Vol. 82, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1978 Pages 492-497

SEPARATION AND CHARACTERIZATION OF TWO BOVINE ALPHA$_1$-FETOPROTEIN MOLECULAR VARIANTS BY CONCANAVALIN A-SEPHAROSE CHROMATOGRAPHY

P.C.W. Lai and F.L. Lorscheider

Division of Medical Physiology, Faculty of Medicine, The University of Calgary and The Alberta Children's Hospital Research Centre, Calgary, Alberta, Canada

Received March 20,1978

SUMMARY. Two molecular variants of bovine alpha$_1$-fetoprotein were separated by affinity chromatography of fetal calf serum on a concanavalin A-Sepharose column. Radialimmunodiffusion assay of bovine alpha$_1$-fetoprotein revealed that 29% of the alpha$_1$-fetoprotein in fetal serum lacked concanavalin A-binding activity whilst 71% of the alpha$_1$-fetoprotein was capable of binding to the lectin. These two bovine alpha$_1$-fetoprotein variants show antigenic identity suggesting that the polypeptide chain rather than the carbohydrate moiety of the alpha$_1$-fetoprotein molecule is the antigenic determinant.

INTRODUCTION

Alpha$_1$-fetoprotein (AFP)*, has a well established clinical significance in the diagnosis of certain types of liver, gastrointestinal and endodermal sinus tumors (1, 2) and as a marker in the prenatal monitoring of the fetus (3, 4). Recent investigations have focused on the structure and function of AFP. There is evidence to suggest that certain mammalian AFPs possess immunosuppressive (5) and estrogen-binding activities (6, 7). Furthermore, AFP has been shown to exist as several molecular variants in man (8, 9) and rat (10-14). Variants of AFP have been demonstrated to possess different immunosuppressive potencies (9, 15) and estrogen-binding parameters (7).

Immunochemical characterization of rat AFP variants, separated by column chromatography of fetal serum or amniotic fluid on insolubilized concanavalin A, has been reported (7, 10, 13, 14). Concanavalin A is capable of binding molecules possessing α-D-mannopyranosyl or α-D-glucopyranosyl end-groups or sterically related residues (16, 17). The objectives of the present study were to separate and identify molecular variants of bovine AFP by concanavalin A-Sepharose chromatography and to characterize these variants by immunochemical methods.

0006-291X/78/0822-0492$01.00/0

MATERIALS AND METHODS

Rabbit anti-bovine AFP. AFP (35 µg) purified from the concanavalin A-reactive fraction of fetal calf serum as previously described (18) was dissolved in 0.4 ml of 0.12 M Tris-HCl, pH 8.1, emulsified with an equal volume of complete Freund's adjuvant (Calbiochem, La Jolla, CA, USA) and injected subcutaneously into a rabbit. Injection was repeated twice at 10-day intervals and the antiserum was obtained 10 days after the final injection.

Immunological techniques. Quantitation of AFP was carried out using radialimmunodiffusion assay (19) as we have previously employed (18). The standardized fetal calf serum used in the assay was calibrated against our purified bovine AFP. Immunological comparison of antigens was carried out in 1% (w/v) agarose gel by Ouchterlony double immunodiffusion method (20) and by Tandem crossed immunoelectrophoresis (21).

Affinity chromatography. Concanavalin A-Sepharose (Pharmacia, Uppsala, Sweden) was packed in a 0.9 x 12 cm column and equilibrated with the starting buffer (0.1 M sodium acetate, 1 mM Ca^{2+}, 1 mM Mg^{2+}, 1 mM Mn^{2+}, 0.02% NaN_3, pH 6.0) at 21°C. Fetal calf serum, (0.5 ml; 55-60 days gestation; GIBCO, Grand Island, NY, USA) estimated to contain 3 mg AFP/ml and 31 mg total protein/ml, was mixed with 1 ml of the starting buffer and chromatographed on the column. After washing with 12 ml of the starting buffer, the column was eluted with 0.1 M α-methyl-D-glucoside (Sigma Chemical Co., St. Louis, MO, USA) dissolved in the starting buffer. The flow rate was maintained at 12 ml/h throughout the chromatographic procedures and 1.1 ml - fractions were collected. Fractions containing proteins not bound to the column were pooled and subsequently rechromatographed on a fresh concanavalin A-Sepharose column under identical conditions to confirm that this protein fraction lacked concanavalin A-binding activity. Fractions from both chromatographic runs were monitored for protein by absorbance at 280 nm or by the method of Lowry et al (22). AFP concentration in each fraction was estimated by radialimmunodiffusion assay as described above and the presence of AFP in each fraction was also identified by analytical disc polyacrylamide gel electrophoresis (23).

