Phytochemistry, 1978, Vol. 17, pp. 1057–1058. © Pergamon Press Ltd. Printed in England

0031-9422/78/0601-1057$02.00/0

SHORT REPORTS

AN ANTI-B SPECIFIC HAEMAGGLUTININ FROM THE SEEDS OF *MUCUNA FLAGELLIPES*

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(*Received* 31 *October* 1977)

Key Word Index—*Mucuna flagellipes*; Leguminosae; phytohaemagglutinin; lectin.

Abstract—Seed fractionation in neutral media produced a globulin fraction which agglutinated B-blood group specifically. Combined application of cellulose acetate electrophoresis and gel chromatography showed two protein bands in this fraction.

INTRODUCTION

A considerable proportion of lectin characterization work in legume seeds [1–4] has been done on the genus *Phaseolus* [5–7] and also on some other bean types [8]. There is no information to our knowledge about lectins in *Mucuna*. Haemagglutinin activity has in many cases been located in both the albumin and globulin fraction [9] of isolated proteins and in many cases, agglutination is regarded as being non-specific. Extracts of the seeds of kidney beans were found to agglutinate erythrocytes and leucocytes [10] from different sources.

The seeds of *Mucuna flagellipes* T. Vogel ex Hook f. which have a crude protein content of 21–22% (wet-weight) are used in many parts of Africa in the preparation of various dishes. These beans form thick slurries in concentrations > 2% in water thereby limiting quantities that can be used. This coupled with the wide applicability of these beans led us to investigate their nutritional characteristics and potential. The following is an account of our work on the extraction of the phytohaemagglutinin components and their characterization.

This lectin should be useful for blood-typing, since few B-specific lectins are recorded in the literature [11].

RESULTS AND DISCUSSION

The globulin fraction extracted was agglutinin-positive in contrast to the albumin fraction which was agglutinin-negative. This globulin fraction showed on cellulose acetate electrophoresis two proteins, the main one of which had on Sephadex gel electrophoresis a MW of about 146000. The minor protein had a MW between 15000 and 20000. Haemagglutinin activity was confined entirely to the larger globulin component. Single component phytohaemagglutinins in the same MW range as the major component had been isolated from navy beans [12] and were shown to be toxic to quail. On subjecting the extracted agglutinin fraction to amino acid analysis on a Beckman 120C Amino Acid Analyzer, a peak which corresponded to glucosamine accounted for 0.5% (w/w) showing this fraction to be a glyco-protein. Definite agglutination was observed at a protein concentration of 2 μg/ml and at lesser concentrations visual agglutination was not detectable. This agglutinating-globulin fraction agglutinated B-type cells giving granular clumps. It showed no activity towards A and O blood groups. On testing the preparation against several A and O cells, no agglutination was observed showing that no anti-H was involved. However, the preparation did agglutinate AB cells as expected and it seems reasonable to classify it as a B-specific lectin. This is not very common and places the isolated glycoprotein within the small group of specific lectins [13]. The albumin fraction (present in solution in the supernatant and obtained by freeze-drying) showed no activity and considering the rather mild extraction procedures employed, it is unlikely that activity was destroyed during preparation. Thus the haemagglutinating activity is apparently concentrated in the globulin fraction, unlike many other isolated phytohaemagglutinins [9] where activity is present in both albumin and globulin. The phytohaemagglutinating component constitutes as much as 14% of the bean protein, taking into consideration the total extractable protein. This could explain the indigenous practice of eating this bean not as a main protein source but usually as a supplement to other dietary proteins. This concentration of haemagglutinins is unusually high being about 1.4 times the amount reported for *Phaseolus vulgaris* [14] and about 5–10 times that of the soya bean [15]. Even though haemagglutinins are known to be heat-labile, it is common knowledge that soya bean and *Phaseolus vulgaris* have a much higher dietary acceptance in the tropics in comparison with *Mucuna*. Work in progress should clarify what effects this high concentration of haemagglutinins has, using the beans as the sole protein source in diets.

EXPERIMENTAL

Materials. The beans were grown in local gardens in Nigeria. The mature seeds were collected traditionally and were stored in a polyethylene bag at 25°. Reagents and reference compounds were the finest grades available.

Trituration and extraction. The seed endosperm was cut into small pieces by a procedure similar to that used for *Pentaclethra* [16] and further comminuted to a powder in a Braun Multimix MX 32 (Braun). The resulting powder was slurried in H_2O to give a 2% suspension which was magnetically stirred for 30 min. This slurry had a pH of 6.7 and was adjusted to pH 7.0 with a few drops of 0.1 M NaOH. The suspension was stirred for a further 2 hr and then centrifuged at 10000 rpm in the Sorvall RC 5 at 15° for 30 min. The supernatant was decanted and the deposit was again slurried with a further 300 ml H_2O, stirred for 30 min and centrifuged as before. All supernatant was pooled and dialyzed against H_2O with daily diffusate changes. Dialysis was for 3 days after which the bags were split open and their contents pooled. The pooled fractions were stirred for 15 min and centrifuged as before. Both the new supernatant and the resultant ppt. were freeze-dried and stored in an evacuated desiccator over H_2SO_4 and NaOH.

