°C .

Allergenicity assays

In vitro IgE binding of concanavalin A-reactive glycoprotein was measured by application of the radioallergosorbent test and radioallergosorbent test inhibition following procedures described previously [2]. Skin prick tests were made to establish the in vivo activity in 12 patients with clinical histories of peanut sensitivity. Controls were provided by testing two non-allergic and three pollen-allergic, but not peanut-sensitive, patients. The tests were carried out by placement of a solution of purified concanavalin A-reactive glycoprotein, (1 mg/ml) in glycerol/water, 1:1, v/v, on the forearm according to the method of Bock et al. [5]. Allergenicity was gauged by comparison of the weal produced with that of the controls.

The allergenic stability of concanavalin A-reactive glycoprotein with respect to both pH and heat was tested in the following way: the allergen was suspended in water by sonication (4 mg/0.5 ml) and 0.1 ml aliquots were placed in Wheaton vials together with 0.9 ml of the appropriate buffer. The buffers were: 0.1 M acetic acid (pH 2.8), 0.1 M ammonium acetate adjusted to pH 5.0, 0.1 M NH_4HCO_3 (pH 8.0) and 0.1 M NH_4OH (pH 10.0). The closed vials were incubated for 30 min at temperatures of 20, 60, 70, 85 and 100°C . The treated solutions were rapidly frozen and then lyophilized to remove the buffers before being redissolved in phosphate-buffered saline (0.8 ml) (pH 7.4). These solutions were tested for their inhibitory activity in the radioallergosorbent test against concanavalin A-reactive glycoprotein discs (50 μg /disc).

Electrophoretic procedures

Sodium dodecyl sulphate polyacrylamide gel electrophoresis was carried out on reduced pro-

teins by the method of Laemmli [6] modified for performance in a Gradipore apparatus with $7 \times 7 \times 0.3$ cm gel slabs (Fig. 2a). Isoelectric focusing in thin layers of agarose was carried out using the high-resolution system of LKB Productor AB (Fig. 2b) [7].

Crossed immunoelectrophoresis, under conditions described by Barnett et al. [2], was applied as shown in Fig. 3. Monospecific anti-concanavalin A-reactive glycoprotein antiserum was prepared in rabbits as described by Barnett and Howden [8]. The IgG fraction was isolated by chromatography on DEAE-Affi-Gel Blue [9]. An aliquot of the IgG fraction was labelled with fluorescein isothiocyanate by the method of Cherry [10] and used as the labelling antibody in a manner analogous to that of the ^{125}I -anti-IgE in the crossed radio-immunoelectrophoretic procedure [2].

N-terminal sequence, amino acid and carbohydrate analysis

The N-terminal sequence of concanavalin A-reactive glycoprotein was determined with an Applied Biosystems Model 470A-01 gas-phase protein sequencer following the procedures of Hewick et al. [11]. Identification of the PTH-amino acids was made with a Waters Associates HPLC system using an IBM Cyano column and buffer system as described by Hunkapiller and Hood [12]. The amino acid composition of concanavalin A-reactive glycoprotein was determined on a Beckman-Spinco 120C amino acid analyser. Hydrolysis was carried out at 115°C for 22 h in methanesulphonic acid [13]. Cysteine SH was determined by the method of Zahler and Cleland [14] before and after reduction of the allergen with dithiothreitol.

Prior to analysis for carbohydrate, the allergen (5 mg) was passed through a 1×30 cm column of Bio-Gel P-6DG in 1% NH_4HCO_3 to remove any contaminating sugars. Total hexose content was measured by the phenol-sulphuric acid method [15] with an equimolar mixture of xylose/mannose as standard. The individual sugars were identified by both paper- and gas-chromatography as described by Baldo et al. [16]. Conditions for hydrolysis of the samples were: 105°C for 1 h in 6 M hydrochloric acid.

An attempt was made to selectively cleave the carbohydrate moiety from concanavalin A-reactive

tive glycoprotein by reaction with trifluoromethanesulphonic acid according to Edge et al. [17]. Residual hexose analysis was carried out as for untreated protein. The allergenicity of the deglycosylated concanavalin A-reactive glycoprotein was tested by radioallergosorbent test inhibition.

Results and Discussion

The purified concanavalin A-reactive glycoprotein separated in the preparative scheme was about 1% by weight of the peanut meal (Table I), a value similar to that quoted by Gleeson and Jermyn [3]. The allergen was found to have many of the properties previously described by them, although certain of our analyses differed markedly.

