Short Communications

Amino acid transfer from sRNA to microsome
I. Activation by sulfhydryl compounds

Incorporation of sRNA-bound amino acid to liver microsomes was described by HOAGLAND *et al*[1] as requiring a factor contained in a particle-free liver supernatant. In attempts to study the function of this fraction, difficulties in obtaining such an effect were encountered. Although a fair amount of protein was removed by washing with aq. sucrose, the washed preparation showed no consistent effect of supernatant (Table I), and sometimes even slightly better incorporation. Ageing the microsomes at between 5–10° for about 2 h, however, caused loss of activity, most of which was recovered on addition of supernatant. Such an effect using the pH-5 precipitate of liver supernatant is illustrated in Table II which shows that nearly full activity may be regained by adding GSH. On the other hand, GSSG was quite inhibitory as can be seen in Table III using freshly prepared microsomes; this inhibition could be partly reversed by pH-5 precipitate. GSH increased transfer slightly, and pH-5 precipitate caused still further stimulation.

In a more systematic study of the SH effect, a microsome preparation was used that had been moderately aged by incubation at pH 7.4 for 30 min at 30° (Table IV).

TABLE I

EFFECT OF WASHING ON THE TRANSFER OF [^{14}C]LEUCINE FROM sRNA TO MICROSOMES

The incubation was for 3.5 min at 25°, and the reaction mixture contained about 7 mg dry wt. of microsomes. Standard conditions used in this and the following tables were as follows: the rat-liver microsomes were isolated according to LITTLEFIELD AND KELLER[2] using the 0.25 *M* sucrose medium, and were washed by resuspending in 0.01 *M* Tris–HCl, pH 7.2, 0.002 *M* $MgCl_2$, and 0.23 *M* sucrose, followed by recentrifugation at 105,000 × *g* for 1 h. The medium contained in a total volume of 1 ml: 0.2–0.5 mg [^{14}C]leucine-RNA, 1000–3000 counts/min; 0.003 *M* ATP; 0.010 *M* CAP; 30 μg of CAP-kinase; 0.0012 *M* GTP; 0.100 *M* Tris–HCl, pH 7.4–7.6; 0.05 *M* KCl; and 0.006 *M* $MgCl_2$. The reaction is stopped by the addition of 5 ml cold 5 % trichloroacetic acid, and the precipitate is washed twice in the centrifuge with 5 ml cold 5 % trichloroacetic acid and once with 5 ml alcohol–ether (3:1). The washed precipitate is now resuspended in 5 ml 5 % trichloroacetic acid, heated for 15 min to 90°, cooled, centrifuged, and the supernatant discarded. The precipitate is washed again with alcohol–ether (3:1), plated, and counted in a Nuclear Chicago windowless gas-flow counter.

Microsomes	*Microsomal supernatant*	*counts/min in protein*
Unwashed	—	285
Washed	—	342
Washed	+	342

Abbreviations: GSH, GSSG, glutathione and its oxidized form; SH, sulfhydryl; TPNH, reduced triphosphopyridine nucleotide; ATP, adenosine triphosphate; GTP, guanosine triphosphate; CAP, carbamyl phosphate; sRNA, soluble ribonucleic acid; Tris, tris(hydroxymethyl)-aminomethane.

TABLE II

THE INFLUENCE OF VARIOUS ADDITIONS ON THE TRANSFER OF AMINO ACIDS FROM sRNA TO AGED MICROSOMES

Incubation was for 3 min at 35°. Every tube contained: 2 μmoles GTP, 100 μmoles Tris–HCl pH 7.6, 5 μmoles $MgCl_2$, 50 μmoles KCl, 6.8 mg of microsomes protein (biuret), and 1900 counts/min of [^{14}C]leucine-RNA and 0.5 mg of sRNA. For other conditions, *cf.* Table I.

Additions	*Counts/min*
None	39
pH-5 ppt., 1 mg protein (biuret)	333
pH-5 supernatant, 4 mg protein	621
20 μmoles GSH	460

TABLE III

THE EFFECT OF GSH AND GSSG ON THE TRANSFER OF [^{14}C]LEUCINE FROM sRNA TO LIVER MICROSOMES

Incubation was for 10 min at 35°. Other conditions were the same as those listed in Table I.

Additions	*Counts/min*
None	968
GSSG, 2 μmoles	58
GSSG, 2 μmoles + pH-5 enzymes	678
GSH, 1 μmole	1210
pH-5 enzymes, 5 mg	1550

TABLE IV

THE REACTIVATION OF AGED MICROSOMES

The microsomes were aged for 30 min at 30° at pH 7.4 in air. With fresh microsomes, 968 counts/min were transferred without additions. The incubation medium is the same as that in Table I.

No.	*Additions*	*Counts/min*
1.	None	191
	pH-5 fraction, 5 mg	1002
	Cysteine, 10 μmoles	910
2.	None	111
	GSH, 1 μmole	259
	GSH, 1 μmole + GSSG reductase + 0.5 μmole TPNH + 5 μmoles glucose 6-phosphate + 5 μg Zwischenferment	798
	pH-5 enzymes, 5 mg	933

Such ageing reduced the transfer to about one-fifth, and the addition of the pH-5 fraction brought the value up to a little above the non-aged preparation; addition of an SH carrier, in this case cysteine, stimulated incorporation almost equally well. In Expt. 2 in Table IV, a similarly aged preparation with a much lower concentration of GSH was only slightly stimulated. If, however, GSSG reductase and a TPNH-generating system were added to regenerate GSH, activity was brought to nearly as high a level as with addition of pH-5 enzyme.

Further confirmation of the oxidative nature of the inactivation caused by ageing was obtained by showing that it could be considerably reduced by keeping the incubation mixture in nitrogen (Table V). The degree of inactivation by ageing was partly dependent on pH, and was greater at pH 8.5 but decreased at pH 6 as might be expected from easier oxidation of GSH at alkaline pH. It was also found that incorporation of amino acid into protein in this system was sensitive to iodoacetate, 60 % inhibition being caused by 0.001 *M* inhibitor.

From these experiments it is concluded that an SH function may be involved in the transfer reactions. It seems to be significant that the inactivation caused by relatively mild manipulation was reversed by the supernatant fraction, and that the same effect could be obtained by small amounts of GSH together with a GSH-

TABLE V

PRESERVATION OF ACTIVITY ON AGEING IN NITROGEN

The incubation medium was the same as in Table I, to which 0.2 ml microsomes (= 0.7 g liver) and 0.1 ml 0.02 *M* Tris–maleate buffer, pH 7.5, were added. After ageing for 30 min at 30°, 0.2 ml Tris–HCl, pH 7.4, and other components were added to a final volume of 1 ml. The further procedure was that described in Table I.

Conditions	*[^{14}C]leucine transfer (counts/min)*
Air, not aged	649
Air, aged	110
N_2, aged	533

regenerating system. Since the supernatant contains both GSH and GSSG reductase, the supernatant effect may, in part, be ascribed to the maintenance of a favorable SH level in the preparation. In view of the difficulties in obtaining a preparation that clearly showed a supernatant effect, for a while we were under the impression that the supernatant effect observed by others[1,3] might be explained by the oxidizability of the system as reversed by GSH. As will appear in the subsequent communication, however, a more specific effect has now been obtained in addition to the SH function.

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