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AFFINITY DIFFERENCES BETWEEN SOME COMMERCIALLY AVAILABLE IMMOBILIZED CON A WITH RAT ALPHA-FETOPROTEIN

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SUMMARY.- Rat alpha-fetoprotein contains three Con A-affinity molecular variants evidenced by affino-immuno-electrophoresis, (a) Con A-non reactive (52 %), (b) Con A-weakly reactive (31 %), (c) Con A-reactive (17 %). Affinity chromatography on several commercially available Con A-linked agarose displays different alpha-fetoprotein affinity patterns. The Con A-weakly reactive variant can be either bound and eluted with the Con A-reactive fraction or unbound and eluted with the Con A-non reactive one. These differences of chromatographic behaviour should be taken into account in structural, biochemical and biological studies dealing with the Con A-affinity molecular variants of alpha-fetoprotein or of other glycoproteins.

INTRODUCTION

Many of glycoproteins prove heterogeneity upon Con A-affinity chromatophy. As first shown by Smith and Kelleher (1) alpha-fetoprotein (AFP) presents this kind of heterogeneity, the molecular basis of which is not yet elucidated. However, recent findings on the changes of the Con A-reactivity of human AFP during fetal development (2) and in pathological pregnancies (3) give rise to a new clinical and biochemical interest on AFP-Con A interaction.

In our experimental conditions, evidences were obtained that rat AFP contains a third molecular variant which is Con A-weakly reactive (7) in addition to the previously described (1, 4-6) Con A-reactive and Con A-non reactive forms. The non-artefactual nature of this Con A-weakly reactive variant was demonstrated using an affino-immuno-electrophoresis technique (8).

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Here we show that the affinity chromatography behaviour of rat AFP and in particular, the separation or not of the Con A-weakly reactive form depend on the commercial origin of the Con A-linked material used.

MATERIALS AND METHODS

Rat AFP was purified from rat amniotic fluid (14-18 days gestation) by immunoadsorption on anti AFP-Sepharose (9).

Immobilized Con A. Five different kinds of immobilized Con A were used. One was our own Con A-Sepharose preparation obtained by coupling Con A from PHARMACIA on Sepharose 4B as described previously (7). The others were purchased from PHARMACIA (Con A-Sepharose), MILES (Glycosylex), SIGMA (Con A-Sepharose), Industrie Biologique Française (Con A-Ultrogel).

Con A-affinity chromatography. The same conditions were used in all column experiments. The Con A columns (1 x 10 cm) were equilibrated with 0.05 M Tris-HCl buffer (pH 7.6) containing 1 M NaCl, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$. The purified AFP samples (1.5 mg in 0.5 ml column buffer) were passed through the columns at a 12 ml/h flow rate. About twenty fractions of 1.6 ml were collected then the Con A-reactive AFP was eluted with 0.1 M O-methyl-α-D-glucose in the same buffer. The AFP elution profile was obtained by rocket immuno-electrophoresis of each fraction on anti AFP impregnated agarose plates (7).

Crossed Affino-Immuno-Electrophoresis. The different Con A-affinity AFP fractions were analysed by Crossed Affino-Immuno-Electrophoresis with free Con A in the first dimension gel as described previously (8). Con A used in these experiments were indifferently from PHARMACIA or Industrie Biologique Française, both gave exactly the same affinity patterns.

RESULTS

Using an home-made Con A-Sepharose i.e. powdered Con A (PHARMACIA) coupled with Sepharose 4B activated by the CNBr procedure, we have shown previously (7) that rat AFP can be divided into three fractions, (a) Con A-non reactive ; (b) Con A-weakly reactive ; (c) Con A-reactive. Fractions a and b are eluted with the equilibration column buffer and c is specifically desorbed by addition of the glucoside. As shown in figure 1, the same Con A-affinity pattern of rat AFP is recovered by affino-immuno-electrophoresis using free Con A in the first dimension gel. With both electrophoretic or chromatographic techniques, the percentages of each AFP variant are approximately : a, 52 % ; b, 31 % and c, 17 %.

