SYNTHESIS AND DEGRADATION OF THE MAJOR HEPATIC PHOSPHOFRUCTOKINASE ISOZYME IN RAT LIVER AND HEPATOMA 3924-A[1]

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SUMMARY. The rates of synthesis were determined for the major phosphofructokinase isozyme in rat liver and the fast growing, poorly differentiated hepatoma 3924-A. Its rate of synthesis, which was measured as rate of incorporation of leucine, was approximately 2.3-fold greater in the hepatoma compared to the normal liver. Using the double labeling technique, which has been demonstrated to estimate degradation rate constants, Glass and Doyle, J. Biol. Chem., 247, 5234 (1972), the $^{3}H/^{14}C$ ratios for this isozyme in the liver and hepatoma were nearly the same, which implied that the degradative process for this isozyme is similar for both tissues. These results indicate for this isozyme that it has attained a steady state level in the hepatoma 3924-A different from the liver, which is described by a 2-3 fold increase in its rate of synthesis and degradation.

Earlier work has demonstrated the presence of phosphofructokinase (E. C. 2.7.1.11) isozymes in normal rat liver (1). The major hepatic phosphofructokinase isozyme (L_2) was purified to homogeneity and its kinetic, physical, and biological properties were examined (1,2). Activity measurements and immunotitration of supernatant fluids and purified L_2 demonstrated that it was elevated 2- to 3-fold in hepatoma 3924-A (3). This increased amount of L_2 in hepatoma 3924-A is representive of a different steady state level which was attained and perhaps maintained as a consequence of a change which affected its rate of synthesis and/or degradation. The contribution of synthesis and degradation in controlling enzyme levels has been previously discussed (4,5).

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METHODS

Rats were maintained as previously described (2,3). Livers and tumors were excised rapidly, homogenized in phosphate buffered saline containing 15 mM L-leucine, and centrifuged as described (2). The antiserum specific for L_2 was taken from rabbits immunized as previously described (1). The incorporation of L-[4,5-^{3}H] leucine (specific activity 30.8 Ci/mM, New England Nuclear, Boston, Mass.) into L_2 and supernate from liver and tumor was performed using previously described methods (2). The methods used for the double labeling experiment have also been described for the liver (2) and these techniques were also used in the hepatoma experiments.

RESULTS AND DISCUSSION

The incorporation of leucine into L_2 and 100,000 x g supernatant protein is shown in Table I. Considering the total supernatant radioactivity, it is apparent that the amounts of labeled leucine maintained in hepatoma 3924-A and liver are statistically identical. Since it has been demonstrated that there is a decreased blood flow in transplanted hepatomas relative to normal liver (6), it is likely that this hepatoma has a greater concentrating capacity than normal liver. Baril and Potter have shown for several hepatomas a diversity of amino acid concentrating abilities (7).

Considering the CCl_3COOH acid soluble radioactivity, it is evident that similar levels of labeled leucine are present in the liver and hepatoma 3924-A; however, this does not indicate the actual leucine pool sizes or the concentration of labeled leucine relative to total leucine pool. Since this hepatoma appears to concentrate leucine, it seems unlikely that its leucine pool size is less than in the liver. If leucine pool sizes are nearly the same or greater in hepatoma 3924-A, the dilution of the labeled leucine into the total leucine pool would be the same or greater. Thus, it is highly probable that L_2 synthesis is elevated at least 2-fold in the hepatoma when leucine pool sizes are the same. If leucine pool sizes are larger in the hepatoma then one would expect more dilution of labeled leucine in the hepatoma, and, consequently, the L_2 synthetic rate in hepatoma 3924-A would be even greater relative to liver.