Examination of our preparation by electrophoresis (Fig. 2a) showed the presence of several contaminants in the affinity-purified allergen. This

TABLE I
RECOVERY OF CONCAVALIN A-REACTIVE GLYCOPROTEIN DURING SEPARATION

Preparation stage	Dry weight (g)
Peanut meal	20.00
Defatted meal	10.00
Tris-HCl + NaCl extract	2.80
Crude allergen	0.42
After guanidinium-HCl and ion-exchange chromatography	0.16 *

* Final 0.16 g represents 39% of the crude allergen and about 1% of the peanut meal.

would be predicted from the lectin staining experiments of Basha and Roberts [18] in which a considerable number of peanut-extract components were bound with ^{125} I-labelled concanavalin A on

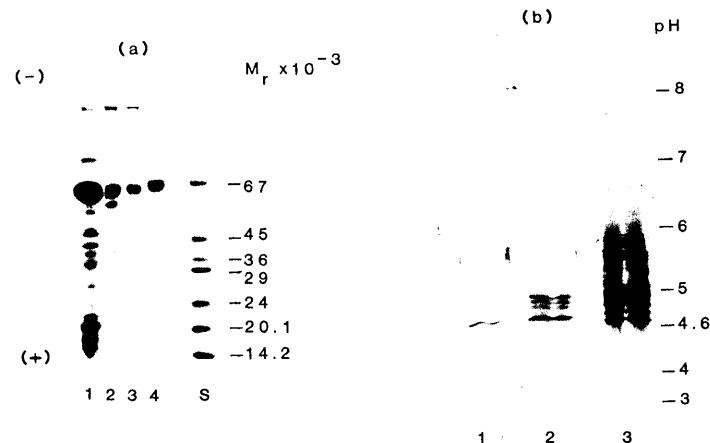


Fig. 2. (a) Sodium dodecyl sulphate polyacrylamide gel electrophoresis (10% total polyacrylamide concentration, 4% cross-linker concentration). Samples were reduced with 2-mercaptoethanol. The gel was stained with Coomassie blue R-250. The samples were: 1, crude peanut extract, 20 μ g; 2, concanavalin A-reactive glycoprotein (CARG) from the concanavalin A affinity column, 10 μ g; 3, CARG after Sephacryl and guanidinium hydrochloride treatment, 7 μ g; 4, bovine serum albumin, 10 μ g; S, molecular weight markers: α -lactalbumin M_r 14200, soybean trypsin inhibitor 20100, trypsinogen 24000, carbonic anhydrase 29000, glyceraldehyde-3-phosphate dehydrogenase 36000, egg albumin 45000 and bovine albumin 67000. (b) Isoelectric focusing in 1% agarose gel of 0.5 mm thickness on Gelbond film, stained with Coomassie blue R-250. Samples 1, CARG after final ion-exchange chromatography, 5 μ g; 2, CARG after Sephacryl and guanidinium hydrochloride treatment, 8 μ g; 3, crude peanut extract, 20 μ g. The running time was 30 min at 4°C using Pharmalytes (pH 3.0–10.0) under constant power of 8 W and E_{max} of 1500 V. The pH gradient was determined with a surface electrode.

two-dimensional gels. The concanavalin A-reactive glycoprotein recovered from gel filtration in guanidinium hydrochloride also showed slight heterogeneity when submitted to ion-exchange chromatography (Fig. 1b), but thereafter appeared homogeneous on both electrophoresis and isoelectric focusing. The apparent relative molecular weight of the allergen, determined by electrophoresis in comparison with the standards, was 65 000. This is in agreement with the earlier reported value [3]. Concanavalin A-reactive glycoprotein was shown (Fig. 2b) to have an apparent pI of 4.6 in agarose isoelectric focusing. This is in contrast to the results of Basha and Roberts [18] wherein the major peanut proteins ($M_r > 60000$) appeared to have pI values near 6.2. However, those results were obtained in the presence of 9.3 M urea and reducing agents, reagents which are known to affect markedly the pI observed on electrofocusing [19].

N-terminal and amino acid analyses

The amino acid composition of concanavalin A-reactive glycoprotein was found to be very similar to the earlier findings [3] and the differences may be attributed to the different hydrolysis methods used. Cysteine SH was found to be 0.2 mol/mol protein in the native allergen and 1.0 after reduction. The composition differs considerably from that reported for α -arachin by Neucere [20] (Table II). Comparison of concanavalin A-reactive glycoprotein results with those obtained by Yamada et al. [21] in their examination of arachin subunits also indicates that the allergen is not an arachin protein. In view of the glycosylation found in concanavalin A-reactive glycoprotein, it may be more appropriately assigned to the conarachins or vicilin-type proteins which are often post-translationally modified [22]. The single N-terminal sequence (15 residues) of the allergen, NH_2 -Gly-Ser-Ala-Pro-Gly-Glu-Arg-Gln-Arg-Gly-Cys-Tyr-Pro-Gly-Asp-, showed no unusual features and the glycine terminal was one of the two (Ala and Gly) previously identified [3] in the apparently heterogeneous crude concanavalin A-reactive glycoprotein. This result, taken with the single bands shown in the electrophoretic analyses, indicates that the allergen consists of a single polypeptide chain.