Agglutination tests. These were carried out on blood samples from 20 human volunteers in our University. The two protein fractions were serially diluted with saline as described [17] and observed after 20–30 min for macroscopical agglutination. Protein content of the relevant (highest) agglutinating dilution was determined according to the Folin–Ciocalteu (Lowry) method [18]. By comparison with standard anti-sera, the material was found to possess anti-B activity.

Cellulose acetate electrophoresis. This was on 6 cm strips of cellulose acetate paper in barbitone [19] buffer (pH 8.6) at a constant current of 2 mA for 30 min. The strips were oven dried and were stained for 15 min in 0.25% Coomasie Brilliant Blue in 10% HOAc. They were destained by successive washing with 10%, 5% and 1% HOAc. Two protein bands of which one was slow-moving and major (globulin I), and the other was much faster and minor (globulin II) were detected in the relevant agglutinating fraction.

Gel electrophoresis. This was on a 1% flat-bed agarose gel in KPi buffer (pH 6.8) using BSA as marker. The gel was stained with Coomasie Brilliant Blue and destained as before. The presence of two proteins bands was confirmed.

Sephadex chromatography. Sephadex G-200, previously calibrated with the following proteins: ribonuclease A (13683), ovalbumin (45000), BSA (65000), creatine phosphokinase (83000), aldolase (150000), was used in 50 mM Tris–HCl buffer (pH 7.6). A column of bed vol. 166 ml was found adequate for determination of MW. The material containing 2.5 mg/cm^3 protein was applied as a vol. of 3.0 cm^3. Fractions of 6.0 cm^3 vol. were collected. The major protein peak was eluted in a vol. of 54 cm^3 corresponding to an apparent MW of 146000 while the minor peak was in the region 15000–20000.

Neutral sugar determination. This was by the PhOH–H_2SO_4 [20] procedure using glucose as standard.

Glycoprotein determination. This was performed by Mr. A. M. C. Davies of A.R.C. Food Research Institute, Norwich, after acid hydrolysis and estimation by Moore and Stein [21] ion exchange chromatography.

Crude protein content. N was determined by a micro-Kjeldahl [22] method and was multiplied by a factor of 6.25.

Acknowledgements—We are grateful to Mrs Sybil Agogbua for supplying us with samples of human anti-sera, Mr A. M. C. Davies for glyco-protein determination, Prof. R. L. M. Synge and Dr A. Pusztai for reading the manuscript. EIM acknowledges grant No. 00132/76 from the University Senate.

REFERENCES

1. Bird, G. W. G. (1959) *Brit. Med. J.* **15**, 165.
2. Liener, I. E. and Kakade, M. L. (1969) in *Toxic Constituents of Plant Foodstuffs* (Liener, I. E. ed.) p. 8. Academic Press, New York.
3. Pusztai, A. (1967) *Nutr. Abstr. Rev.* **37**, 1.
4. Jaffe, W. G., Levy, A. and Gonzalez, D. I. (1974) *Phytochemistry* **13**, 2685.
5. Rigas, D. A., Head, C. and Rigas-Egnitis, C. (1972) *Physiol. Chem. Phys.* **4**, 153.
6. Dahlgren, K., Porath, J. and Lindahl-Kiessling, K. (1970) *Arch. Biochem. Biophys.* **137**, 306.
7. Oh, Y. A. and Conrad, R. A. (1971) *Biochem. Biophys. Acta* **146**, 525.
8. Ikegwuonu, F. I. and Bassir, O. (1976) *Toxicol. Appl. Pharmacol.* **37**, 211.
9. Pusztai, A., Grant, G. and Palmer, R. (1975) *J. Sci. Fd Agric.* **26**, 149.
10. Nordman, C. T., de la Chapelle, A. and Grasbeck, R. (1964) *Acta Med. Scand. Suppl.* **412**, 49.
11. Osawa, T. and Akiya, S. (1961) *Bull Tokyo Univer.* **8**, 287.
12. Andrews, A. T. and Jayne-Williams, D. J. (1974) *Brit. J. Nutr.* **32**, 181.
13. Sharon, N. and Lis, H. (1972) *Science* **177**, 949.
14. Pusztai, A. and Watt, W. B. (1974) *Biochim. Biophys. Acta* **32**, 181.
15. Liener, I. E. and Pallansch, M. (1952) *J. Biol. Chem.* **197**, 29.
16. Laird, W. M., Mbadiwe, E. I. and Synge, R. L. M. (1976) *J. Sci. Fd Agric.* **27**, 127.
17. Pusztai, A. and Watt, W. B. (1970) *Biochim. Biophys. Acta* **207**, 413.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265
19. Sargent, J. R. (1969) in *Methods in Zone Electrophoresis* p. 53. BDH Chemicals Ltd., Poole, England.
20. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956) *Anal. Chem.* **28**, 350.
21. Spackman, D. H., Stein, W. H. and Moore, S. (1958) *Anal. Chem.* **30**, 1190.
22. Jennings, A. C. and Watt, W. B. (1967) *J. Sci. Fd Agric.* **18**, 527.