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A CONCANAVALIN A-LIKE LECTIN IN THE DEVELOPING SEED OF *CANAVALIA ENSIFORMIS*

MALLIKA RAYCHAUDHURI and MANORANJAN SINGH

Biochemical Engineering Division, Indian Institute of Chemical Biology, 4 Raja S C Mullick Road, Calcutta 700032, India

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Key Word Index—*Canavalia ensiformis*; Leguminosae; jackbean; concanavalin A; concanavalin A-like lectin; biosynthesis; precursor; hemagglutination; peptide mapping.

Abstract—During the early stages of seed development (Stage A) in *Canavalia ensiformis* (jackbean), the presence of an α-D-mannoside specific lectin(s) is shown by hemagglutination assays, although the usual subunit of concanavalin A (M_r 26 000), is not detectable by SDS-polyacrylamide gel electrophoresis. By employing affinity chromatography on Sephadex G-50 we have identified a Con A-like lectin with specificity to α-D-mannoside and α-D-glucoside in the seeds at early stages of development. Its native M_r has been estimated by gel filtration to be *ca* 122 000 with subunits of *ca* 28 500 as determined by SDS-polyacrylamide gel electrophoresis. It accounts for the hemagglutinating activity in the immature seeds of jackbean. In the later stage of seed development (Stage B), the high hemagglutination titre values are consistent with the presence of significant amounts of both this Con A-like lectin and Con A, whereas the equally high titre values in the dry mature seeds (Stage C) may be attributable essentially to Con A since the M_r 28 500 polypeptide is present only as a very minor component. Immunological and peptide mapping studies reveal a very close structural relationship between this lectin and Con A. These similarities and the inverse relationship in the relative abundance of these two proteins during different stages of seed development indicate an apparent precursor–product relationship between these two lectins.

INTRODUCTION

Lectins are proteins or glycoproteins of non-immune origin, with specific carbohydrate-binding properties, and due to this unique specificity they are frequently used as cell-surface probes or reagents for the isolation of glycoproteins and glycolipids [1–5]. Concanavalin A (Con A) is a plant lectin specific for α-D-mannosides and α-D-glucosides and it has been extensively studied and widely used as a reagent. It is of non-glycoprotein nature and its amino acid sequence as well as the three dimensional structure has been elucidated [6].

Marcus *et al.* [7] have recently reported the presence of several forms of Con A in the developing seeds of jackbean (*Canavalia ensiformis*). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) followed by Western blot analysis using anti-Con A sera as the probe and [^{125}I]-Con A overlay, revealed the presence of a polypeptide of M_r 33 000 in the immature jackbean seeds only. This led them to suggest that this polypeptide may be a glycosylated precursor for Con A and subsequent processing by deglycosylation may give rise to the final form of Con A. In the case of some other plant lectins, the existence of precursor forms which are subsequently processed to the normal storage forms by a post-translational modification mechanism, has been reported [8–11].

In course of our studies on the lectins in the developing jackbean seeds, a Con A-like lectin (CLL) is identified in the immature seeds. Its structural and immunological characterizations have been carried out. CLL disappears on the maturation of the seeds and its possible relationship vis-a-vis the biosynthesis of Con A is discussed.

RESULTS

The level of lectin in the developing jackbean seeds

The lectin content (expressed in μg/ml of Con A) as determined by the hemagglutination (Hgg) activity of the seed homogenates is found to be low at stage A, but the level rises considerably and remains constant at Stage B and Stage C (Table 1). α-Methyl-D-mannoside, which specifically binds to Con A, completely inhibits the Hgg activity of the seed extracts. Furthermore, the Hgg activity of the seed extracts of all three stages can be completely removed by specific anti-Con A sera (Table 2).

Table 1. Lectin content in the developing jackbean seeds

Sample	Protein (mg/ml)	Titre*	Con A (μg/ml)	Con A (μg/mg of protein)	Con A (μg/gm seed)
Stage A	2.6	2	0.96	0.4	9.6
Stage B	4.8	1024	492	102	4920
Stage C	5.8	1024	492	84	4920

*Reciprocal of dilution.

The Hgg activity was determined by incubation of equal volumes of serially diluted seed extracts with 2% rabbit erythrocyte suspension for 1 hr at room temperature. For quantitation of the lectin content of the seed extracts, standard, purified Con A was simultaneously assayed and the lectin content is expressed as equivalent to Con A (μg protein). The proteins were estimated according to Warburg and Christian [24].

