Isolation and microheterogeneity of rat α-fetoprotein

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Rat α-fetoprotein (AFP) was isolated from the fetus by combined techniques of Rivanol-ammonium sulfate precipitation, preparative electrophoresis and immune absorption and variant forms of AFP with a difference in molecular weight and isoelectric point were found to be present in the fetus.

The principle of the isolation of rat AFP was based on the technique of immune absorption against adulterant proteins using anti-rat serum protein antibody. The process of purification of the AFP is outlined as follows. In step 1, crude AFP was precipitated from saline extracts of whole tissues of fetus around 15th day of gestation by Rivanol-ammonium sulfate fractionation. In step 2, crude AFP was partially purified by preparative polyacrylamide gel disc electrophoresis. Following this partial purification, in step 3 the adulterant proteins of the partially purified preparation were eliminated by immune absorption using anti-rat serum protein, rabbit γ-globulin and the separation of purified AFP from surplus γ-globulin followed as step 4.

The agar gel immunoelectrophoretic pattern of the purified preparation after step 4 formed no precipitin line against either anti-rat serum protein

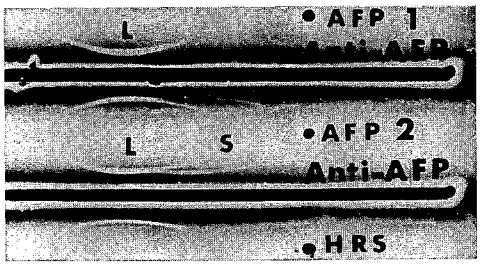

Fig. 1. Agar gel immunoelectrophoretic patterns of variants of AFP. AFP-1 is highly pure AFP fractionated at isoelectric point lower than 5.0 by isoelectric focusing. AFP-1 contains only AFP-L. AFP-2 is highly pure AFP (AFP-L and AFP-S) fractionated at a pI of more than 5.0. Anti-AFP is a monospecific AFP-antiserum obtained from rabbit immunized with AFP-L. HRS is the serum of rat with 3′-Me-DAB induced hepatoma.

rabbit antiserum or anti-rabbit serum protein goat antiserum, and partially fused precipitin lines located in the α_1 and α_2-zone against anti-rat AFP rabbit antiserum were seen. These partially fused precipitin lines differing in electrophoretic mobility were termed AFP-L and AFP-S, respectively as shown in Fig. 1. These characteristic findings are due to the presence of variant forms of rat AFP. Further the micro-Ouchterlony test for purified AFP revealed no adulterant proteins. Therefore, the prepared AFP-preparation was found to be highly pure. Further separation of these variant AFP in step 5 was carried out by isoelectric focusing. AFP-L alone was isolated from AFP-S. As shown in Fig. 1, isolated AFP-1 is observed. Monospecific AFP-antiserum was obtained from a rabbit immunized with the isolated AFP-L.

The mobility of AFP-L in the polyacrylamide gel disc electrophoretic pattern was slower than that of AFP-S. The discrepancy of electrophoretic mobility in agar gel and polyacrylamide gel was considered to be presumably due to the difference in molecular weight between AFP-L and AFP-S. The molecular weights of AFP-L and AFP-S were estimated by SDS polyacrylamide gel disc electrophoresis. AFP-L and AFP-S had molecular weights of 69,000 and 23,000, respectively as shown in Fig. 2. Moreover,

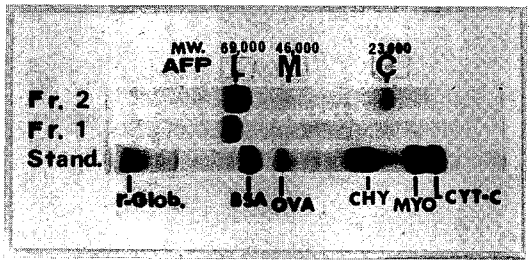

Fig. 2. SDS disc electrophoretic pattern.
Fr. 1: AFP fraction at pI less than 5.0 by isoelectric focusing.
Fr. 2: AFP fraction at pI more than 5.0 by isoelectric focusing.

another slight band of AFP with a molecular weight of 46,000 was observed. To ascertain the difference of molecular weight, separation was carried out by application on a Sephadex G-100 column and the variant AFP was labeled with ^{125}I. ^{125}I-AFP of the variant form was divided into ^{125}I-AFP-L and ^{125}I-AFP-S. Both ^{125}I-AFPs also reacted against antiserum obtained from rabbit immunized with AFP-L. The isoelectric points of AFP-L and AFP-S were 5.0 and 5.4, respectively. No cross reaction between human AFP and rat AFP against the opposite AFP-antiserum was recognized in agar gel immunodiffusion or radioimmunoassay.

From above results, microheterogeneity of rat AFP in difference of molecular weight and isoelectric point was clearly demonstrated. Two forms of rat AFP, with molecular weights of 69,000 or 46,000 may be presumably polymers of another AFP with molecular weight of 23,000.