COMPARISON OF AMINOACYL-tRNA SYNTHETASES FROM RAT LIVER AND HEPATOMAS

U. DEL MONTE and Grazia CINI
Institute of General Pathology, University of Firenze, 50134 Firenze, Italy

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1. Introduction

Amino acid activation is catalyzed by a family of enzymes, the aminoacyl-tRNA synthetases, each of which is specific for one amino acid [1]. The lack of detailed studies of aminoacyl-tRNA synthetases in tumours prompted us to investigate comparatively these enzymes in normal and tumour tissue to gain information about the capability for amino acid activation in tumour cells [2]. The present report summarizes results of a nearly complete analysis of aminoacyl-tRNA synthetases carried out with rat liver, with the 2 well-differentiated, slow-growing Morris hepatomas 5123C and 7793, and with the highly anaplastic, fast-growing Yoshida ascites hepatoma AH130. The analysis showed that these enzymes are more active in the fast-growing tumour than in the 2 slow-growing ones and that the enzyme patterns of all the tumours examined are different from that of liver.

2. Materials and methods

The Morris hepatomas 5123C and 7793 were grown subcutaneously in female Buffalo rats for 8–10 weeks while the Yoshida hepatoma AH130 was grown intraperitoneally in Wistar rats for 5–6 days. Normal livers were taken from female Buffalo rats of the same age as those bearing the tumours. Prior to the experiment all the animals were fasted overnight.

Liver and Morris hepatomas were cut in small pieces and then rapidly rinsed with cold medium containing 0.25 M sucrose, 0.02 M potassium phosphate buffer (pH 7.4), 0.01 M $MgCl_2$ and 1 mM EDTA. The ascites cells were collected by centrifugation of the ascitic fluid at low speed and then washed twice with the medium indicated above. Tissues and ascites cells were homogenized in 3.5 vol of medium. Both liver and Morris hepatomas were homogenized with a Potter-Elvehjem Teflon-glass homogenizer while a stainless steel Dounce-type homogenizer fitted with a Teflon ball suited better to break the ascites hepatoma cells. Experiments comparing the 2 homogenizers revealed that the enzyme activity found in the liver, used as reference tissue in this study, was independent of the homogenizer used.

To prepare the enzyme fraction, the particulate matter was removed from the homogenate by centrifugation (first at 10,000 g for 15 min, and then at 35,000 rpm for 75 min in the Spinco rotor 50) and the resulting supernatant recovered quantitatively. An aliquot of this supernatant (5 ml) was then freed from amino acids, nucleotides and tRNA by filtration through a column of Sephadex G-75 (2.7 × 35 cm) equilibrated in the cold with a solution containing 0.15 M KCl, 0.1 M Tris-Cl (pH 7.5) and 10% (v/v) glycerol. Fractions were collected and those corresponding to the protein peak (containing all the aminoacyl-tRNA synthetase activity and 90% of the protein) were pooled and then divided in 2 parts which were stored in ice overnight, one in the presence and one in the absence of 4.5 mM 2-mercaptoethanol. The fraction to be tested for asparagyl-, asparaginyl-, glutamyl- and prolyl-tRNA synthetase activity was stored and assayed in the absence of 2-mercaptoethanol. All the enzyme determinations were carried out the next day. The enzyme fractions were suitably diluted before each assay with the same solution used for gel-filtration, to which bovine serum albumin had been added (1 mg/ml).

The standard reaction mixture contained (in addition to enzyme) per ml: 130 μmoles Tris-Cl (pH 7.5); 14 μmoles KCl; 3 μmoles ATP; 3 μmoles 2-mercaptoethanol (when present); 2.4 A_{260} units rat liver tRNA (omitted from "blank" tubes); 4 μCi ^{14}C-L-amino acid; 1.8–6 μmoles $MgCl_2$, depending on the amino acid (1.8 μmoles with leu, 3 μmoles with ala, arg, asp, gln, glu, gly, ile, pro, thr, and 6 μmoles with all other amino acids). The incubation was carried out at 26°. The reaction was started by adding the enzyme and lasted for 2–4 min in a final volume of either 125 or 250 μl. The ^{14}C-aminoacyl-tRNA formed was measured in 50–100 μl samples by the filter paper disc method [3] and was proportional to the amount of enzyme added and to the incubation time. Amino acid incorporation in the N-terminal position of proteins [4] was ruled out by testing the discs for hot trichloroacetic acid-insoluble radioactivity [5]. Radioactivity was determined in a Nuclear Chicago liquid-scintillation spectrometer with a counting efficiency of 50%.

Rat liver tRNA was prepared essentially according to Yang and Novelli [6] but it was discharged from attached amino acids by incubation in 0.3 M Tris-Cl (pH 8.5) for 90 min at 37°. The integrity of the 3′OH pCpCpA terminus of tRNA was checked enzymatically [7].

Radioactive glutamic acid, isoleucine and leucine where obtained from New England Nuclear Co., Frankfurt/M, Germany. All other labelled compounds were purchased from the Radiochemical Centre, Amersham, Buckshire, England. Except for histidine and glutamine (specific radioactivities 50 and 41 mCi/mmole, respectively), amino acids had specific radioactivities higher than 100 mCi/mmole.

3. Results and discussion

Enzyme patterns of liver and hepatomas were obtained by measuring comparatively all the enzyme activities in each experiment.

The enzyme pattern of normal liver is reported in table 1. Clearly, in liver preparations the majority of the aminoacyl-tRNA synthetases display rather similar activities, a finding which does not exclude that *in vivo* the relative activities of the various enzymes might be very different. Indeed, the aminoacylation reaction is markedly influenced by the conditions [1, 8], and we adopted different storage and assay conditions depending on the optimum of the various enzymes.

