CHANGES OF ISOELECTRIC POINTS OF CONCANAVALIN A INDUCED BY THE BINDING OF CARBOHYDRATES

Hitoshi Akedo, Yoichi Mori, Mayumi Kobayashi and Masako Okada

Department of Biochemistry and Department of Cell Biology, The Center for Adult Diseases, Osaka, Osaka 537, Japan

Received August 22,1972

SUMMARY

The effects of certain sugars on the isoelectric points of several molecular species of concanavalin A are reported. Pre-incubation with D-mannose, α-methyl-D-glucoside or D-glucose resulted in extensive shifts of isoelectric points of these species of the protein towards alkaline pH. D-Galactose which binds poorly to concanavalin A had little effect. The shifts of isoelectric points occurred predominantly in those molecular species with isoelectric points ranging between pH 6.6 and 7.1.

Concanavalin A, a carbohydrate-binding protein from jack beans, has been reported to possess hemagglutinating and mitogenic activities (1,2). It was also shown to regulate the growth of transformed fibroblasts by restoring density-dependent phenomena (3). Since the protein is known to bind to cell surfaces (4), studies on those actions of concanavalin A could help us in understanding of possible mechanisms of an apparatus which could reside in cell membranes and which could regulate cell growth and differentiation. In that line, we have succeeded in isolating two receptor proteins from rat erythrocyte stroma (5). Receptors for concanavalin A were also isolated from Novikoff ascites tumor cells (6) and from pig lymphocytes (7).

We have also undertaken physicochemical studies of concanavalin A crystallized in our laboratory from nata beans (5) in an effort to elucidate the mechanism of its biological actions. Recently we reported that the crystallized concanavalin A was separated into several molecular species by electrofocusing (5). In the present paper we discuss an evidence from a study of electrofocusing that suggests conformational changes of concanavalin A upon the binding of specific carbohydrates.

MATERIALS AND METHODS

Concanavalin A : Concanavalin A was purified from nata beans (Canavalia gladiata) by the method of Agrawal and Goldstein (8) with slight modification. The protein preparation was crystallized as previously reported (5). The crystallized concanavalin A was dissolved in 1 M NaCl for experimental use.

Electrofocusing : Electrofocusing in 4 % polyacrylamide gel using 2 % ampholyte solution (pH 3-10) was performed by the method of Righetti and Drysdale (9). Gels, 0.5 cm in diameter and 7 cm in length, were used. In order to fractionate concanavalin A preincubated with sugars, polyacrylamide gels were prepared in the presence of the same concentrations of sugars used in the preincubation. Preliminary tests indicated that the addition of sugars in polyacrylamide gel did not interfere with the formation of a pH gradient. After focusing, the gels were immersed in 12.5 % trichloroacetic acid to precipitate proteins and to remove the ampholytes from the gels. The proteins were then stained with 0.1 % Coomassie Blue.

Densitometric quantitation : Quantitation by densitometry of the stained gels was performed using Densitron Model SP-3, Jooko Sangyo Co., Japan.

Estimation of pH gradient : The pH gradients were estimated from eluates of gel sections (0.5 cm length) using H_2O as a solvent according to the method of Righetti and Drysdale (9). The pH of gel eluates was measured at room temperature (22°C) with pH-Meter 22, Radiometer, Copenhagen.

Sources of chemicals : Ampholyte solution (40 % w/v) with buffering capacities in the pH range 3-10 was purchased from LKB Producter AB, Sweden. Acrylamide and N,N'-methylene bisacrylamide were obtained from Seikagaku Kogyo Co., Japan. N,N,N',N'-Tetramethylethylenediamine and D-glucose were obtained from Katayama Chemicals, Japan ; D-mannose, α-methyl-D-glucoside from Nakarai Pure Chemicals, Japan. D-Galactose was purchased from Sigma, U S A.

RESULTS AND DISCUSSION

Electrofocusing of concanavalin A preincubated with sugars.

Although the crystallized concanavalin A appeared to be homogeneous in ultracentrifugation and in polyacrylamide gel electrophoresis, it was separated into at least eight proteins by electrofocusing (5). When concanavalin A was preincubated with 0.06 M D-mannose for 30 minutes at 37°C, the main protein bands with isoelectric points ranging between pH 5.9 and 7.3 almost disappeared and two protein bands appeared, as indicated by the arrows in Fig. 1. The broader band was found to be isoelectric between pH 7.8 and 8.0, and the isoelectric point of the other band was around pH 7.6. The shift of isoelectric points of concanavalin A towards the range between pH 7.8 and 8.0 seems to be the major change induced by this sugar.