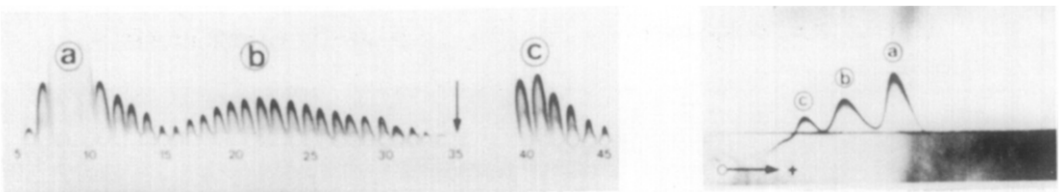

Figure 1.- Separation of the three Con A-affinity molecular variants of rat AFP by affinity chromatography and affino-immuno-electrophoresis.
a : Con A-non reactive ; b : Con A-weakly reactive ; c : Con A-reactive AFP.

It is noteworthy that the affino-electrophoresis derived values do not depend on the commercial origin of the Con A, whereas important variations can be observed in the chromatographic results as it is shown in figure 2. Using identical experimental conditions we have compared the elution profile of rat AFP chromatographied on four different immobilized Con A commercially available and we have characterized each chromatographic fraction by affinity electrophoresis. Two kinds of patterns can be directly distinguished : those obtained with Con A-Sepharose from PHARMACIA and SIGMA (Fig. 2, patterns A and B) which retain 50 % of rat AFP and those obtained with IBF and MILES, Con A-linked materials (Fig. 2, patterns C and D) which bind a much lower percentage (17 %) of rat AFP. These differences of binding capacity are related to the behaviour of the Con A-weakly reactive AFP variant on the Con A-columns as it can be observed by the affino-electrophoresis analyses. Indeed, with the two high affinity columns (A and B) the weakly-reactive fraction (b) is retained and eluted with the reactive fraction (c). In the opposite, with the low affinity columns (C and D) the weakly-Con A reactive AFP variant is eluted with the equilibration column buffer so that the bound AFP only consists of the Con A-reactive variant.

In this case, the elution profile of the unbound fraction is more trailing than with the high affinity Con A-columns. A sequential affino-electrophoretic analysis of the eluted fractions from a low affinity column shows (Fig. 3) that the trailing material corresponds to the elution of the Con A-weakly reactive AFP. Only the first column fractions contains pure Con A-non reactive AFP and then, there is a progressive enrichment in the Con A-weakly reactive AFP.

