THE UNIQUE IDENTITY OF RAT HEPATOMA PHENYLALANINE HYDROXYLASE

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SUMMARY: Rat hepatoma H4-II-E-C3 culture phenylalanine hydroxylase is unique and differs from any of the three isozymes of liver or the one of kidney. Isoelectric focusing results in a single isozyme with an isoelectric point of 5.20 compared to 5.20, 5.30, 5.60 for the liver and 5.35 for the kidney. Hepatoma phenylalanine hydroxylase cross reacts with antibody #2 of rat liver but differs from isozyme #2 by a tenfold difference in the ratio of antigen to antibody at equivalence on immunotitration. Kidney phenylalanine hydroxylase which also cross reacts with antibody #2 of liver can quantitatively (at 2 mU per mg immunoglobulin) absorb antibody against hepatoma enzyme, liver isozyme #2 and itself without having any effect on antibody #1 and #3 of liver.

INTRODUCTION

Phenylalanine hydroxylase [L-phenylalanine tetrahydropteridine O_2 oxidoreductase (4-hydroxylating) EC 1.14.3.1] activity in mammalian tissues is limited to liver, kidney and pancreas (1). Immunologic data from immunotitration, immunodiffuion and immunoabsorption argue for the existence of three unique and non-cross reacting isozymes of liver and one of kidney which cross reacts with antibody #2 of liver (2). Phenylalanine hydroxylase activity is not expressed in tissue culture except in minimal deviation hepatoma cell lines H4-II-E-C3 and MH_4 (3). Recent studies on the expression of H4-II-E-C3 culture phenylalanine hydroxylase and its nutritional and hormonal regulation have been used as a model for the study of the genetic and epigenetic control mechanisms of mammalian phenylalanine hydroxylase synthesis (4-7). Thus it becomes important to determine the identity and number of H4-II-E-C3 culture isozymes with respect to those of rat liver. Isoelectric focusing and immunologic properties of H4-II-E-C3 culture phenylalanine hydroxylase point to a single isozyme which differs from any of the three liver and the kidney isozymes.

METHOD AND MATERIALS

The preparation of purified rat liver phenylalanine hydroxylase and antibody production followed by the purification of immunoglobulin was done as described (2). Immunotitration of hepatoma phenylalanine hydroxylase in addition to immunoabsorption of the immunoglobulin with kidney phenylalanine hydroxylase were done as described (2).

H4-II-E-C3 cells were cultured, harvested and processed and phenylalanine hydroxylase activity determined as described (7).

Isoelectric focusing was done by preparing 6% acrylamide gels with 1% each of Ampholine carriers (LKB, Bromma, Sweden) pH 5-8 and pH 3.5-10 and 0.15% riboflavin. The gels were run at 1mA/tube for 1 hour and 50 minutes. A dialysed and concentrated sample of partially purified extract that contained all of the initial extract activity (8), or samples from the purification steps of phenylalanine hydroxylase from rat liver by the method of Kaufman and Fisher (9), were used. The pH gradient and phenylalanine hydroxylase activity were determined directly on 3 mm gel slices.

One mU of enzyme activity is defined as 1 nanomole tyrosine formed per minute in 0.1 M K phosphate buffer pH 7.2 at 25°C.

Chemicals, media and supplies for cell culture were obtained from the same sources as cited previously (2, 7).

RESULTS

Isoelectric focusing of phenylalanine hydroxylase. Isoelectric focusing of H4-II-E-C3 hepatoma, liver and kidney results in a single peak with a pI of 5.20 for H4-II-E-C3 and of 5.20, 5.30 and 5.60 for rat liver and 5.35 for kidney (Fig. 1A). Purification of rat liver phenylalanine hydroxylase by the method of Kaufman and Fisher (9) resulted in no changes in the isoelectric points of the progressively purified fractions (Fig. 1B).