Table 2. Removal of hemagglutination activities of seed extracts by Con A-specific antisera

Treatment	Hgg titre		
	Stage A	Stage B	Stage C
1. PBS	2	256	256
2. Normal rabbit sera	2	256	256
3. Con A-specific antisera	0	0	0

200 μl of the seed extracts of different developmental stages, A, B and C (total protein contents 6.1 mg/ml, 5.0 mg/ml and 5.6 mg/ml respectively) were incubated with 100 μl of anti-Con A sera, normal rabbit sera or PBS in the presence of 0.2 M α-methyl-D-mannoside for overnight at 4°. All the IgG was removed by passing through Protein A-Sepharose and then dialysed extensively to remove α-methyl-D-mannoside. The Hgg activity of the dialysed supernatant was determined as in Table 1.

In order to find out whether this Hgg activity is reflected in the protein profile of the seed extracts, analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. The polypeptide of M_r 26000, which is the usual subunit of Con A, is not detectable at Stage A but sufficient amounts are present at other stages. Instead, a polypeptide of about M_r 28500 is observed at Stage A (indicated by an arrow in lane a) and Stage B in significant amounts and it is virtually absent in Stage C (Fig. 1).

Isolation of the Con A-like lectin from the developing seeds of jackbean by affinity chromatography

In order to identify the compound which possesses Con A-like lectin properties, affinity chromatography on Sephadex G-50 is carried out with the extracts of stage B seeds; and stage C seed extracts are used as the control. Bound proteins are eluted with 0.2 M α-methyl-D-mannoside and the analysis of the column eluates by SDS-PAGE, reveals the presence of the M_r 28500 polypeptide (indicated by an arrow in lane d) in the case of Stage B and the M_r 26000 polypeptide (lane c) in the case of Stage C (Fig. 2). Two faster moving bands are observed in both the cases and these are commonly observed in the case of Con A [12, 13].

Both the M_r 28500 and 26000 polypeptides of CLL and Con A respectively as well as their fragments did not show the presence of carbohydrates on PAS staining of the affinity purified lectins, whereas some other polypeptides (M_r *ca* 33000 and higher) present in the homogenate of Stage B seeds were identified as glycoproteins (data not shown).

Immunological relationship between Con A and the Con A-like lectin

Distinct precipitin bands are observed in all the cases when the seed extracts of the three developmental stages are subjected to immunodiffusion test against rabbit anti-Con A sera; and spur formation with the precipitin band of standard Con A is detectable only in the case of the Stage A seed extract (Fig. 3).

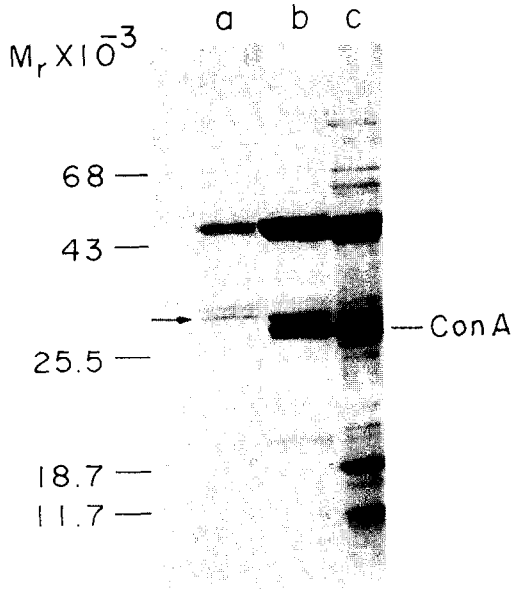

Fig. 1. SDS-PAGE analysis of proteins extracted from mature and immature seeds. Seeds of different stages were homogenized and after centrifugation the supernatant solutions were subjected to SDS-PAGE (12% acrylamide). Proteins were stained with Coomassie Blue. (a) Early (Stage A, 60 μg protein), (b) middle (Stage B, 75 μg of protein) (c) dry mature (Stage C, 98 μg of protein). The numbers in the left margin represent various standard protein M_r markers.

