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MICROHETEROGENEITY OF HUMAN GALACTOSE-1-PHOSPHATE URIDYLYL TRANSFERASE. ISOELECTROFOCUSING RESULTS

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SUMMARY

Using thin-layer acrylamide gel isoelectrofocusing, several bands of galactose-1-Phosphate uridylyl transferase were found in various human tissues. Liver transferase, as well as that of some other tissues, was resolved into several bands with pHi between 5.30 and 5.80 ; red cell enzyme was resolved into five bands with pHi between 5.0 and 5.45. The comparison of erythrocytes with their precursors, reticulocytes and erythroblasts, showed a striking difference : the pHi of the erythroblast enzyme was between 5.55 and 5.90 and that of reticulocytes between 5.30 and 5.50. It is possible that molecular aging is the cause of the anodisation of the erythrocyte transferase and the microheterogeneity of the enzyme observed in other tissues.

INTRODUCTION

Galactose-1-phosphate uridylyl transferase (EC 2.7.7.12) ("transferase") catalyses the reaction : Galactose-1-phosphate + UDP Glucose $\rightleftarrows$ Glucose-1-Phosphate + UDP Galactose. Kalckar (1) has shown that "galactosemia", a genetic disease with autosomal recessive transmittance, is due to the absence of transferase activity in liver. This enzyme is also present in red blood cells where its deficiency may be easily detected. The work of Tedesco and Mellman (2) on the existence of a "Cross Reacting Material" in erythrocytes of patients with galactosemia, and that of Nadler et al. (3) on the appearance of transferase activity after hybridization of fibroblasts from different patients, have given evidence for structural gene mutations as a cause of the disease.

The tissue distribution of transferase has been studied by kinetic and electrophoretic methods (4). The enzyme is present, not only in liver and in erythrocytes, but also in leukocytes, in cultured fibroblasts (5) and amnio-

Abbreviation : Gal 1 P, Galactose-1-Phosphate.

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tic cells (6), in intestinal mucosa (7), in heart and in kidney (4). Some of its developmental aspects were also studied in human and rat tissues (8, 9, 10).

The exact structure of the enzyme is not known with certainty. Ng et al. (11) were able to resolve transferase from Duarte variant individuals into three bands, suggesting that the enzyme was made up of subunits. Tedesco (12), by purifying transferase from human liver and erythrocytes, suggested that the enzyme possesses a trimeric or a tetrameric structure ; but, more recently, Dale and Popjak (13) suceeded in extensively purifying human erythrocyte enzyme, and concluded that transferase would be a dimer. Connellan and Buchanan (4) studying several human tissues showed that after prolonged migration on starch gel, liver transferase was resolved into a single band, heart transferase into two bands and kidney transferase into five bands. These authors concluded that a dimeric form of enzyme may explain the electrophoretic pattern of liver and heart transferases ; but an additional locus would be necessary to explain the pattern observed with kidney extracts.

Consequently, many obscurities persist, and it seemed of interest to apply to this problem the very resolutive method of isoelectrofocusing on thin-layer acrylamide. Moreover, this technique has enabled the microheterogeneity of several presumably pure enzymes to be demonstrated (14). Several cases of modifications of charge as a consequence of molecular aging have been described (15 - 18). Consequently, we have searched for such a modification of transferase in red cells where no biosynthesis occurs. We have compared the electrofocusing pattern of mature red cells with that of their precursors, reticulocytes and erythroblasts, and with other tissues.

MATERIALS AND METHODS

Preparation of blood cells and extracts.

Leukocytes were prepared from normal blood according to Kampine et al. (19). Circulating erythroblasts from patients were collected as described in ref. (20). Red blood cells were washed three times and then lysed in water. They were stored some days at -75°C. Fibroblasts were obtained at confluence from human embryonic skin. Liver biopsies were taken during various surgical

interventions ; heart, kidney and other human tissues were obtained from autopsies performed a few hours after death. Samples were stored at -75°C. Tissues were extracted in 1 to 20 volumes of water, the volume of which was calculated in order to obtain about the same final activity.

Electrophoretic methods.

Electrophoresis on starch gel and the detection of transferase were performed according to Mathai and Beutler (21) with some modifications previously described (22).

Isoelectrofocusing on acrylamide gel was performed using Vesterberg's method with modifications. The LKB Multiphor system was used ; the cathodic tank was filled with 1 M NaOH and the anodic tank with 0.25 M H_3PO_4. The gel contained a final concentration of 2.5% of ampholines of various pH (pH 4.0-6.0 and pH 6.0-8.0). After migration at 4°C to a final potential of 500 volts, the gel was covered by an agar layer containing a specific staining mixture. After a variable period of time, fluorescent bands of NADPH corresponding to the enzyme activities were seen. They were then photographied under long-wave ultraviolet light. In some other experiments phenazine-methosulfate and nitroblue tetrazolium were added to the reagent mixture : tetrazolium salts were reduced into blue-violet formazan which precipitated. It should be noted that this coloration method is less sensitive but more precise than fluorescence. Blanks were prepared by omitting substrates (UDP Glu or Gal 1 P) in the reaction mixture.

RESULTS

Using electrophoresis on starch gel we found several bands of liver, heart and kidney transferases. As pointed out by Connellan and Buchanan (4), fluorescent spots often appear even in the absence of the substrates Gal-1-P or UDPG. We found that these false reactions occured not only in heart and muscle, but also sometimes in liver and in kidney. For this reason we decided to only perform experiments using the isoelectrofocusing technique.