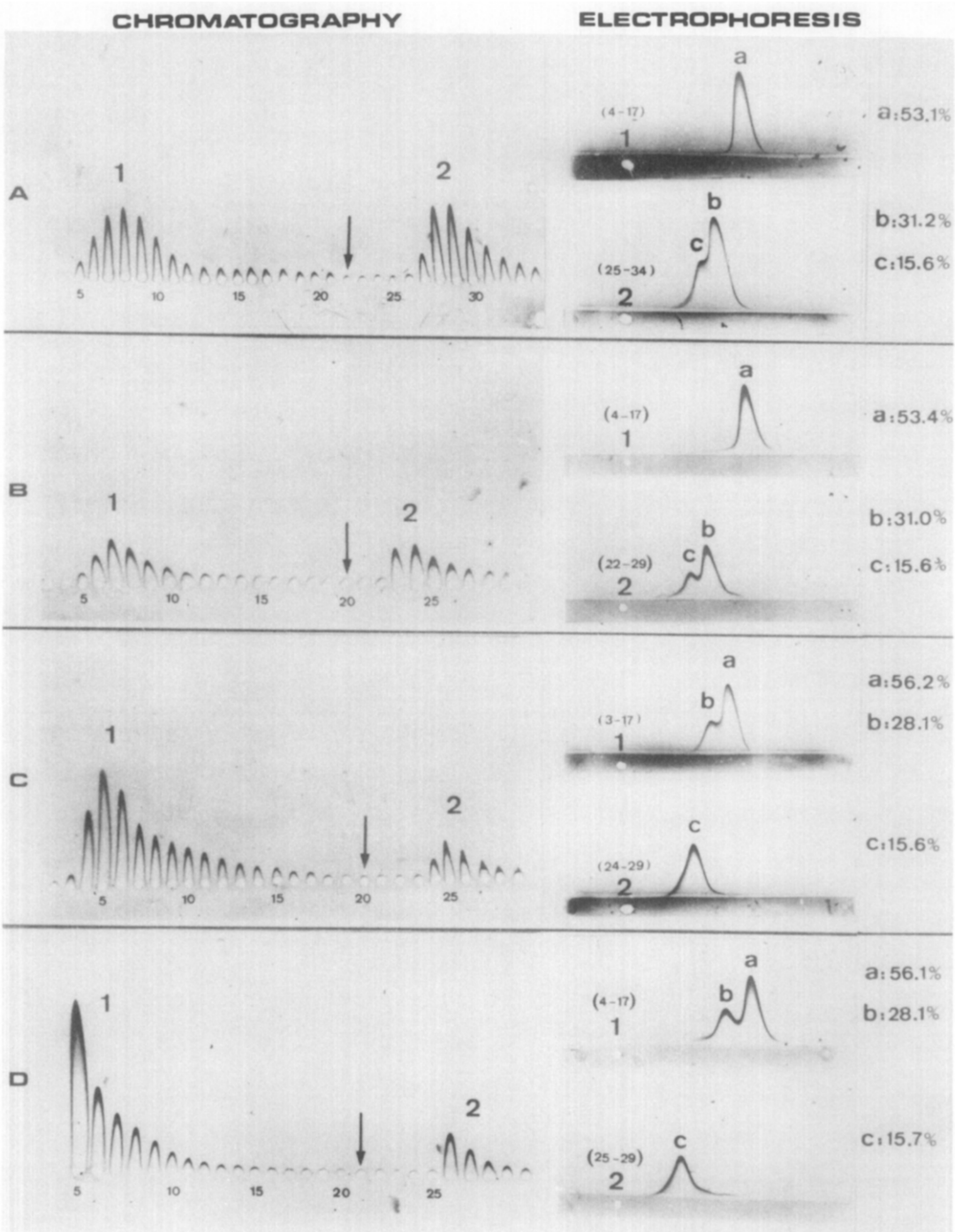

Figure 2.- Con A-affinity pattern of rat AFP with different commercial immobilized Con A column (left) and affino-immuno-electrophoretic analysis of pooled unretained (1) and retained (2) column fractions (right).