Preincubation with the same concentration of α-methyl-D-

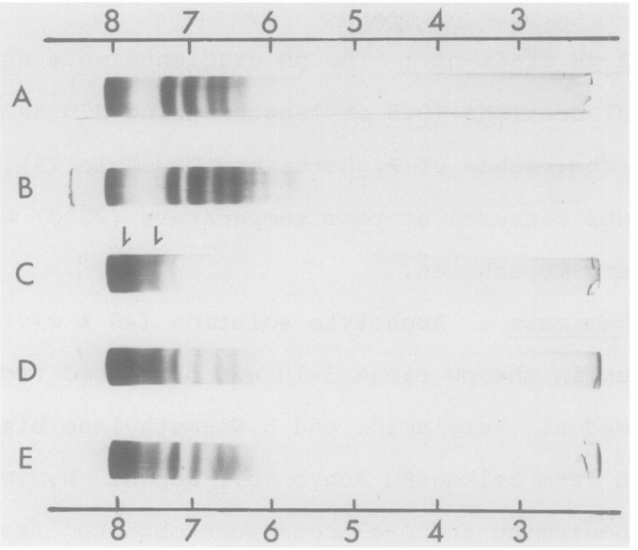

Fig. 1. Electrofocusing of concanavalin A preincubated with sugars. Concanavalin A (1.2 mg/ml) was preincubated with sugars (0.06 M) for 30 minutes at 37°C. A : with D-galactose, B : without any sugar (control), C : with D-mannose, D : with α-methyl-D-glucoside, E : with D-glucose. Proteins were stained with Coomassie Blue. Numbers in the figure indicate pH gradients performed. Arrows indicate protein bands appeared by preincubating concanavalin A with specific sugars.

glucoside or D-glucose resulted in similar changes in isoelectric points, although some proteins still remained in the isoelectric point range between pH 5.9 and 7.3 (Fig. 1). The ability of the sugars to induce shifts of isoelectric points seems to be proportional to binding affinities of these sugars for concanavalin A (10). D-Galactose which was reported to bind poorly to concanavalin A (11) caused considerably less shifting of isoelectric points, as shown in Fig. 1.

Electrofocusing of concanavalin A preincubated with various concentrations of D-mannose.

Fig. 2 shows the electrofocusing of concanavalin A preincubated with various concentrations of D-mannose for 30 minutes at 37°C. As is seen, the shift of isoelectric points of the proteins towards the pH range, 7.8-8.0 was enhanced by increasing concentra-

