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Compartmentation of Albumin and Ferritin Synthesis in Rat Liver *in vivo*

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Infusion of rats with [U-^{14}C]glycine resulted in labelling of glycine and serine in plasma albumin and liver ferritin. The patterns of labelling in these two proteins were not similar, suggesting that each is synthesized from a different pool of free amino acids.

In studies of protein synthesis it is generally accepted that aminoacyl-tRNA exists as a homogeneous pool within the cell, serving both membrane-bound and free ribosomes. This acceptance has arisen largely through lack of any contradictory evidence. However, it has been reported (Ilan & Singer, 1975) that the specific radioactivities of leucine in nascent peptide chains isolated from membrane-bound and from free ribosomes are appreciably different, suggesting that precursor leucine, and therefore leucyl-tRNA, is contained in more than one compartment. We have investigated this question by a method previously employed (Fern & Garlick, 1973, 1974) in which the metabolic interconversion of glycine and serine has been used to label differentially the free pools of these amino acids in liver and in plasma. By comparing the specific radioactivity of serine with that of glycine in two pure proteins synthesized in the liver, namely albumin and ferritin, some further possible evidence of cellular compartmentation of aminoacyl-tRNA has been obtained.

Methods

Two separate experiments were carried out, both of which measured and compared the specific radioactivity of serine with that of glycine in the free and protein-bound pools of liver and in plasma albumin. In addition, the first experiment measured the specific radioactivities of these amino acids in the free pools of arterial and hepatic portal plasma, and the second experiment measured them in liver ferritin.

In both studies female hooded rats maintained *ad libitum* on a 10.9%-casein diet were used, although the size of the animals differed in the two cases. In Experiment 1 the mean body weight at the time of infusion was 108g, whereas in Experiment 2 it was about 200g. The use of larger rats in the second instance was to ensure the isolation of adequate amounts of ferritin. The method of infusion and that of determining the specific radioactivities of glycine and serine were identical with those described before (Fern & Garlick, 1973). Essentially the animals were infused continuously through the lateral tail vein with a solution of 0.9% NaCl containing [U-^{14}C]glycine (10mCi/mmol) at a rate of 0.48ml/h. Each rat received an equivalent dose of approx. 25μCi over the infusion period. Specific radioactivities of glycine and serine were determined by automated amino acid analysis and liquid-scintillation counting of effluent fractions.

Experiment 1

A total of twelve rats were infused for either 3 or 6h, at the end of which the animals were anaesthetized with ether. After the hepatic portal vein had been exposed, the infusion was stopped, and a 2ml sample of blood was withdrawn, together with a second sample of blood (1ml) from the left ventricle of the heart. Plasma was separated by centrifugation, and a 0.3ml sample of each was deproteinized with an equal volume of 5% (w/v) sulphosalicylic acid. A sample (1g) of liver was also taken, rinsed in 0.9% NaCl and homogenized in 5ml of ice-cold 5% sulphosalicyclic acid. The resulting protein precipitate was separated by centrifugation. Albumin was isolated from mixed hepatic portal and arterial plasma by precipitation with 1% (w/v) trichloroacetic acid in ethanol and with diethyl ether and by gel filtration on Sephadex G-200 (column dimensions 50cm×1.6cm) (Judah *et al.*, 1973).

Experiment 2

Twelve rats were randomly divided into two equal groups. Rats in one group were injected subcutaneously with 10mg of iron (as iron sorbitol) 14 days before infusion. The second group acted as controls. Liver ferritin was isolated from both groups by the ultracentrifugation technique described by Bjorklid

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& Helgeland (1970). Total liver protein and plasma albumin were isolated as described for Experiment 1. The purity of liver ferritin and plasma albumin was checked by disc gel electrophoresis. In all cases hydrolysis of proteins was achieved by incubation with 6M-HCl for 24h at 105°C.

Results and Discussion

Experiment 1 (Table 1) confirmed previous observations (Fern & Garlick, 1973, 1974) that, although the specific radioactivities of free glycine and serine rose between 3 and 6h of infusion, their ratio (serine/glycine) remained virtually constant. The specific-radioactivity ratio in total liver protein was also constant and much closer to that of the free amino acids in liver than in either arterial or hepatic portal plasma. As before, we interpret this to mean that the precursor pool for protein synthesis under these experimental conditions is more directly related to intracellular amino acids than to those in plasma.

With albumin, also, the specific-radioactivity ratio indicated synthesis from an intracellular pool, again because the ratio was better related to the free pool in liver rather than to that in either plasma. However, there was a significant difference between the specific-radioactivity ratios in albumin and mixed liver protein. Theoretically the relative specific radioactivities of two amino acids in any single protein should be an accurate reflection of the specific-radioactivity ratio of the same two amino acids in the pool that serves as precursor for its synthesis. This relationship should not be affected by the concentration, the amino acid composition or the rate of turnover of the protein. It need not, though, be true for a mixture of proteins with different turnover rates, such as total tissue protein. Consequently the difference in specific-radioactivity ratio between albumin and total liver protein does not necessarily indicate the existence of more than one precursor pool.

We therefore compared the labelling of albumin with that of another purified protein, liver ferritin (Experiment 2; Table 2). Results were obtained from normal rats and others in which the synthesis of ferritin had been induced previously with iron. This comparison was made because the chances of contamination of the small amount of ferritin from normal rats would be minimized by the much greater yield of ferritin from the induced animals. The results from these two groups were similar (Table 2). The specific-radioactivity ratio in liver ferritin was very close to that in the total liver protein, whereas, as before, that in albumin was lower.