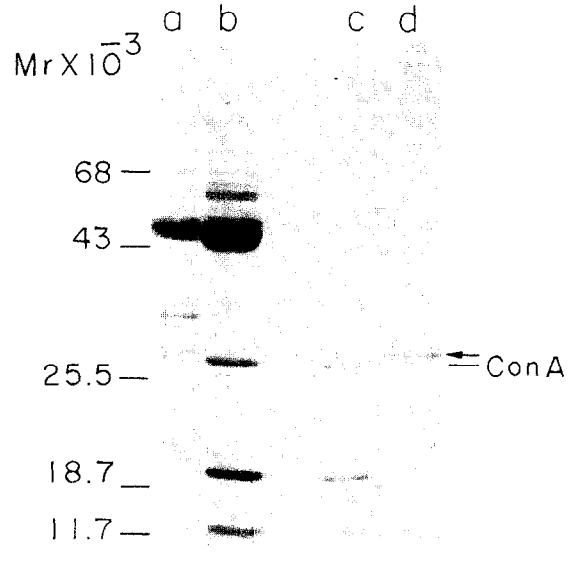

Fig. 2. SDS-PAGE of the affinity purified proteins. Lectins isolated by affinity chromatography on Sephadex G-50 by eluting with 0.2 M α-methyl-D-mannoside were analysed by SDS-PAGE (12% acrylamide). Proteins were stained with Coomassie Blue. (a) Total extract of Stage B seed, 75 μg protein; (b) total extract of Stage C seed, 90 μg protein; (c) eluted fraction from Stage C seed with highest protein concentration, 25 μg protein; (d) eluted fraction from Stage B seed with highest protein concentration, 25 μg protein. The numbers in the left margin represent various standard protein M_r markers.

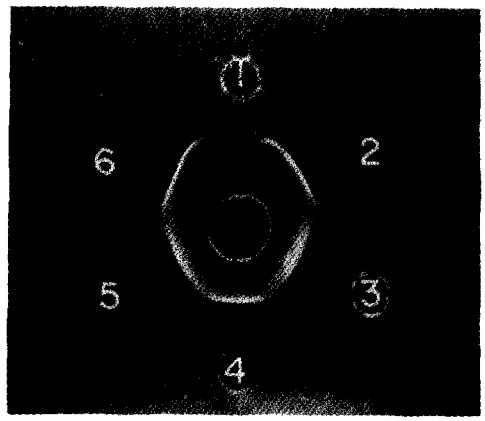

Fig. 3. Immunodiffusion studies in developing seeds. Immunodiffusion was carried out in 1.5% agarose containing 0.5 M α-methyl-D-mannoside using specific anti-Con A sera and seed extracts of three stages along with standard Con A. Central well: Rabbit anti-Con A sera (50 μl). (1) Stage A seed extract (26 μg protein). (2) Standard Con A (10 μg). (3) Stage C seed extract (58 μg protein). (4) Standard Con A (10 μg). (5) Stage B seed extract (48 μg protein). (6) Standard Con A (10 μg).

The radio-iodinated extract of Stage B seed is immunoprecipitated with rabbit anti-Con A sera and analysed by SDS-PAGE and autoradiographed. Two major bands and a minor band corresponding to M_r 28 500, 26 000 and 43 000 respectively are observed (Fig. 4).

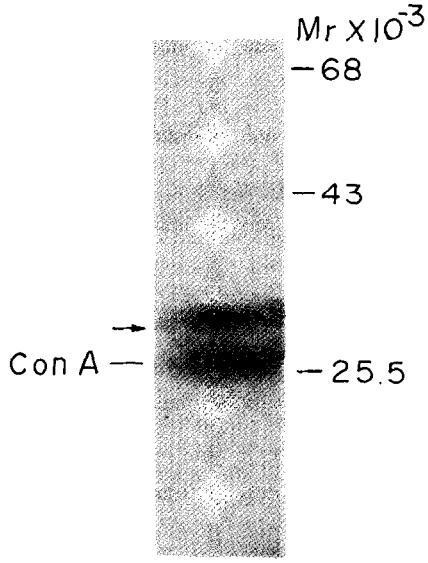

Fig. 4. Immunoprecipitation of Con A and CLL. Stage B seed extract was radio-iodinated and then immunoprecipitated with Con A-specific rabbit antisera. After washing, the immunoprecipitate (28 000 cpm) was analysed by SDS-PAGE (12% acrylamide). The gel was dried and autoradiographed.

