Acta Medica Okayama

Volume 29, *Issue* 1 1975 *Article* 4

FEBRUARY 1975

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Abstract

A purified and homogeneous preparation of rat alpha-fetoprotein (AFP) was separated into two components, AFPa and AFPb, by polyacrylamide gel electrophoresis. These two components had a definite difference in electrostatic net charge and gave only a single band on sodium dodecyl sulfate-electrophoresis. Neuraminidase-treated AFP gave clearly separable, slower moving four to six and finally two components depending on the time of incubation with neuraminidase. The time-dependent conversion of each AFPa and AFPb into slower migrating components upon neuraminidase treatment was confirmed by re-electrophoresis of separated and similarly treated AFPa and AFPb.

KEYWORDS: Microheterogeneity, alpha-fetoprotein

Acta Med. Okayama **29**, 29—32 (1975)

MICROHETEROGENEITY OF RAT ALPHA-FETOPROTEIN

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Received for publication, August 14, 1974

Abstract: A purified and homogeneous preparation of rat alpha-fetoprotein (AFP) was separated into two components, AFPa and AFPb, by polyacrylamide gel electrophoresis. These two components had a definite difference in electrostatic net charge and gave only a single band on sodium dodecyl sulfate-electrophoresis. Neuraminidase-treated AFP gave clearly separable, slower moving four to six and finally two components depending on the time of incubation with neuraminidase. The time-dependent conversion of each AFPa and AFPb into slower migrating components upon neuraminidase treatment was confirmed by re-electrophoresis of separated and similarly treated AFPa and AFPb.

During the course of biochemical studies of rat fetal and cancer AFP to investigate the mechanism of AFP production by liver injuries, we have recently found microheterogeneous forms of rat AFP on conventional polyacrylamide gel electrophoresis. In this communication, some physicochemical and immunological properties of microheterogeneous forms of AFP were examined in an attempt to elucidate the nature of the observed microheterogeneity.

MATERIALS AND METHODS

AFP of Sprague-Dawley rats was purified from a pooled fetal serum by using an immunoadsorbent column of Sepharose coupled with specific rabbit antiserum to rat AFP by means of cyanogen bromide as described previously (1, 2). For incubation of AFP with neuraminidase (type VI, purified from *Clostridium perfringens*) at 37°C up to 6 hr, 0.64 unit of the enzyme per mg AFP at a final enzyme concentration of 0.5 mg/ml was sufficient. For a longer incubation (12 hr), a further necessary amount (20 mU) of fresh enzyme was added after 6 hr of the treatment. Vertical disc electrophoresis on 5% polyacrylamide gel was performed at 4°C for 70 min with a constant current of 4 mA per tube (3). Sodium dodecyl sulfate-disc electrophoresis and isoelectric focusing in polyacylamide gel were performed according to the methods of WEBER and OSBORN (4) and WRIGLEY (5), respectively.

RESULTS

The purified AFP was confirmed to be homogeneous by both electrophoresis on Cellogel and immunoelectrophoresis. However, disc electrophoresis of the purified preparation revealed two components (Fig. 1). These components had relative mobilities against bromophenol blue (BPB) as a marker, R_{BPB}, of 0.88 and 0.85, the relative amounts being 35 and 65% of of the total protein, respectively. On re-electrophoresis of the individual components, which were separated and extracted from the gel, each produced a single band with its original mobility. The fast and slow migrating components are referred to as AFPa and AFPb.

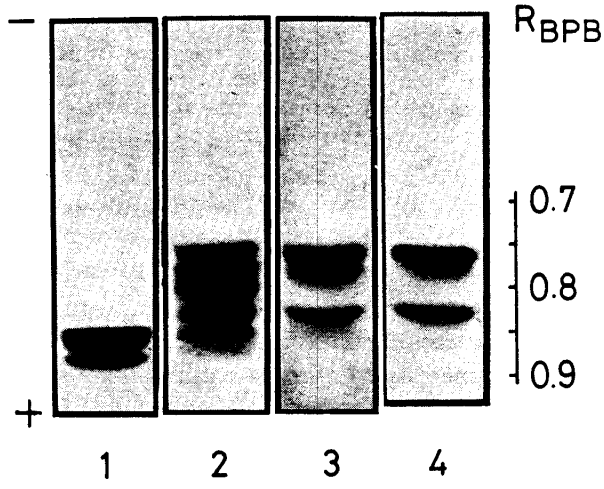

Fig. 1. Disc electrophoretic patterns of AFP treated for various periods of time with neuraminidase. 1, untreated AFP ; 2, 6 min ; 3, 6 hr ; and 4, 12 hr. Five percent polyacrylamide gels were used for electrophhoresis of each 20 μg of purified AFP. Neuraminidase ran off the gels after electrophoresis.

Plots on logarithmic scale of R_{BPB} values for two components of AFP against varying concentrations of acrylamide monomer gave nearly parallel straight lines for these components. The definite differences in R_{BPB} value among the two components present at the extraporated zero concentration of acrylamide monomer suggested that these two components of the fetoprotein had similar molecular sizes but differed from each other in net charge (6). This was further confirmed by the fact that sodium dodecyl sulfate-disc electrophoresis of the purified AFP containing two components gave only a single band corresponding to an approximate molecular weight of 74, 000.