It has been demonstrated that the ratio of initial leucine incorporation (^{3}H leu) relative to leucine incorporation (^{14}C) after a set period of time (3 days in this case) can be used to estimate degradation rate constants (8). These $^3H/^{14}C$ ratios for L_2 and supernatant protein in liver and hepatoma

Table I

Incorporation of L-Leucine into L_2 and Supernatant Protein of Liver and Hepatoma 3924-A[a]

Tissue	Total supernatant radioactivity (DPM/mg protein)	Supernatant protein incorporation (DPM/mg protein)	Soluble radioactivity (DPM/mg protein)	L_2 incorporation (DPM/mg protein)
Liver (6)	7168 ± 811	2443 ± 264	4724 ± 480	9.8 ± 1.0
Hepatoma 3924-A (5)	6423 ± 782	1558 ± 192	4865 ± 498	21.7 ± 2.3

[a]Means ± standard deviations are given, and the number in parenthesis indicates the number of rats used. The units are reported in DPM per mg of supernatant protein. Normal and tumor bearing rats were injected (i.p.) with 75 μCi of [4,5-^{3}H] leucine in 0.3 ml of phosphate buffered saline. The incorporation of leucine into protein was determined by precipitation with CCl_3COOH and washing onto glass filter discs, and incorporation of leucine into L_2 was determined by immunoprecipitation of L_2 (see ref. 2 for more complete description of methods). The soluble radioactivity is the difference between the total supernatant radioactivity and supernatant protein incorporation.

3924-A are shown in Table II. Considering the ratios for supernatant protein, it appears that the soluble protein is less stable in liver. However, since the hepatoma 3924-A expresses protein not present in the liver (or vice versa) as well as maintains different levels of mutual proteins a clear interpretation is not possible with a mixture of proteins. A clearer interpretation should be possible with individual proteins such as L_2. Such a comparison of the $^3H/^{14}C$ ratios for L_2 from liver and hepatoma 3924-A indicates they are statistically identical which tempts one to speculate that the degradative mechanism for L_2 is quite similar in both tissues. Although degradative rate constants may be identical, it is likely that the rate of degradation of L_2 is increased the same magnitude as the synthetic rate in order to maintain steady state conditions. Using the rate equation that the rate of enzyme degradation is the product of the rate constant times the steady level of the enzyme (4,5), it is easily seen that if the degradation rate constants are identical for liver and hepatoma L_2 then the ratio of degradation rates equals the ratio of the steady state levels of L_2 in liver and hepatoma 3924-A. Prior

Table II

Ratios of ^{3}H to ^{14}C DPM in Supernatant Protein and L_2 in Liver and Hepatoma 3924-A[a]

Tissues	(^{3}H) Leucine incorporation (DPM x 10^{3}/ml supernate)		(^{14}C) Leucine incorporation (DPM x 10^{3}/ml supernate)		(^{3}H) Leucine incorporation / (^{14}C) Leucine incorporation	
	Supernate	L_2	Supernate	L_2	Supernate	L_2
Liver (6)	4791 ± 489	1058 ± 110	1219 ± 131	499 ± 54	3.93 ± 0.40	2.12 ± 0.22
Hepatoma 3924-A (7)	1826 ± 202	793 ± 83	922 ± 95	402 ± 44	1.98 ± 0.22	1.97 ± 0.20

[a] Means ± standard deviations are given, and the number in parenthesis indicates the number of rats used. Normal and tumor bearing rats were injected (i.p.) with 25 μCi of [^{14}C] leucine in 0.2 ml of phosphate buffered saline; three days later the same rats were injected with 75 μCi of [4,5-^{3}H] leucine in 0.3 ml. The rats were handled as described (2,3). See legend of Table I for more details.

work has demonstrated that the levels of L_2 in hepatoma and liver are 10.2 and 3.9 units/g tissue, respectively (3). Thus, it can be calculated that the steady state level of L_2 in hepatoma 3924-A is 2.6-fold greater than in liver and logically the rate of L_2 degradation should be 2.6-fold greater in hepatoma 3924-A relative to normal liver. Comparatively, there seems to be good agreement between the increased rates of L_2 synthesis (2.2) and increased degradation (2.6) in hepatoma 3924-A compared to normal liver; and the closeness of the two numbers seem to support their validity.

These data describe the new steady state for L_2 in hepatoma 3924-A as a 2-3 fold increase in its rate of synthesis and degradation. It is not unreasonable to speculate that at some point in the early history of the hepatoma or hyperplastic liver an increase in genic expression occurred, which created an imbalance in steady state conditions for L_2 such that the rate of L_2 synthesis was favored. Further, the new steady state conditions for L_2 were established when the new level of this isozyme reached a level which was increased relatively the same amount as the new rate of synthesis.

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