Structural comparison between Con A and the Con A-like lectin by peptide mapping

Further evidence for the structural similarity of these two proteins is obtained by peptide mapping studies of the M_r 28 500 and 26 000 dalton radio-iodinated polypeptides employing trypsin as the protease. A similar fragmentation pattern is observed for the two polypeptides; and the autoradiograph (Fig. 5) shows four bands each. The two faster moving fragments have similar mobilities and the M_r 28 500 polypeptide yields a fragment which is slightly larger than the corresponding fragment from the M_r 26 000 polypeptide. The slowest moving bands are the intact polypeptides. In a similar experiment, chymotrypsin also generates very similar peptide fragments from the two lectins. Of the four fragments in each case, only the largest fragment of CLL is slightly larger than the corresponding one of Con A (data not shown).

DISCUSSION

During the seed development in jackbean, the Hgg activity is low at the early stages, and significantly higher activities are observed at later stages. The complete inhibition of this activity by α-methyl-D-mannoside indicates that the Hgg activity in the seeds at all stages of development is due to the presence of an agglutinin(s) with sugar specificity for α-D-mannosides. Moreover, since the Hgg activities in the seeds at all stages of development can be completely removed by specific anti-Con A serum; these are expected to be due to Con A only. However, our inability to detect the M_r 26 000 polypeptide, which is the authentic subunit of Con A, by SDS-

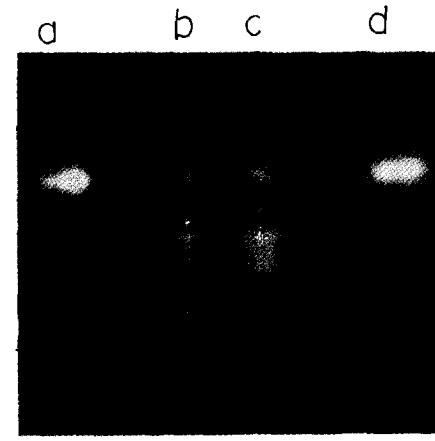

Fig. 5. Peptide mapping of Con A and CLL. Affinity purified Con A and CLL were labeled with ^{125}I and the iodinated proteins were diluted with respective cold proteins to yield approximately 10^4 cpm/μg protein. These proteins were subjected to SDS-PAGE and visualized by rapid staining and destaining. The protein bands were cut out and gel slices were loaded on another slab gel (5 cm long stacking gel, 5% acrylamide; 7 cm long separating gel, 12% acrylamide). Trypsin solution (8 μg/gel slice) was overlayed (as indicated) and electrophoresis was carried out immediately. Autoradiography was done with the dried gel. (a) Con A (15 000 cpm) − trypsin; (b) Con A (16 000 cpm) + trypsin; (c) CLL (17 800 cpm) + trypsin; (d) CLL (12 000 cpm) − trypsin.

PAGE in the immature (Stage A) seeds led us to look for other agglutinin(s) having α-D-mannoside specificity. The identification of such a protein with a subunit of M_r 28 500 by affinity chromatography on Sephadex G-50 accounts for the Hgg activity in Stage A seeds. The activity in Stage B seeds may be attributed to CLL and Con A, whereas in the dry mature (Stage C) seeds, the activity is essentially due to the latter.

The presence of smaller polypeptides along with standard Con A subunit (M_r 26 000) in SDS-PAGE (Fig. 2, lane C) is consistent with the earlier reports [12, 13] and is probably due to some kind of endopeptidase activity in jackbean seeds as reported by Dalkin and Bowles [14].

The affinity purification of the new lectin on Sephadex also establishes that it binds both α-D-glucoside and α-D-mannoside, and in this respect it has close resemblance to Con A. Our present finding of distinct precipitin bands along with spur formation, when CLL and Con A are subjected to immuno-diffusion against anti-Con A sera (Fig. 3) shows that CLL is closely related to, but not identical with, Con A. This is further substantiated by the finding that two major polypeptides (M_r 28 500 and 26 000 respectively) are identified on SDS-PAGE analysis of the immunoprecipitate from Stage B seed extract with Con A antiserum (Fig. 4).

A polypeptide of M_r *ca* 43 000 represents one of the major proteins in all the seed extracts (Fig. 2); and the observation of a faint band of the same size in the immunoprecipitates (Fig. 4) may be due to some minor peptide homology between this polypeptide and Con A. On the other hand the homology between Con A and CLL has to be quite extensive.