Table 1
Activity of aminoacyl-tRNA synthetases of normal rat liver.

Amino acid	pmoles of aminoacyl-tRNA formed/min/mg tissue nitrogen*
Glycine	54 ± 11.5
Alanine	70 ± 11.1
Glutamic acid	48 ± 7.6
Glutamine	70 ± 10.8
Aspartic acid	6 ± 0.8
Asparagine	58 ± 12.3
Arginine	403 ± 48.6
Lysine	422 ± 39.5
Methionine	70 ± 12.7
Valine	48 ± 6.4
Leucine	73 ± 12.3
Isoleucine	49 ± 12.5
Hystidine	174 ± 29.9
Proline	79 ± 9.7
Threonine	221 ± 16.3
Phenylalanine	40 ± 4.3
Tyrosine	152 ± 15.3

* Mean values of 15 experiments ±S.E.M.

Fig. 1 compares the activities of aminoacyl-tRNA synthetases in the 3 hepatomas. They are expressed as per cent differences from the same activities found in the normal liver, used as reference tissue following Weber and Morris [9]. To take into account differences in the composition of the various materials (tumours are known to have a higher water content than normal tissues), all values were calculated on the classical basis of tissue nitrogen [10]. It should be said, however, that even if comparisons were based on specific activities of the enzyme fractions and if the liver of tumour-bearing rats were used as reference tissue instead of normal liver, the conclusions which can be drawn are practically the same.

Briefly, from the above comparison 2 main points emerge. First, all 3 hepatomas have aminoacyl-tRNA synthetase patterns which are different from that of liver. It is remarkable, however, that the patterns of the 2 slow-growing Morris hepatomas 5123C and 7793 are rather similar to each other. Second, the fast-growing Yoshida hepatoma AH130 has much higher enzyme activities than either the 2 slow-growing Morris hepatomas or the normal liver.

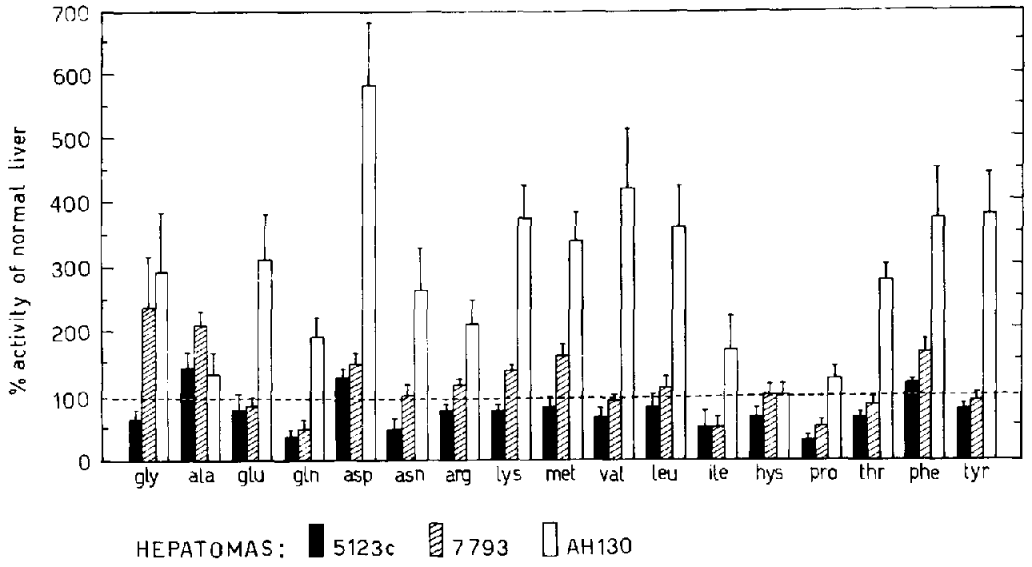

Fig. 1. Activity of aminoacyl-tRNA synthetases of hepatomas (columns) given as the % of the activity of normal liver (dotted line). Number of experiments: hepatoma 5123C, 12; hepatoma 7793, 15; hepatoma AH130, 8. Vertical lines represent the S.E.M.

On the whole, the fast-growing hepatoma has about 2.5 times more total aminoacyl-tRNA synthetase activity than the 2 slow-growing ones, which suggests the existence of a relationship between its greater ability to activate the amino acids and its higher net rate of protein synthesis. It is apparent, however, that a direct relationship between the total enzyme activity and the rate of protein synthesis in hepatomas can be only hypothesized at the present time as the rate of reaction is strictly related to the amount of enzyme only in a fully supplemented system, in which only the enzyme concentration is limiting.

Apparently, the total aminoacyl-tRNA synthetase activity of the 2 slow-growing hepatomas 5123C and 7793 approximates that of normal liver, but in hepatomas the relative proportion of the various enzymes markedly differs from that found in normal liver. It is tempting to suppose that this situation is relevant to the problem of cellular control. Indeed, if the changes of enzyme activities found in the hepatomas influence the extent of acylation *in vivo* of the tRNA's required for the readout of some particular mRNA's, it may change the production of individual proteins. For instance, in liver tumours an accelerated synthesis of proteins necessary for growth could accompany the reduced production of non-essential proteins (e.g. serum albumin).

Briefly, it is conceivable that the observed changes of aminoacyl-tRNA synthetases, possibly associated with changes of tRNA's known to occur in tumours [11–16], may effect growth regulation by modifying the translational capacities of the cells. The increasing evidence that "the amounts and relative concentrations of the enzymes, the free tRNA's and the aminoacylated tRNA's may play a significant rate-controlling function in protein biosynthesis" [8] may be brought in support of this hypothesis.

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