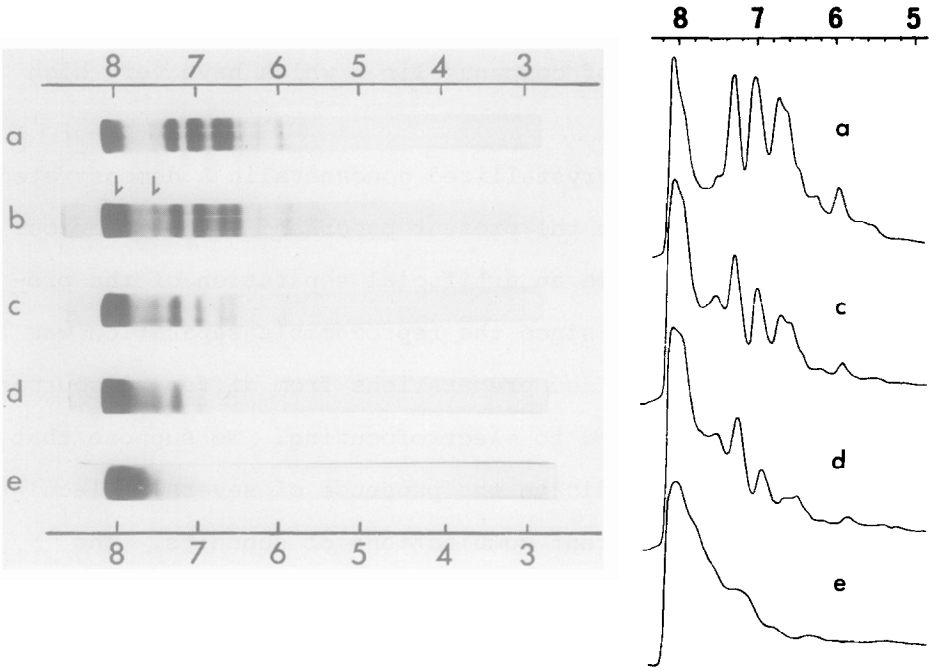

Fig. 2. Electrofocusing of concanavalin A preincubated with various concentrations of D-mannose. Right : densitometric scanning of the gels at 600 mμ. Concanavalin A (1.2 mg/ml) was preincubated with D-mannose for 30 minutes at 37°C. a : without D-mannose, b : 1.6 mM D-mannose, c : 8 mM D-mannose, d : 40 mM D-mannose, e : 200 mM D-mannose. Numbers in the figure indicate pH gradients performed. Arrows show protein bands appeared by preincubating concanavlin A with D-mannose.

tions of D-mannose. Densitometric quantitation of four major protein bands revealed that those proteins, isoelectric between pH 6.6 and 7.1, shifted location more rapidly than the one isoelectric at pH 7.3. This indicates that the former proteins could have greater affinities for the sugar than the latter protein, as judged by the shift of their isoelectric points.

Although the resolution of the protein band at pH 7.6 was not satisfactory, the shift of isoelectric points towards pH 7.6 induced by D-mannose seems to have reached a maximum at the lowest concentration of the sugar (Fig. 2). The shift towards pH 7.6 may

correspond to the change of isoelectric point(s) of one or several of some molecular species of concanavalin A which have very high affinities for that sugar.

The heterogeneity of crystallized concanavalin A demonstrated by using electrofocusing in the present paper and in our previous report (5) appears not to be an artificial separation of the protein upon electrofocusing, since the reproducible separation was obtained when several purified preparations from different sources of nata beans were subjected to electrofocusing. We suppose that the separation may well indicate the presence of several molecular species consisted of different combinations of subunits. The presence of heterogeneous subunits in the concanavalin A molecule has previously been suggested (5, 12, 13). Although the relationship between each molecular species is not clear at present, the isolation and characterization of each protein band after electrofocusing are in progress in our laboratory and the results will be presented in a forthcoming paper.

The increase in net possitive charge of concanavalin A molecules as demonstrated by the shifts of isoelectric points of its species suggests conformational changes of concanavalin A upon the binding of specific carbohydrates. Changes in conformation of concanavalin A from jack beans upon the binding of α-methyl-D-mannoside was noticed by Pflumm and others when measuring circular dichroic spectrum (14). Since the changes are induced by a group of sugars which is known to bind specifically to concanavalin A and to inhibit the biological actions of the protein, such changes may relate closely to the mechanism by which concanavalin A functions in hemagglutination and in mitogenesis of animal cells.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Cancer

Research from the Ministry of Welfare and from the Ministry of Education, Japan.

REFERENCES

1. Sumner, J. B. and Howell, S. F., J. Immunol., 29, 133 (1935).
2. Powell, A. E. and Leon, M. A., Exp. Cell Res., 62, 315 (1970).
3. Burger, M. M. and Noonan, K. D., Nature, 228, 512 (1970).
4. Nicolson, G. L. and Singer, S. J., Proc. Natl. Acad. Sci. U.S.A., 68, 942 (1971).
5. Akedo, H., Mori, Y., Tanigaki, Y., Shinkai, K. and Morita, K., Biochim. Biophys. Acta, 271, 378 (1972).
6. Wray, V. P. and Walborg, Jr., E. F., Cancer Res. 31, 2072 (1971).
7. Allan, D., Auger, J. and Crumpton, M. J., Nature New Biol., 236, 23 (1972).
8. Agrawal, B. B. L. and Goldstein, I. J., Biochim. Biophys. Acta, 133, 376 (1967).
9. Righetti, P. and Drysdale, J. W., Biochim. Biophys. Acta, 236, 17 (1971).
10. So. L. L. and Goldstein, I. J., Biochim. Biophys. Acta, 165, 398 (1968).
11. Goldstein, I. J., Hollerman, C. E. and Smith, E. E., Biochemistry, 4, 876 (1965).
12. Wang, J. L., Cunningham, B. A. and Edelman, G. M., Proc. Natl. Acad. Sci. U.S.A., 68, 1130 (1971).
13. Abe, Y., Iwabuchi, M. and Ishii, S., Biochem. Biophys. Res. Commun., 45, 1271 (1971).
14. Pflumm, M. N., Wang, J. L. and Edelman, G. M., J. Biol. Chem., 246, 4369 (1971).