Peptide mapping studies employing trypsin and chymotrypsin firmly establish that there is indeed a close homology in the primary sequences of CLL and Con A. Of the three peptides generated by limited digestion with trypsin, the slowest moving fragment of CLL is slightly larger than the corresponding one of Con A. Similarly, in the case of chymotrypsin also, only the largest fragment of CLL moves slightly slower than the corresponding one of Con A. These results suggest that the primary difference between the subunit of Con A and that of CLL is the presence in the latter, of an extra segment of about 20–24 amino acid residues, corresponding to the difference of M_r 2500 in the sizes of these two polypeptides. This polypeptide segment is unlikely to be a signal peptide since CLL has quaternary structure and also possesses lectin activity. However, this needs confirmation by other studies such as mRNA translation, which is being presently undertaken.

It has been reported that favin, pea lectin and castor bean lectin are synthesized as larger precursor polypeptides which are subsequently processed after translation [8–11]. In these studies, the isolation of a precursor having lectin activity, has not been clearly demonstrated. However, in the present studies we have isolated a lectin larger than Con A, having similar sugar specificity as the latter. The immunological and structural similarities have been well established. These findings, taken together with the observation that CLL disappears on maturation of seeds with a concomitant rise in Con A as shown by SDS-PAGE studies, suggest a precursor-product relationship between CLL and Con A. Our preliminary pulse–chase studies also indicate CLL to be a precursor of Con A [Raychaudhuri, M. and Singh, M., unpublished results].

The present status of Con A biosynthesis is quite confusing. On the basis of [^{125}I]-Con A overlay and immunoblot studies a glycosylated polypeptide of M_r 33 000 has been postulated as a precursor of Con A [7]. On the other hand, in a recent preliminary report by Carrington *et al.* [15], another glycosylated polypeptide of M_r 37 000 has been suggested as a precursor of Con A; and this group has further indicated a unique polypeptide ligation step in the mechanism of biosynthesis and processing of the Con A precursor. It is not certain whether the two glycopolypeptides of M_r 33 000 and 37 000 as postulated by these two groups are the same or not. Hence, the present status is such that the mechanism proposed for Con A biosynthesis by Marcus *et al.* [7] involves post-translational glycosylation, deglycosylation and proteolytic processing whereas the other mechanism [15] requires post-translational polypeptide transposition and ligation over and above glycosylation, deglycosylation and proteolytic processing. In view of the fact that Con A is not a glycoprotein, the elucidation of both the nature and the role of the carbohydrate moiety in the postulated precursor will be needed before the mechanism of Con A biosynthesis and processing is settled.

EXPERIMENTAL

Materials. Jackbean was cultivated in the Institute gardens and tagging was carried out as soon as the pods were formed. Sodium deoxycholate, Triton X-100, α-methyl-D-mannoside, Coomassie Brilliant Blue R-250, standard protein M_r markers, trypsin, chymotrypsin and lactoperoxidase were purchased from Sigma. Phenyl methyl sulphonyl fluoride was from Boehringer and Freund's complete and incomplete adjuvants were from Difco. Carrier free [^{125}I]-NaI and [^{14}C]-amino acid mixture (a *Chlorella* protein hydrolysate, 40 mCi/milliatom of C) were supplied by Bhabha Atomic Research Centre, Trombay, India; Sephadex G-50 and Protein A-Sepharose, by Pharmacia and Biogel P-4, by Biorad. Other chemicals were analytical grade reagents.

Purification of Con A and CLL by affinity chromatography. Con A and CLL were purified from dry mature (Stage C) and fresh immature (Stage B) seeds respectively by affinity chromatography on Sephadex G-50 as in ref. [16] and the purity of the preparations were checked by SDS-PAGE [17].

Preparation of antibody and immunodiffusion tests. Antibody was raised in rabbits by three weekly subcutaneous injections of the purified Con A emulsified in complete Freund's adjuvant (a total of 1.5 mg protein per animal) followed by another injection in incomplete Freund's adjuvant. Immunodiffusion tests [18] were carried out in 1.5% agarose containing 0.5 M α-methyl mannoside, using purified Con A and seed extracts.