TABLE II

AMINO ACID ANALYSIS OF CONCAVALIN A-REACTIVE GLYCOPROTEIN AND COMPARISON WITH α -ARACHIN

n.d., not determined.

Amino acid	Residues per 100 residues		
	this study	Gleeson and Jermyn [3]	α -arachin [20]
Lys	5.0	5.2	2.14
His	2.5	2.5	2.00
Arg	10.1	11.2	9.42
Trp	0.5	n.d.	n.d.
Asx	12.5	12.4	12.70
Thr	2.9	2.7	2.47
Ser	6.9	6.5	4.11
Glx	18.5	19.5	19.51
Pro	5.1	6.0	2.83
Gly	8.0	7.6	2.49
Ala	5.6	5.0	3.79
Cys ^a	trace	n.d.	0.00
Val	5.6	4.8	4.03
Met	1.6	1.0	0.07
Ile	3.9	3.9	3.31
Leu	6.8	5.8	6.23
Tyr	1.9	0.9	3.30
Phe	4.7	4.0	5.34
GlcN	0.2	n.d.	n.d.

^a Cysteine SH was determined separately at 1 mol/mol concanavalin A-reactive glycoprotein.

Carbohydrate analyses

The carbohydrate content of concanavalin A-reactive glycoprotein was previously reported to be 12% by weight and to consist mainly of galactose [3]. In our analyses, the total hexose content determined was shown to be only 2.4%. The sugars found were xylose, mannose and glucose, in the ratio 2:1:1. *N*-Acetylglucosamine was detected as glucosamine in the amino acid analyses (Table II). However, the conditions of hydrolysis are likely to lead to lowered values for this sugar. No galactose was detected by either paper- or gas-chromatography. This major difference may be reconciled by considering the possibility of contamination of the samples of Gleeson and Jermyn [3] with free sugar, perhaps derived from the Sepharose 4B used in the gel filtration step of their preparation. A major hydrolysis product of Sepharose would be galactose. We guarded against such a possibility by passage of the sample through

a polyacrylamide gel filtration medium (Bio-Gel P-6DG) immediately before analysis. Our finding of a relatively low carbohydrate content may account for two other earlier observations [3]. Concanavalin A-reactive glycoprotein failed to behave anomalously in dodecyl sulphate electrophoresis as might have been expected from a glycoprotein with apparently more than 10% carbohydrate content [23], and also did not react positively with Schiff's stain.

Deglycosylated concanavalin A-reactive glycoprotein was analysed to determine the effectiveness of the reaction. Only 0.3% total hexose remained and this was shown by chromatography to be glucose. This small trace could be the result of sample contamination, but overall the removal of carbohydrate from the protein appeared to be almost complete.

Allergenicity

Concanavalin A-reactive glycoprotein was earlier [2] shown to bind specific IgE but in vivo tests were not made in that study. Table IIIA records the result of such tests, showing that 11 of the 12 peanut-sensitive patients tested gave positive skin-prick reactions to the allergen. None of the controls gave a positive reaction, indicating that the results were not due to a non-specific reaction to concanavalin A-reactive glycoprotein. The in vitro (radioallergosorbent test) results with serum from the test patients correlate well for crude peanut extract discs, concanavalin A-reactive glycoprotein discs and in vivo reaction. The patient (Table IIIA) giving a negative skin-prick test also gave a relatively low radioallergosorbent test result. Radioallergosorbent test inhibition data for eight of the sera are also recorded in Table IIIB and show that six of the eight sera required 9 µg or less of concanavalin A-reactive glycoprotein to give 50% inhibition of crude peanut extract discs (100 µg/disc). The radioallergosorbent test results for concanavalin A-reactive glycoprotein discs (50 µg/disc) showed close correspondence those for crude peanut extract for all the sera. The values obtained indicate that, for these patients, concanavalin A-reactive glycoprotein is responsible for a significant part of the total allergic response to peanut.