Disc electrophoresis of the neuraminidase-treated AFP yielded four (R_{BPB} ; 0.85, 0.83, 0.78 and 0.76) to six (R_{BPB} ; 0.88, 0.85, 0.83, 0.80, 0.78

and 0.76) and finally two (R_{BPB} ; 0.83 and 0.76) bands depending on the time of incubation with neuraminidase (Fig. 1). The time-dependent conversion of faster into slower migrating components of both AFPa (R_{BPB} ; 0.88→0.85→0.83) and AFPb (R_{BPB} ; 0.85→0.80→0.78→0.76) upon neuraminidase treatment was confirmed by re-electrophoresis of separated and neuraminidase-treated AFPa and AFPb (Fig. 2). This confirmed that no interconversion occurred between the two components of AFP.

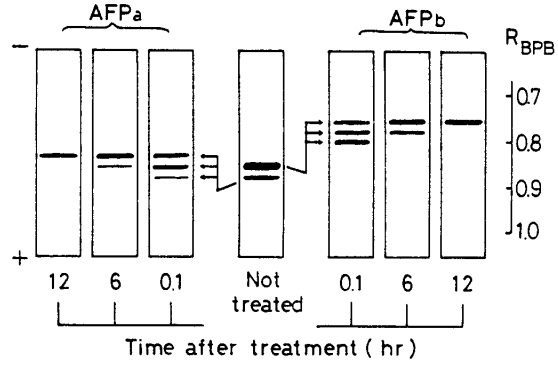

Fig. 2. Effect of neuraminidase treatment on disc electrophoretic mobilities of separated AFPa and AFPb. Forty μg of AFP were electrophoresed on 5% polyacrylamide gels. AFPa and AFPb discs were separately cut out from the gels according to each R_{BPB} value, minced and extracted at 37°C with mechanical stirring in a small volume of 50 mM Tris-HCl, pH 7.5. The extracted solutions from identical discs were pooled, concentrated by ultrafiltration through a collodion membrane and treated with neuraminidase for the identical periods of time. Re-electrophoresis of the treated AFPa and AFPb was performed similarly on 5% polyacrylamide gels. A schematic illustration of disc electrophoretic patterns is shown.

Isoelectric focusing of untreated AFP yielded two bands with a higher isoelectric point (measured pI, 4.82) for the less intense band, AFPa, and a lower (pI, 4.61) for the more intense band, AFPb. After removal of sialic acid residues from AFP by treatment with neuraminidase for 12 hr, the resulting desialized AFP still gave two bands with higher pI values (pI, 5.10 and 5.38 for treated AFPa and AFPb, respectively), the order of band positions being thus reversed upon desialization.

AFPa and AFPb treated with or without neuraminidase gave single fused precipitin lines against the anti-AFP serum in Ouchterlony double-diffusion analysis.

DISCUSSION

A possibility that the observed microheterogeneity of purified rat AFP arises from its partial denaturation or proteolysis during purification is un-

likely, because the native fetal serum also exhibited the corresponding two bands on disc electrophoresis with R_{BPB} values and relative amounts of each component identical to those of AFP purified from serum and also liver. Another possibility that the electrophoretic microheterogeneity might results from aggregation of AFP molecules could be eliminated by the fact that two components have similar molecular sizes.

The changes in electrophoretic mobility upon neuraminidase treatment, *i.e.* conversion of faster into slower migrating components of both AFPa (R_{BPB} 0.88→0.85→0.83) and AFPb (R_{BPB} 0.85→0.80→0.78→0.76), suggest a stepwise removal of sialic acid residues from each component. The removal of sialic acid from AFPa and AFPb rather revealed a more distinct separation of these fetoproteins, suggesting that structural features other than those involving sialic residues play an important role in determining these two components. Furthermore, two protein bands of AFP were still observed on electrofocusing even after desialization, although they had higher pI values and the order of their band positions was reversed upon desialization. These results suggest that additional negative charges other than the carboxyl groups of sialic acid are required for AFPa to move faster than AFPb at an alkaline pH on disc electrophoresis and are donated by a dissociable group(s) with a pK_a above the isoelectric points.

Loss of definite electrophoretic separation of AFP into components and of their immunological reactivity upon treatment with β-mercaptoethanol with or without employing a denaturing agent such as urea (unpublished observation) may indicate the importance of subtle differences of certain secondary or tertiary structures unaffected by urea treatment as a contributory factor to microheterogeneity of the fetoprotein.

REFERENCES

1. NISHI, S. and HIRAI, H.: Purification of human, dog and rabbit α-fetoprotein by immunoadsorbents of sepharose coupled with anti-human α-fetoprotein. *Biochim. Biophys. Acta* 278, 293-298, 1972
2. WATANABE, A., TAKETA, K. and KOSAKA, K.: Immunological properties of two fetus-specific globulins of rat in experimentally induced hepatic lesions. *Acta. Med. Okayama* 28, 321-332, 1974
3. DAVIS, B. J.: Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* 121, 404-427, 1964
4. WEBER, K. and OSBORN, M.: The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244, 4406-4412, 1969
5. WRIGLEY, C. W.: Analytical fractionation of plant and animal proteins by gel electrofocusing. *J. Chromat.* 36, 362-365, 1968
6. TAKETA, K. and WATANABE, A.: Interconvertible microheterogeneity of glucose 6-phosphate dehydrogenase in rat liver. *Biochim. Biophys. Acta* 235, 19-26, 1971