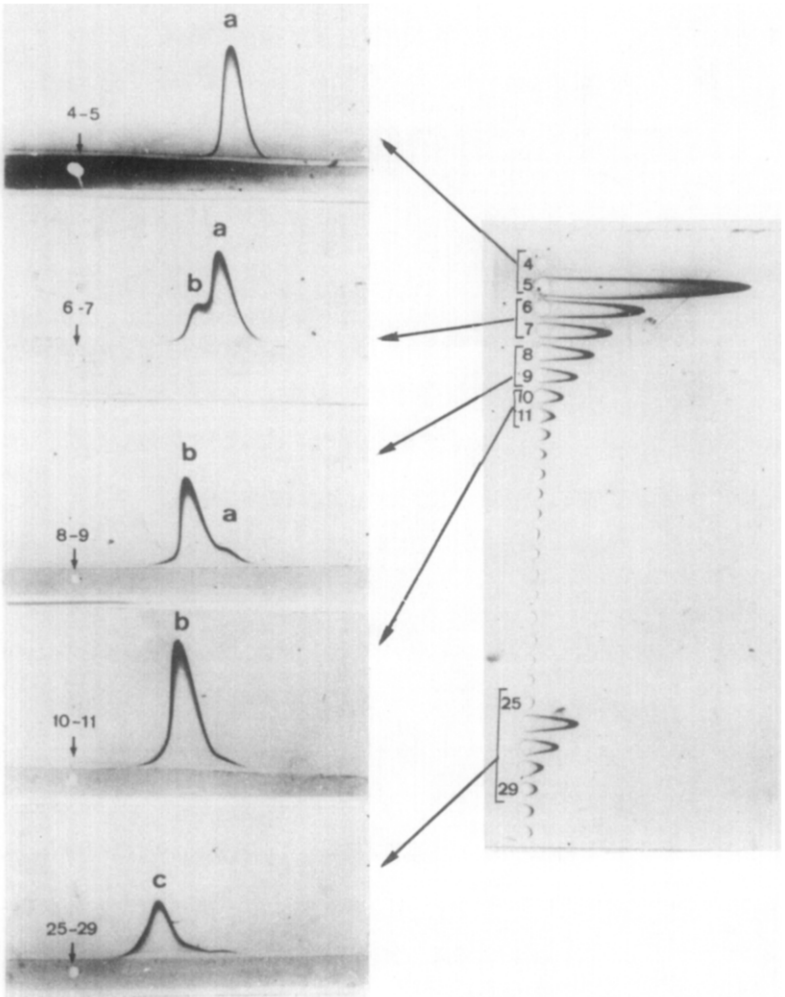

Figure 3.- Right : Elution pattern of rat AFP on Con A-Sepharose (glycosylex from MILES). The first peak (fractions 4-11) was eluted with 0.05 M Tris-HCl (pH 7.6) 1 M NaCl/1 mM $MgCl_2$/1 mM $MnCl_2$/1 mM $CaCl_2$. Addition of 0.1 M methyl-α-D-glucose resulted in the elution of the Con A-reactive fraction c. Left : Affino-immuno-electrophoresis sequential analysis of the chromatographic fractions eluted from the glycosylex column.

DISCUSSION

Like many other glycoproteins, rat AFP was known to contain two molecular variants, bound and unbound on Con A-columns (1, 4-6). Our previous studies indicated that a third molecular population, Con A-weakly reactive,

A : Con A-Sepharose from PHARMACIA ; B : Con A-Sepharose from SIGMA ; C : Con A Ultrogel from IBF and D : glycosylex from MILES. The percentages of the affino-electrophoretic AFP fractions have been determined by measuring peak height. a, b and c correspond respectively to the Con A-non reactive, Con A-weakly reactive and Con A-reactive AFP.

exists in rat AFP as evidenced by affinity chromatography (7) and more reliably by affinity electrophoresis (8). From the present work it appears that the Con A-weakly reactive variant which accounts for 30 % of the whole AFP, can be recovered either in the unbound or in the bound column fractions, depending on the commercial origin of the immobilized Con A. Thus, when the Con A-weakly reactive AFP is eluted with the Con A-reactive fraction (Pharmacia and Sigma Con A-Sepharose columns), a higher binding capacity (50 % AFP binding) is obtained as compared to the Con A-columns from other manufacturers (IBF and MILES), which bind only the Con A-reactive AFP (17 %). Consequently, this difference of chromatographic behaviour should be taken in terms of Con A-affinity rather than Con A-binding capacity.

The reasons why some Con A-linked materials can retain more tightly the Con A-weakly reactive AFP remain to be determined. Hydrophobic interactions of the lectin itself (references in 10) as well as non specific interactions of the gel matrix could be invoked and investigated. However, the constant occurence of the Con A-weakly reactive AFP variant in affino-immuno-electrophoresis makes gel matrix effects unlikely. In the electrophoretic affinity technique, the separation only depends on the presence of the free Con A (8) whereve this Con A is purchased (unpublished observations).

In conclusion, the dependence of the Con A-affinity patterns of rat AFP with the commercial origin of the immobilized Con A, should be taken into account in structural or biochemical studies of the molecular variants not only of AFP but probably also of others microheterogeneous glycoproteins.

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