RESULTS AND DISCUSSION

The elution profile of fetal calf serum chromatographed on a concanavalin A-Sepharose column is illustrated in Fig. 1 (left). The first protein peak consisted of concanavalin A-nonreactive proteins not bound to the column and the second peak represented concanavalin A-reactive proteins which were bound to the column and subsequently eluted with α-methyl-D-glucoside. Two distinct AFP molecular variants separated by affinity chromatography could be detected by radialimmunodiffusion assay of AFP. analytical disc polyacrylamide gel electrophoresis revealed that the two AFP molecular variants do not differ in electrophoretic mobility. The analytical recoveries of total protein and AFP from the column were 91% and 90% respectively. The presence of AFP in the first protein peak was not due

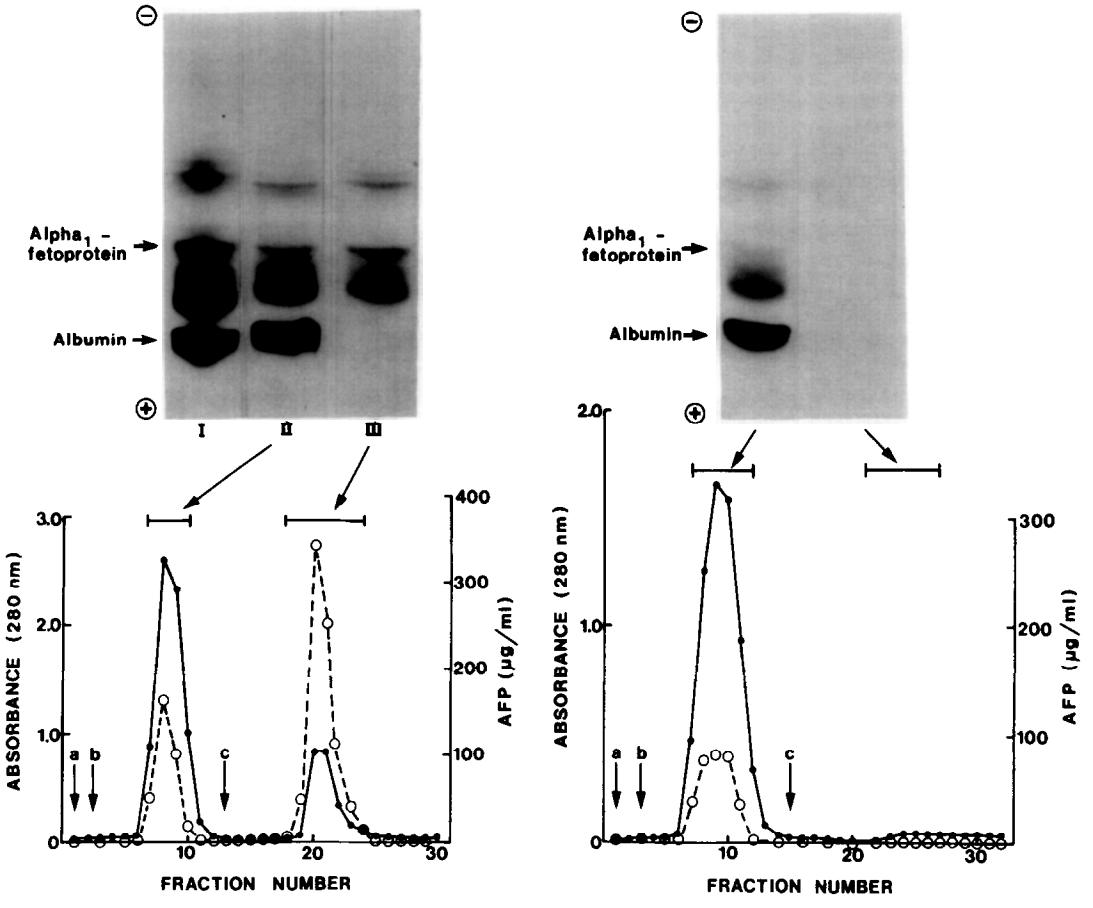

Fig. 1. (*Left*) Elution profile of the affinity chromatography of fetal calf serum on a concanavalin A-Sepharose column. Closed circles: absorbance at 280 nm; open circles: AFP concentration. Abbreviations: a, sample application; b, elution with starting buffer; c, elution with 0.1 M α-methyl-D-glucoside added to starting buffer. Analytical disc polyacrylamide gel electrophoresis: I, fetal calf serum; II, concanavalin A-nonreactive fraction; III, concanavalin A-reactive fraction. Horizontal bar represents fractions pooled. (*Right*) Elution profile of the rechromatography of pooled concanavalin A-nonreactive fractions. Disc polyacrylamide gel electrophoresis of the fractions are inserted. Abbreviations a-c, same as above.