Table IIIB presents the results of radioallergo-

sorbent test inhibition by concanavalin A-reactive glycoprotein before and after deglycosylation. The tests were carried out with a pooled serum and

TABLE III

(A) ALLERGENICITY OF CONCAVALIN A-REACTIVE GLYCOPROTEIN AS INDICATED BY BOTH IN VIVO (SKIN-PRICK TESTS) AND IN VITRO (RADIOALLERGOSORBENT TEST) METHODS

Results for the radioallergosorbent test (RAST) are given as % of radioactive uptake. Values for inhibition are given as the amounts (µg) of concanavalin A-reactive glycoprotein (CARG) which gave 50% inhibition of the radioallergosorbent test on crude peanut extract (CPE) discs (100 µg/disc). The procedure was as described by Barnett et al. [2]. n.d., not determined.

Peanut-sensitive patients	Skin-prick test	RAST		Inhib.
		CPE	CARG	
A	+	16.5	13.9	5.9
B	+	4.5	4.8	42.0
C	+	33.1	33.9	2.6
D	++	12.0	10.1	9.0
E	+	11.1	8.3	48.1
F	—	4.7	2.9	
G	+	19.1	13.7	5.0
H	++	26.0	25.9	3.0
I	+	15.0	14.4	
J	+	16.2	17.1	
K	+	28.3	12.2	
L	++	26.7	28.4	4.8
Controls				
pollen-sensitive				
M	—	0.8	n.d.	
N	—	1.3	n.d.	
O	—	0.6	n.d.	
non-allergic				
X	—	0.8	n.d.	
Y	—	0.5	n.d.	

(B) EFFECT OF DEGLYCOSYLATION ON THE ALLERGENICITY OF CONCAVALIN A-REACTIVE GLYCOPROTEIN (CARG)

Inhibition was carried out in quadruplicate with 100 µg crude peanut extract/radioallergosorbent test disc using a pool of sera A, B, C, D and E (See Table IIIA). Variation of percent inhibition within a quadruplicate averaged ±10%.

Inhibitor (µg)	RAST inhibition (%)	
	CARG	deglycosylated CARG
5	49.8	25.0
10	56.0	38.0
20	59.0	42.0
50	66.0	50.0

appear to show that deglycosylation does somewhat reduce allergenicity. However, during deglycosylation the allergen became partially insoluble and required urea for redissolution [17]. Consequently the protein may have suffered irreversible denaturation and possibly some amino acid degradation. Alternatively, its reduced solubility or simply the almost complete removal of the carbohydrate moiety – even without denaturation – may have affected its capacity to bind specific IgE.

In Table IV, results of the tests to examine the allergenic stability of concanavalin A-reactive glycoprotein with respect to pH and temperature are presented. In these experiments the same pooled serum, as in the deglycosylation tests above, was used but the inhibition was of radioallergosorbent test discs of native allergen. The relatively small losses in inhibitory activity at neutral pH and over the range of temperatures show the extraordinary thermal stability of this allergen. This confirms the result of a previous study which demonstrated the antigenic stability of concanavalin A-reactive glycoprotein in severely heat-treated peanut foods [8]. The greatest change in allergenicity was produced at extremes of pH and temperature. Alkaline treatment was most marked in effect, suggesting that deamidation or other hydrolytic processes may be taking place. An allergic requirement for amide groups may be involved.

TABLE IV

ALLERGENIC STABILITY OF CONCAVALIN A-REACTIVE GLYCOPROTEIN WITH TEMPERATURE AND pH (30 min)

Values are given as percent inhibition in the concanavalin A-reactive glycoprotein radioallergosorbent test (50 µg/disc) using pooled serum and 50 µl of the reconstituted treated concanavalin A-reactive glycoprotein solution (1 µg/µl). The blank value of inhibition by 50 µg of the untreated allergen was 74.9%. All determinations were in duplicate; the blank was performed in quadruplicate.

Sample pH	Temperature (°C)				
	20	60	70	85	100
2.8	67.5	68.1	66.1	68.0	69.0
5.0	75.0	72.7	74.1	71.0	67.9
8.0	73.5	73.1	72.2	73.3	68.2
10.0	73.0	71.0	70.0	66.4	60.2

Identification of concanavalin A-reactive glycoprotein in the crossed immunoelectrophoresis pattern