Immunotitration of H4-II-E-C3 culture phenylalanine hydroxylase with immunoglobulin. Immunotitration of H4-II-E-C3 culture phenylalanine hydroxylase results in a single slope. The extrapolation of this slope to the abscissa results in an equivalence value of 2 mU (of enzyme activity) per mg of immunoglobulin. All of the antibody against H4-II-E-C3, kidney and isozyme #2 of liver phyenylalanine hydroxylase can be quantitatively absorbed with kidney. Kidney phenylalanine hydroxylase quantitatively cross reacts with antibody #2 of liver (2) when it is incubated at a ratio of 2 mU/mg immunoglobulin (Fig. 2). It has been previously shown that the immunoglobulin that remains after kidney immunoabsorption maintains all of its antibody activity towards

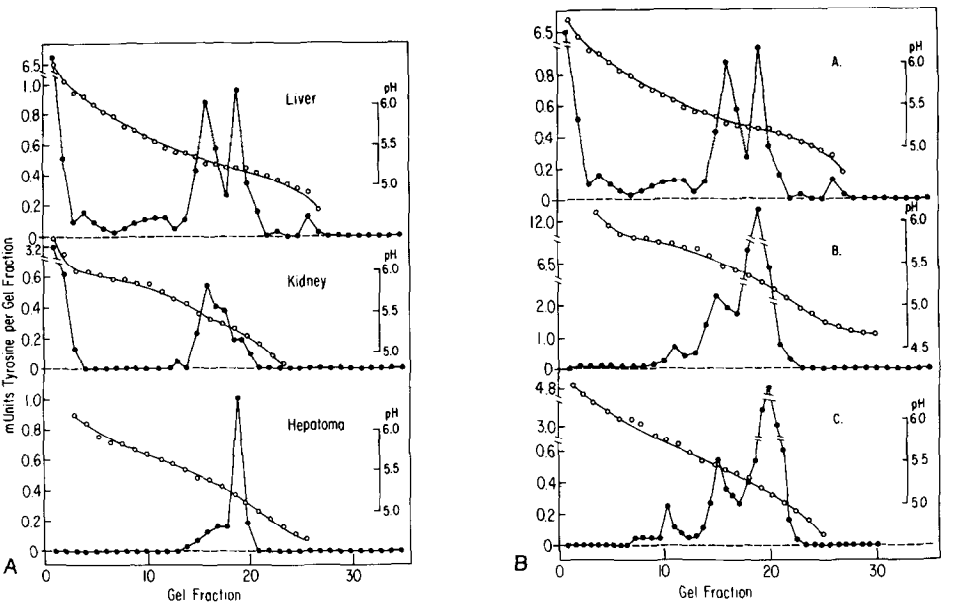

Figure 1.

Isoelectric focusing of tissue extracts and progressively purified fractions of phenylalanine hydroxylase.

A. 1.1, 0.27 and 0.1 mU of phenylalanine hydroxylase (dialised ammonium sulfate fraction (8)) from liver, kidney and hepatoma respectively were applied to the gels and run at 1 mA per tube for 1 hour and 50 minutes.

B. 1.1 mU of phenylalanine hydroxylase (ammonium sulfate fraction) by the method of Gillam et al. (8), 1.3 mU of phenylalanine hydroxylase (ammonium sulfate fraction) by the method of Kaufman and Fisher (9) and 0.8 mU of Sephadex C-200 purified by the same method were applied to acrylamide gels and treated as above.

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antigen #1 and #3 of liver phenylalanine hydroxylase (2).

Double immunodiffusion of H4-II-E-C3 culture phenylalanine hydroxylase.

Double immunodiffusion of H4-II-E-C3 culture phenylalanine hydroxylase at a ratio of 2 mU/mg immunoglobulin, and a ten-fold variation of either the antigen or the antibody by increasing or decreasing this titre, resulted in no observable immunoprecipitin line (not shown).