to overloading of the column because rechromatography of the concanavalin A-nonreactive fraction on a fresh concanavalin A-Sepharose column confirmed that this protein fraction indeed lacked concanavalin A-binding activity (Fig. 1, right).

Table 1 shows the percentage distribution of total serum protein and

Table 1. Relative distribution of total fetal calf serum proteins and AFP respectively in concanavalin A-reactive and concanavalin A-nonreactive fractions eluted from the affinity chromatography of the serum on a concanavalin A-Sepharose column. Protein concentration was determined by the method of Lowry et al (22).

	% eluted in concanavalin A-nonreactive fraction	% eluted in concanavalin A-reactive fraction
total serum proteins	68	32
AFP	29	71

AFP in the concanavalin A-reactive and nonreactive fractions.

Fig. 2 demonstrates that the concanavalin A-reactive and nonreactive AFPs show antigenic identity by Ouchterlony double diffusion method. This finding suggests that in contrast to bovine fetuin (24) the polypeptide chain rather than its carbohydrate moiety is the antigenic determinant of the bovine AFP molecule. The antiserum was specific for AFP and did not contain precipitating antibodies to non-pregnant cow serum. The reaction of identity between the two AFP molecular variants was confirmed by Tandem crossed immunoelectrophoresis.

The ability of AFP to bind to concanavalin A varies among mammalian species. Human AFP is almost entirely concanavalin A-reactive (7). In contrast, rat AFP consists of as many as three forms, namely concanavalin A-nonreactive, concanavalin A-weakly-reactive and concanavalin A-reactive variants (10, 13, 14). In the present study, we have demonstrated the existence of only two forms of bovine AFP, i.e. concanavalin A-reactive and concanavalin A-nonreactive variants. The species difference with respect to AFP-concanavalin A interaction is not known.

Vallette et al (7) suggested that the carbohydrate moiety of the rat AFP molecule may have a role in estrogen-AFP interaction. Their hypothesis was based on the finding that the concanavalin A-reactive or "high

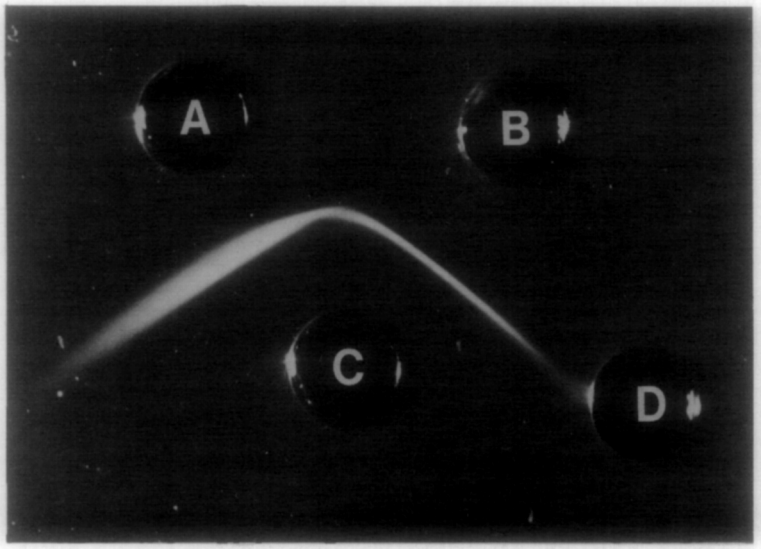

Fig. 2. Ouchterlony double immunodiffusion gel plate. Antigens: A, pooled concanavalin A-nonreactive fractions; B, pooled concanavalin A-reactive fractions; A and B obtained from affinity chromatography (see text); C, non-pregnant cow serum. Antiserum: D, rabbit anti-bovine AFP.

carbohydrate" form has low affinity for 17β-estradiol while the concanavalin A-nonreactive or "low carbohydrate" form has high affinity for this steroid hormone. Their hypothesis was supported by the correlation of the lack of estrogen-binding activity of human AFP with the finding that human AFP consists mostly of the "high carbohydrate" or concanavalin A-reactive form. The present study shows that such a hypothesis is not applicable to the bovine species inasmuch as bovine AFP is not a significant estrogen-binding protein in fetal calf serum (25) and yet we have demonstrated that this glycoprotein consists of substantial amounts of a concanavalin A-nonreactive or "low carbohydrate" form.