Hemagglutination and its inhibition by α-mannoside. Jackbean seeds of three different stages, A (early, 26 days after pod formation), B (middle, 38–40 days) and C (mature dry seed) were homogenized in 10 vols of 10 mM Tris–HCl, pH 7.5 containing 1 M NaCl and 1 mM phenyl methyl sulphonyl fluoride. The supernatant soln obtained after centrifugation at 15 000 *g* for 30 min was used or stored at −20°, if necessary. Hemagglutinating activity of the seed extracts was determined [19], using 25 μl of diluted extracts and 25 μl of 2% fresh rabbit erythrocyte suspension in saline.

SDS-PAGE of proteins and peptide mapping. Proteins were analysed by SDS-PAGE according to ref. [17] and stained with Coomassie Blue for protein. Periodic acid-Schiff staining was carried out for carbohydrate [20]. Peptide mapping of Con A and CLL was done according to ref. [21].

Iodination and immunoprecipitation. Iodination of proteins was carried out by lactoperoxidase method [22] and free ^{125}I was removed by gel filtration using Biogel P-4. The immunoprecipitation was carried out using Con A-specific rabbit antisera in the presence of 0.5 M α-methyl-D-mannoside in a buffer containing 0.3% sodium deoxycholate and 0.6% Triton-X-100 and the ppt was washed by centrifuging it through a discontinuous (0.5 M and 1.0 M) sucrose gradient at 3000 *g*. for 20 min [23].

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REFERENCES

1. Goldstein, I. J. and Hayes, C. E. (1978) *Adv. Carbohydr. Chem. Biochem.* **35**, 128.
2. Lis, H. and Sharon, N. (1977) in *The Antigens* (Sela, M., ed.) Vol. 4, pp. 429–529. Academic Press, New York.
3. Goldstein, I. J. (1972) *Methods Carbohydr. Chem.* **6**, 106.
4. Lis, H. and Sharon, N. (1973) *Ann. Rev. Biochem.* **42**, 541.
5. Surolia, A., Bachhawat, B. K. and Poddar, S. K. (1975) *Nature (London)* **257**, 802.
6. Reeke, G. N. Jr., Becker, J. W., Cunningham, B. A., Wang, J. L., Yahara, I. and Edelman, G. M. (1975) *Adv. Exp. Med. Biol.* **55**, 13.
7. Marcus, S. E., Burgess, J., Maycox, P. R. and Bowles, D. J. (1984) *Biochem. J.* **222**, 265.
8. Hemperly, J. J., Mostov, K. E. and Cunningham, B. A. (1982) *J. Biol. Chem.* **257**, 7903.
9. Roberts, L. M. and Lord, J. M. (1981) *Eur. J. Biochem.* **119**, 31.
10. Higgins, T. J. V., Chandlar, P. M., Zurawski, G., Button, S. C. and Spencer, D. (1983) *J. Biol. Chem.* **258**, 9544.
11. Higgins, T. J. V., Chrispeels, M. J., Chandlar, P. M. and Spencer, D. (1983) *J. Biol. Chem.* **258**, 9550.
12. Abe, Y., Iwabuchi, M. and Ishii, S. I. (1971) *Biochem. Biophys. Res. Commun.* **45**, 1271.
13. Wang, J. L., Cunningham, B. A. and Edelman, G. M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1130.
14. Dalkin, K. and Bowles, D. J. (1983) *Planta* **157**, 531.
15. Carrington, D. M., Auffret, A. and Hanke, D. E. (1985) *Nature (London)* **313**, 64.
16. Agrawal, B. B. L. and Goldstein, I. J. (1967) *Biochim. Biophys. Acta* **147**, 262.
17. Laemmli, U. K. (1970) *Nature (London)* **227**, 680.
18. Ouchterlony, O. (1948) *Acta Pathol. Microbiol. Scand.* **25**, 186.
19. Sarkar, M., Wu, A. M. and Kabat, E. A. (1981) *Arch. Biochem. Biophys.* **209**, 204.
20. Kapitany, R. A. and Zebrowski, F. J. (1973) *Analyt. Biochem.* **56**, 361.
21. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102.
22. Marchalonis, J. J. (1969) *Biochem. J.* **113**, 299.
23. Cladaras, C. and Cottam, G. L. (1980) *J. Biol. Chem.* **255**, 11499.
24. Warburg, O. and Christian, W. (1942) *Biochem. Z.* **310**, 384.