A monospecific antiserum to concanavalin A-reactive glycoprotein [8] was used in the tests to locate the allergen in the crossed immunoelectrophoresis pattern and the results are illustrated in Fig. 3. For reference, Fig. 3a is an example of peaks obtained with crude peanut extract and its corresponding antiserum [2]. Fig. 3b is a demonstration of the apparent antigenic homogeneity of the allergen when tested in this system. The migration distance of the single peak corresponded closely with the main peak of Fig. 3a. In Fig. 3c the interposing of an intermediate gel containing monospecific concanavalin A-reactive glycoprotein antiserum served to define the position and identity of the allergen with regard to the main peak. However, the main peak was not removed from the upper pattern, as might be expected from this treatment. The result could be due to an insufficient titre in the intermediate gel or the possibility that concanavalin A-reactive glycoprotein is part of a complex of antigenically similar proteins. The main peak was not removed when the monospecific antiserum concentration was increased 5-fold and it thus seems that the allergen may be one of a complex of proteins. The photograph in Fig. 3d, taken in ultraviolet light, shows the labelling effect of an overlay of the pattern of Fig. 3a with fluorescein isothiocyanate-tagged monospecific antiserum to concanavalin A-reactive glycoprotein. The antiserum appeared to have bound to the major peak and also to a second peak within the main one. These peaks may represent different proteins or may result from concanavalin A-reactive glycoprotein in the crude protein extract migrating both as a monomer and as a disulphide-linked dimer. The dimer was described in the work of Gleeson and Jermyn [3]. Its probable presence in our concanavalin A-reactive glycoprotein preparation was suggested by our cysteine SH analysis. Notwithstanding this complication, concanavalin A-reactive glycoprotein does appear to represent a major allergenic component of the main crossed immunoelectrophoresis antigen peak.

Concanavalin A-reactive glycoprotein appears to exhibit many of the characteristics of allergens as described in the review of Aas [24]. The allergen

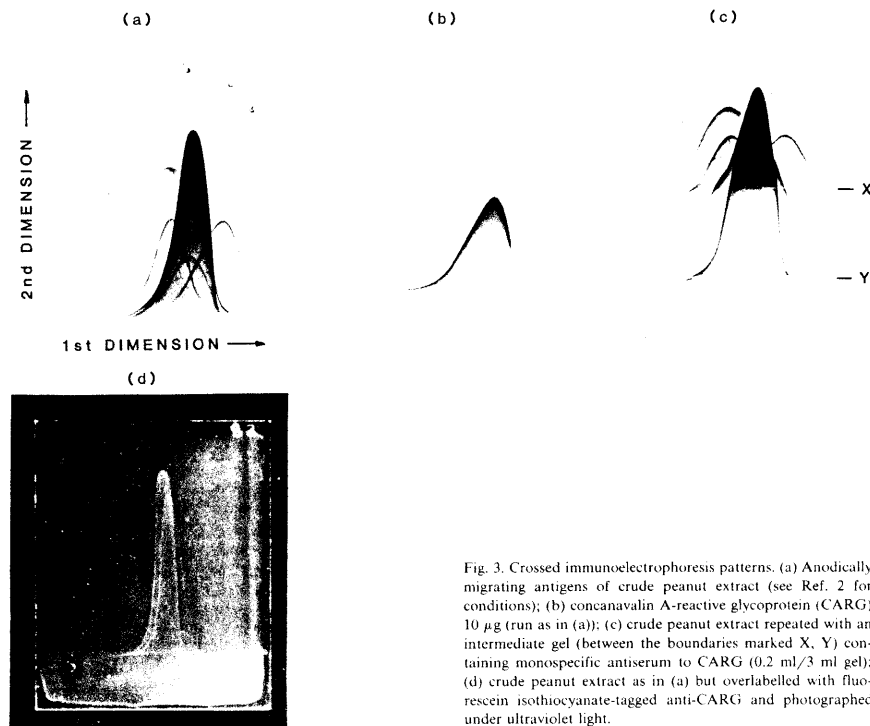


Fig. 3. Crossed immunoelectrophoresis patterns. (a) Anodically migrating antigens of crude peanut extract (see Ref. 2 for conditions); (b) concanavalin A-reactive glycoprotein (CARG) 10 µg (run as in (a)); (c) crude peanut extract repeated with an intermediate gel (between the boundaries marked X, Y) containing monospecific antiserum to CARG (0.2 ml/3 ml gel); (d) crude peanut extract as in (a) but overlabelled with fluorescein isothiocyanate-tagged anti-CARG and photographed under ultraviolet light.

is highly resistant to extremes of both heat and pH, a property which is consistent with the hypothesis that the allergenic determinants may consist of linear sequences of amino acids rather than more labile topological features. Allergen M from codfish was shown to have such reactive sequences that were able to mimic the whole molecule in both in vitro and in vivo tests [4]. Further characterisation of concanavalin A-reactive glycoprotein and location of such determinants is in progress. In the complex field of food allergy, greater knowledge of the allergenic structures involved is required so that studies may be placed on a more rational basis.

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