These results suggest that albumin and ferritin are not synthesized from the same pool of free amino acids. This apparent compartmentation can be explained in two ways. The first considers that the differences arise from compartmentation at the tissue level of organization. Even though albumin and ferritin are almost exclusively associated with hepatocytes (Chandrasakharam *et al.*, 1967; Van Wyk *et al.*, 1971; Cook *et al.*, 1974), it has been shown that functional variations exist among apparently homogeneous populations of these cells (Hamashima *et al.*, 1964; Tanikawa, 1968; Drochmans *et al.*, 1975), and thus all hepatocytes may not simultaneously synthesize both proteins.

The second possibility is that synthesis of albumin and ferritin occurs at different sites within the cell. The most obvious way in which this can occur is through localization of ribosomes. There are many studies that have indicated that albumin is almost entirely synthesized by membrane-bound ribosomes (see Rolleston, 1974). The situation with regard to the synthesis of ferritin is not as clear. Although studies by Hicks *et al.* (1969), Redman (1969), Puro &

Table 1. *Experiment* 1: *Serine/glycine specific-radioactivity ratios in free amino acid pools, total liver protein and plasma albumin in the rat*

Rats (108g) were infused for either 3 or 6h with [U-^{14}C]glycine. Each result is the mean±S.E.M. from six animals. Statistical comparisons were made by using Student's *t* test for paired differences; values marked with the same letter (^{a}, ^{b}) differ significantly at the $P<0.01$ level.

	Specific radioactivity (d.p.m./nmol)				Serine/glycine specific-radioactivity ratio	
	Serine		Glycine			
Time of infusion ...	3h	6h	3h	6h	3h	6h
Free pools						
Arterial plasma	34.2±2.0	44.6±2.2	130.3±9.0	142.2±4.4	0.266±0.016	0.314±0.011
Hepatic portal plasma	32.0±1.8	43.6±1.5	103.4±7.6	125.4±5.4	0.319±0.033	0.351±0.020
Liver homogenate	59.2±3.4	75.2±4.5	64.9±4.9	77.9±3.2	0.919±0.020	0.963±0.031
Protein-bound pools						
Total liver protein	2.32±0.09	5.16±0.16	2.71±0.09	5.76±0.14	0.861±0.038^a	0.896±0.018^b
Plasma albumin	2.60±0.09	6.22±0.36	3.51±0.13	8.31±0.42	0.739±0.007^a	0.748±0.019^b

Table 2. *Experiment 2: Serine/glycine specific-radioactivity ratios in liver free amino acids, total liver protein, liver ferritin and plasma albumin in the rat*

Rats (normal group 220g; induced group 185g) were infused for 6h with [U-^{14}C]glycine. Each result is the mean±S.E.M. from six animals. Statistical comparisons of groups were made by using the paired *t* test; values marked with the same letter differ significantly at the following levels: [a], [b], [c], [e] ($P<0.01$); [d] ($P<0.001$); [f] ($P<0.02$); [g] ($P<0.05$).

	Specific radioactivity (d.p.m./nmol)							
	Liver free amino acids		Total liver protein		Liver ferritin		Plasma albumin	
	Serine	Glycine	Serine	Glycine	Serine	Glycine	Serine	Glycine
Normal group	24.81±0.57	25.30±0.51	1.89±0.10	1.77±0.11	0.57±0.04	0.55±0.04	1.97±0.11	2.53±0.13
Induced group	23.25±0.59	23.44±0.69	1.84±0.07	1.78±0.07	0.64±0.05	0.68±0.05	1.67±0.09	2.01±0.10

	Serine/glycine specific-radioactivity ratio			
	Liver free amino acids	Total liver protein	Liver ferritin	Plasma albumin
Normal group	0.981±0.021[a]	1.076±0.050[b]	1.035±0.039[c]	0.783±0.016[a,b,c]
Induced group	0.993±0.016[d]	1.037±0.031[e,g]	0.950±0.025[f,g]	0.828±0.016[d,e,f]

Richter (1971) and Shafritz & Isselbacher (1974) have all shown a predominant synthesis of ferritin by free ribosomes, it has been proposed (Niitsu *et al.*, 1973; Konijn *et al.*, 1973) that rat liver ferritin contains not one, as previously thought, but two species of subunit differing both in molecular weight and in the site of synthesis. Konijn *et al.* (1973) suggested that the larger subunits are synthesized by membrane-bound ribosomes whereas the smaller ones are synthesized by free ribosomes. It is also thought that the induction of ferritin synthesis with iron leads to a change not only in the proportion of each subunit synthesized but also in the relative rates of their turnover (Konijn *et al.*, 1973; Linder *et al.*, 1974), a finding that may account for the small difference between the specific-radioactivity ratios of ferritin in the normal group and in the induced group (Table 2).

A distinction between free and bound ribosomes was suggested by the work of Ilan & Singer (1975), when they showed that in newt liver the specific radioactivity of [^{3}H]leucine in nascent peptides from free ribosomes was twice that from membrane-bound ribosomes. However, because of the uncertainty with regard to the site of ferritin synthesis, we cannot say that this is the reason for the difference in labelling between albumin and ferritin.

Nevertheless the present results, although unable to define the exact nature of precursor compartmentation related to the synthesis of these proteins, do show that they are not synthesized from a mutual pool of intermediates. If this compartmentation does not arise purely from differences in the site of synthesis at the tissue level, then there must be some cellular distinction. There are several studies (see review by Kisselev & Favorova, 1974) that have shown that aminoacyl-tRNA synthetases, and in some cases tRNA, are associated with ribosomal complexes. It is therefore no longer unreasonable to suppose that, under conditions *in vivo*, different ribosomal populations may exist as independent synthetic entities, each obtaining the necessary intermediates for protein synthesis from a localized environment.

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