We have demonstrated that bovine AFP is heterogeneous by affinity chromatography on a concanavalin A-Sepharose column. It is interesting to note that carcinoembryonic antigen, another carcino-fetal protein isolated from human hepatic metastases of rectal and pancreatic adenocarcinoma was also reported to contain concanavalin A-reactive and nonreactive variants

(26). At present, the significance and the chemical nature of the heterogeneity of AFP in the development of bovine fetuses remain to be elucidated.

ACKNOWLEDGEMENTS

This investigation was supported by the Medical Research Council of Canada grant no. MA 5292 and a grant from The Alberta Children's Hospital Research Foundation.

REFERENCES

1. Alpert, E. (1976) Prog. Liver Dis. 5, 337-349.
2. Nørgaard-Pedersen, B. (1976) Scand. J. Immunol. Suppl. 4, 24-35.
3. Brock, D.J.H. (1976) Child's Brain 2, 1-23.
4. Seppälä, M. (1977) Clin. Obstet. Gynecol. 20, 737-757.
5. Tomasi, T.B. (1977) Ann. Rev. Med. 28, 453-465.
6. Swartz, S.K., Soloff, M.S., and Suriano, J.B. (1974) Biochim. Biophys. Acta 359, 273-281.
7. Vallette, G., Benassayag, C., Belanger, L., Nunez, E.A., and Jayle, M.F. (1977) Steroids 29, 277-289.
8. Alpert, E., and Perencevich, R.C. (1975) Ann. N.Y. Acad. Sci. 259, 131-135.
9. Lester, E.P., Miller, J.B., and Yachnin, S. (1976) Proc. Natl. Acad. Sci. (USA) 73, 4645-4648.
10. Smith, C.J., and Kelleher, P.C. (1973) Biochim. Biophys. Acta 317, 231-235.
11. Belanger, L., and Dufour, D. (1974) L'alpha fetoproteine (Masseyeff, R., ed.), pp. 25-36, INSERM, Paris.
12. Watabe, H. (1974) Int. J. Cancer 13, 377-388.
13. Lai, P.C.W., Forrester, P.I., Hancock, R.L., Hay, D.M., and Lorscheider, F.L. (1976) Onco-developmental Gene Expression (Fishman, W.H. and Sell, S., eds.), pp. 685-690, Academic Press, New York.
14. Bayard, B., and Kerckaert, J.P. (1977) Biochem. Biophys. Res. Commun. 77, 489-495.
15. Lester, E.P., Miller, J.B., and Yachnin, S. (1977) Proc. Natl. Acad. Sci. (USA) 74, 3988-3992.
16. Goldstein, I.J., Hollerman, C.E., and Merrick, J.M. (1965) Biochim. Biophys. Acta 97, 68-76.
17. Goldstein, I.J., Hollerman, C.E., and Smith, E.E. (1965) Biochemistry 4, 876-883.
18. Lai, P.C.W., Peters, E.H., and Lorscheider, F.L. Biochim. Biophys. Acta (in press).
19. Mancini, G., Carbonara, A.O., and Heremans, J.F. (1965) Immunochemistry 2, 235-254.
20. Ouchterlony, O. (1968) Handbook of Immunodiffusion and Immunoelectrophoresis, Ann Arbor Science Publishers, Ann Arbor.
21. Axelsen, N.H., Brock, E., and Kroll, J. (1973) Scand. J. Immunol. Suppl. 2, 91-94.
22. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
23. Lai, P.C.W., Forrester, P.I., Hancock, R.L., Hay, D.M., and Lorscheider, F.L. (1976) J. Reprod. Fert. 48, 1-8.
24. Jalanko, H., and Rouslahti, E. (1976) Protides of Biological Fluids (Peeters, H., ed.), pp. 303-306, Pergamon Press, Oxford.
25. Pineiro, A., Olivito, A.M., Perdices, A., and Uriel, J. (1976) Protides of Biological Fluids (Peeters, H., ed.), pp. 239-242, Pergamon Press, Oxford.
26. Kosaki, G., and Yamamoto, T. (1975) Ann. N.Y. Acad. Sci. 259, 366-376.