[Agr. Biol. Chem., Vol. 31, No. 7, p. 874~876, 1967]

Identification of the Hexosamine in a 7S Protein of Soybean Globulins*

By Ikunori KOSHIYAMA

Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan

Received March 6, 1967

Only a few informations have been obtained on glycoprotein from plants. Wada et al.¹⁾ reported that soybean hemagglutinin, which was prepared from the supernatant fraction of water-extractable soybean proteins at pH 4.4~4.6, was a glycoprotein containing glucosamine.

Recently, Lis et al.^{2,3)} showed that soybean hemagglutinin is a glycoprotein resembling some animal glycoproteins, such as ovalbumin^{4,5)} and ribonuclease B^{6,7)} in its carbohydrate composition and type of carbohydrate-protein linkage, and isolated a glycopeptide from the purified preparation by enzymatic digestion.

Further, Wrench⁸⁾ has shown that hexosamine-containing glycopeptide is present in wheat flour and glycoproteins containing hexosamine occurs extensively in various

plants as well as in animals.

In a previous paper⁹⁾ an ultracentrifugally and electrophoretically purified 7S protein^{10,11)} of soybean globulins was reported to contain a hexosamine (approximately 1.2% of dry weight) with mannose (approximately 3.8% of dry weight) as the constituents of carbohydrate.

The present paper deals with the identification of the hexosamine in the protein.

As the first step for identifying the hexosamine, the purified 7S protein was hydrolysed

FIG. 1. A Portion on a Chromatogram of a Hexosamine in the 7S Protein and Synthetic Mixture of Hexosamines.

Column: 0.8×43 cm of Dowex 50×8 (200~400 mesh). Eluent: 0.33 N HCl. Fraction volume: 0.97 ml. Flow rate: 2 ml/hr.

a: Glucosamine. b: Galactosamine

* This paper represents Part VI of Studies on Soybean Proteins. Presented at the 52nd Annual Meeting of American Association of Cereal Chemists, Los Angeles, California, April 1967.

1) S. Wada, M. J. Pallansch and I. E. Liener, *J. Biol. Chem.*, **233**, 395 (1958).

2) H. Lis, N. Scharon and E. Katchalski, *Biochim. Biophys. Acta*, **83**, 376 (1964).

3) H. Lis, N. Scharon and E. Katchalski, *J. Biol. Chem.*, **241**, 684 (1966).

4) P. G. Johansen, R. D. Marshall and A. Neuberger, *Biochem. J.*, **78**, 518 (1961).

5) A. P. Fletcher, G. S. Marks, R. D. Marshall and A. Neuberger, *ibid.*, **87**, 265 (1963).

6) T. H. Plummer, C. H. W. Jr. and Hirs, *J. Biol. Chem.*, **238**, 1396 (1963).

7) T. H. Plummer, C. H. W. Jr. and Hirs, *ibid.*, **239**, 2530 (1964).

8) P. M. Wrench, *J. Sci. Fd. Agric.*, **17**, 403 (1966).

9) I. Koshiyama, *This Journal*, **30**, 646 (1966).

10) I. Koshiyama, *This Journal*, **29**, 885 (1965).

11) I. Koshiyama, *Cereal Chemistry*, in press.

in vacuo for 6 hours at 110°C with 3.5 N hydrochloric acid. After concentration under reduced pressure, the hydrolysate was put on a Dowex 50×8 column (0.8×43 cm, H⁺-form, 200~400 mesh) and eluted with 0.33 N hydrochloric acid according to the method of Randle and Morgan.¹²

As shown in Fig. 1, glucosamine (2-amino-2-deoxy-D-glucose) and galactosamine (2-amino-2-deoxy-D-galactose) were separately eluted by this column chromatography and the elution position of the hexosamine in this protein corresponded to that of glucosamine. The assay of the eluted hexosamine was performed by the modified method of Boas.¹³ The

FIG. 2. Paper Chromatograms of the Hexosamine in the 7S Protein, Standard Hexosamines, N-Acetylglucosamine and N-Acetyl Neuraminic Acid.

Solvent (1):¹⁴ *n*-butanol: pyridine: water=6:4:3 (by volume)

Solvent (2):¹⁵ phenol: water added one drop of conc. NH₄OH=4:1 (by volume)

The sample (b) was prepared by column chromatography with Dowex 50×8 after hydrolysis with 3.5 N HCl for 6 hours at 110°C in vacuo. The spots a, c, d and e are authentic glucosamine, galactosamine, N-acetylglucosamine and N-acetyl neuraminic acid respectively. The filter paper (Toyo No. 50) were developed at room temperature and stained with the Ehrlich's reagent.¹⁶

12) C. J. M. Randle and W. T. J. Morgan, *Biochem. J.*, **59**, 13p (1955).

13) N. F. Boas, *J. Biol. Chem.*, **204**, 553 (1953).

14) M. J. Crumpton, *Biochem. J.*, **72**, 479 (1959).

15) C. E. Dent, *ibid.*, **43**, 169 (1948).

16) S. M. Partridge, *Nature*, **158**, 270 (1946).

17) J. L. Ressig, J. L. Strominger and L. F. Leloir, *J. Biol. Chem.*, **217**, 959 (1959).

FIG. 3. Absorption Spectra of the Colored Products Obtained on Reaction of the Compound from the 7S Protein (A), Glucosamine (B), Galactosamine (C), N-Acetyl glucosamine (D) and N-Acetyl Neuraminic Acid (E) with Acetylacetone and Ehrlich's Reagent.¹³

results of paper chromatography exhibited also the presence of glucosamine as shown in Fig. 2.

The same result was also obtained by staining the filter papers with ninhydrin reagent.

Furthermore, the hexosamine isolated with a Dowex 50 column was ascertained by spectrophotometric assay with a Hitachi recording spectrophotometer as shown in Fig. 3.

Negative reaction of N-acetylhexosamine in this 7S protein shows that this protein contains only glucosamine. By the way, sialic acid was not found in the protein by the method of thiobarbituric acid.¹⁸

Pusztai¹⁹ recently isolated a glycoprotein containing mannose and glucosamine as well as those in soybean hemagglutinin from kidney beans. These facts show that these constituents of carbohydrate in the glycoprotein, mannose and glucosamine, exist not only in animals,^{6,20} but also in plants.

Moreover, it is very much interesting that mannose and glucosamine are present in nearly the same amounts as found in soybean hemagglutinin by Lis and coworkers.³¹

18) L. Warren, *J. Biol. Chem.*, **234**, 1971 (1959).

19) A. Pusztai, *Biochem. J.*, **95**, 3c (1965).

20) R. G. Spiro, *New Engl. J. Med.*, **269**, 566, 616 (1963).

Acknowledgements. The author wishes to express his sincerest thanks to Prof. Y. Sakurai of the Japan Women's University and Prof. M. Fujimaki of the University of Tokyo for their kind guidance and encouragement throughout this work. Great indebtedness is acknowledged to Prof. K. Arima and Prof. Y. Ikeda of the University of Tokyo, and Dr. D. Fukushima of our laboratory for their valuable advice and suggestions. Thanks are due to Dr. M. Mogi and Dr. N. Iguchi for their encouragement and Miss. T. Ichikawa for her kind assistance.