Fig. 1 shows the electrofocusing pattern of transferase from various tissues stained by the reduction of nitro-blue-tetrazolium. No bands appeared when the reaction was performed without UDPG or Gal-1-P. With substrates one fact is striking. Liver transferase is resolved into about 6 bands with pHi 5.30 to 5.80. The resolution of the most anodic bands is poor. In contrast, only two or three erythrocyte transferase bands are visible. Their pHi (between 5.30 and 5.45) is the same as the most anodic liver bands. Reticulocytes display an intermediary pattern with a pHi between 5.30 and 5.50. Leukocyte transferase is resolved into at least three bands corresponding to three bands of liver enzyme, with pHi between 5.50 and 5.60.

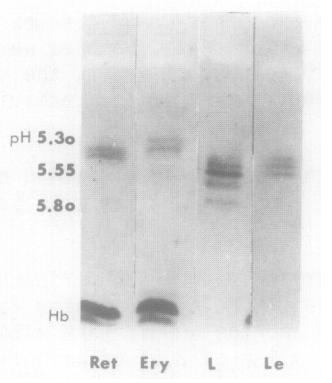
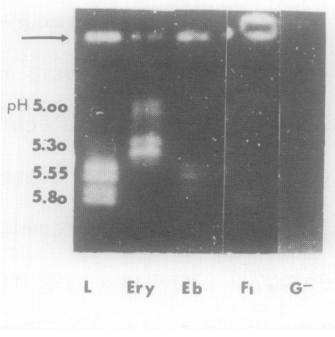


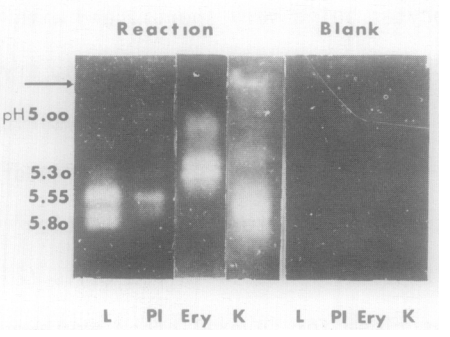
Fig. 1 : Electrofocusing of transferase from human tissues, stained by the reduction of nitro-blue-tetrazolium.
Supporting medium : acrylamide 4% with ampholines in pH range 4.0-8.0. Ret, reticulocytes - Ery, erythrocytes - L, liver - Le, leukocytes.

Fig. 2 gives the electrofocusing pattern of erythroblast transferase detected by fluorescence of the NADPH formed : its activity was too slight to be detected by the nitro-blue-tetrazolium technique, due to the small amount of available material. Its pHi was between 5.45 and 5.80. This is more basic than either the erythrocyte or the reticulocyte enzymes. Red cell transferase is resolved by fluorescence into three bands (between pHi 5.30 and 5.45) and two additional anodic bands become visible between 5.0 and 5.15 (fig. 2 and fig.3). We have submitted several hemolysates of known galactosemic patients to isoelectrofocusing and have not been able to detect enzyme activity. Liver extract transferase is resolved into at least six bands between pH 5.30 and 5.80. The very weak bands of fibroblast enzyme appeared at pH between 5.50 and 5.80.

Fig. 3 gives the pattern of transferase from different tissues, detected also by fluorescence. The kidney transferase pattern appears similar to that of the liver enzyme with several bands between pH 5.30 and 5.80, but with a different relative intensity. We were able to study placenta transferase for the first time. Its pHi appears to be comparable to that of liver, with several bands of weaker intensity. Blanks without substrates showed a very



(2)



(3)

Fig. 2 : Comparison between the transferase patterns of erythroblasts and erythrocytes from normal patients, and of hemolysates from normal and galactosemic patients.
L, liver - Ery, erythrocytes - Eb, erythroblasts - Fi, fibroblasts - G^-, galactosemic hemolysate.

Fig. 3 : Electrofocusing of transferase from human tissues, detected by fluorescence of the NADPH formed ; with and without substrates.
L, liver - Pl, placenta - Ery, erythrocytes - K, kidney.

weak band corresponding to pH 5.0.

DISCUSSION

By using the thin layer isoelectrofocusing technique we have shown that transferase of all tissues tested may be resolved into several bands. The results of Dale and Popjak (13) are in favor of a dimeric structure of transferase, but the presence of multiple bands cannot be explained only by a different charge of two subunits. Another hypothesis seems more likely : a microheterogeneity due to a post-synthetic and progressive modification of the charge of some subunits, as a consequence of their aging. Our results with erythrocytes are in favor of this hypothesis : the pHi of red cell transferase is much more anodic than that of other tissues, and corresponds closely to that of the most anodic liver bands. It is known from the studies on galactosemia, that liver and erythrocyte transferase are coded by the same gene. But it cannot be excluded that they have only one subunit in common.It is especially interesting to compare the pHi of the enzyme of red cells (whose life-span is 120 days) with that of the enzyme of white cells (the life-span of

granulocytes being very short), and with that of the transferase of precursors of red cells - reticulocytes and erythroblasts - (where biosynthesis is active). If the "anodisation" of erythrocyte transferase was due to molecular aging, it could be compared to the "anodisation" of some chains of aldolase A in muscle (15), and to the "anodisation" of glucose-6-phosphate dehydrogenase and of aldolase A itself in red cells (23, 24). Analogous phenomena have also been described for several other erythrocyte and lens enzymes (17, 25). The exact mechanism is known only for aldolase A : deamidation in vivo of an asparagine residue near the C-terminal (26). In any case, it is probable that these modifications have some physiopathological consequences.

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