DISCUSSION

H4-II-E-C3 hepatoma phenylalanine hydroxylase cross reacts with anti-

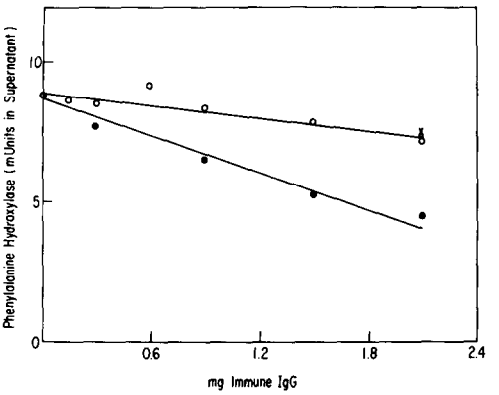

Figure 2.

Immunotitration of H4-II-E-C3 hepatoma phenylalanine hydroxylase with rat liver phenylalanine hydroxylase antibody and kidney absorbed immunoglobulin.

Nine mU of hepatoma phenylalanine hydroxylase was added to increasing amounts of immunoglobulin ●——● or to kidney absorbed immunoglobulin O——O or to control non-immune globulin X in a final 150 µl volume. Control non-immune globulin was added to equalize the immunoglobulin of each incubation which also contained 0.1M K phosphate pH 6.8. After 3 hours at 0°-4°C, the precipitated antigen-antibody complex was centrifuged at 13,400x g for 20 minutes and 50 µl of the supernatant was assayed in duplicate for phenylalanine hydroxylase activity.

body to isozyme # 2 of liver phenylalanine hydroxylase. However it differs from antigen # 2 of liver and from kidney isozyme, which also cross reacts with the same liver antibody, in a number of important characteristics. The equivalence ratios of antigen # 2 of liver and kidney are 21 mU and 2 mU respectively compared to 2 mU of enzyme activity per mg of immunoglobulin for H4-II-E-C3 culture phenylalanine hydroxylase. Liver antigen # 2 and kidney give very strong immunoprecipitin lines at the above equivalence ratios (2). H4-II-E-C3 culture phenylalanine hydroxylase does not result in any observable immunoprecipitin line at equivalence or at any other antigen or antibody ratio tested. Immunoglobulin absorption by kidney removes all of the antibody activity against liver antigen # 2, kidney and H4-II-E-C3 culture phenylalanine hydroxylase but none against antigen # 1

(61 mU/mg immunoglobulin) and antigen #3 (2 mU/mg immunoglobulin) (2). And lastly, kidney and hepatoma isoelectric points are different, pI 5.35 and 5.20 respectively. Taken together these results argue for a unique isozyme of H4-II-E-C3 hepatoma phenylalanine hydroxylase, different from any of the three isozymes of rat liver or the one of kidney.

The identity of H4-II-E-C3 culture phenylalanine hydroxylase will have to be taken into consideration in the interpretation of data relating to the regulation of phenylalanine hydroxylase synthesis and the extrapolation of this data to mammalian liver.

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REFERENCES

1. Tourian, A., Goddard, J., and Puck, T.T. (1969) J. Cell. Physiol. 73, 159-170.
2. Tourian, A., Treiman, L., and Abe, K. (1975) Bioch. 14, 4055-4059.
3. Haggerty, D.F., Young, P.L., Popjack, G. and Carnes, W.H. (1973) J. Biol. Chem. 248, 223-232.
4. Haggerty, D.F., Young, P.L., and Buese, J.V. (1974) Develop. Biol. 40, 16-23.
5. McClure, D., Miller, M., and Shirnau, R. (1975) Exp. Cell Res. 90, 31-39.
6. Haggerty, D.F., Young, P.L., and Buese, J.V. (1975) Develop. Biol. 44, 158-168.
7. Tourian, A. (1975) J. Cell. Physiol. (in press).
8. Gillam, S.S., Woo, S.L.C., and Woolf, L.I. (1974) Bioch, J. 139, 731-739.
9. Kaufman, S., and Fisher, D.B. (1970) J. Biol. Chem. 245, 4745-4750.