

ref.⁸ and ⁹). It has been stated previously^{8,9} that cytidine probably has the amino form, and the close similarity of the poly C bands indicates that it also is probably in the amino form. The same data show that the interaction of the polymers causes no change of tautomeric form in the inosine units and probably none in the cytidine units*.

It has been proposed that the observed increase in frequency of the poly I peak (a large decrease would be required if there had been a change to the enol form) and decrease in intensity of the poly C peak upon interaction of the polymers may be due primarily to a decrease in the dielectric constant of the immediate environment of the vibrating groups¹⁰. If this proposal is correct, then it would follow that all or most of the hypoxanthine rings are in approximately the same dielectric environment.

It is also apparent that no hydrogen-bonding scheme which involves placing a charge on the hypoxanthine or cytosine rings is possible, in view of the absence of any strong bands near 1600 cm⁻¹ or 1711 cm⁻¹ (Table I; it is possible, though unlikely, that the 1711 cm⁻¹ band might be shifted to lower frequency and obscured by the inosine band).

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* In the case of poly A + poly U, the facts that the structure of the helix is known² and that the keto form of uridine is definitely established^{8,9} makes it possible to say that the adenine units are definitely in the amino form (even in the absence of the desirable imino model compound) since only this tautomer could form a hydrogen-bonding scheme in a helix of this structure. In the case of poly I + poly C, however, such a definite statement cannot be made about poly C since the detailed structure of the helical interaction product is not known.

Transfer of RNA-bound amino acids to microsomal proteins

Evidence for incorporation of amino acids into soluble RNA has been reported¹⁻⁶. Recently, HOAGLAND *et al.*⁶ demonstrated that isolated RNA-bound amino acids are transferred to peptide-bound material in microsomes. The present communication describes some studies on the rat-liver soluble fraction which catalyzes incorporation

Abbreviations: RNA, ribonucleic acid; ATP, adenosine 5'-triphosphate; GTP, guanosine 5'-triphosphate.

of [^{14}C]amino acids from RNA-bound amino acid into ribonucleoprotein particles. RNA- ^{14}C leucine was isolated from incubations of the pH 5-precipitable fraction of rat-liver supernatant with ATP and randomly labelled [^{14}C]L-leucine⁶. Incubation of RNA- ^{14}C leucine with microsomes, ATP, GTP, and an ATP-generating system did not result in labeling of the microsomal proteins in absence of the soluble fraction (Table I). Most of the activity appeared to reside in the supernatant of the isoelectric precipitation at pH 5 (pH 5 supernatant). Transfer of radioactivity from RNA to microsomes was proportional to the amount of pH 5 supernatant added (Fig. 1). ATP was essential; GTP and an ATP-generating system were necessary for optimal labeling.

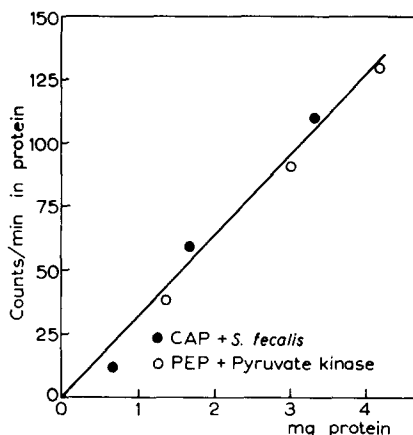


Fig. 1. Effect of pH 5 supernatant on the transfer of [^{14}C]leucine from RNA to microsomes. Incubation conditions as described in Footnote (**), Table I. Carbamyl phosphate (CAP, 10 μmoles) and a crude extract of *Streptococcus faecalis* (250 μg) or phosphoenolpyruvic acid (PEP) and crystalline pyruvate kinase was used as the ATP-generating system. Additions of pH 5 supernatant are presented as mg protein in the preparation.

Although pH 5 supernatant material was stable at room temperature for 18 h, dialysis at 4° for 12 h against distilled water, 0.02 *M* potassium phosphate, pH 7.4, or 10⁻³ *M* glutathione resulted in complete loss of activity; concentrated dialysates were also inactive and activity was not restored on recombination of dialysates and dialyzed material. Preparations dialyzed against sucrose, however, retained approximately 40 % of the initial activity and addition of the dialysate to these preparations led to complete restoration of activity (Table I). The dialysate alone did not catalyze the transfer of radioactivity to proteins.

A preliminary separation of the non-dialyzable component in the pH 5 supernatant has been obtained by precipitation with $(\text{NH}_4)_2\text{SO}_4$. Approximately 70 % of the precipitated activity appears to reside in the 20–40 % (w/v) precipitate (Table I); the rest of the activity was present in the fraction precipitated with 20 % $(\text{NH}_4)_2\text{SO}_4$ while other fractions were inactive. Only a slight transfer of radioactivity from RNA to microsomes was observed when active $(\text{NH}_4)_2\text{SO}_4$ fractions were tested in absence of a dialysate obtained from pH 5 supernatant. It was not possible to assay accurately the activity precipitated with $(\text{NH}_4)_2\text{SO}_4$ since a rapid loss of activity has been observed in these partially purified preparations when stored frozen at -15°. These results indicate the presence of two components in the soluble fraction of rat liver

which catalyze the transfer of amino acids from soluble RNA to protein. Purification of the non-dialyzable component and characterization of the dialyzable factor are in progress.

TABLE I

TRANSFER OF [^{14}C]AMINO ACID FROM RNA TO PROTEIN IN THE PRESENCE OF RAT-LIVER SOLUBLE PREPARATIONS

Incubation conditions	Counts/min in protein*
Incubation mixture**	0
+ homogenate supernatant***	75
+ pH 5 precipitate§	32
+ pH 5 supernatant§§	112
+ pH 5 supernatant§§ (dialyzed)§§§	49
+ pH 5 supernatant§§ (dialyzed)§§§ + dialysate†	115
+ dialysate†	4
+ 20-40% $(\text{NH}_4)_2\text{SO}_4$ fraction††	18
+ 20-40% $(\text{NH}_4)_2\text{SO}_4$ fraction†† + dialysate†	50

* Total counts/min in protein isolated from microsomes.

** The incubation mixture contained the following: RNA- ^{14}C leucine, approx. 0.1 mg (1000 counts/min); microsomes, washed once by sedimentation at $102,000 \times g$, resuspended in buffered salt-sucrose media, approx. 4 mg protein; ATP, 0.5 μmole ; GTP, 0.5 μmole ; phosphoenolpyruvic acid, 10 μmoles ; and crystalline pyruvate kinase, 30 μg . Final vol., 1.5 ml.

*** Supernatant obtained at $102,000 \times g$ from a 20% rat liver homogenate in buffered salt-sucrose media; approx. 23 mg protein.

§ Prepared from the homogenate supernatant*** by isoelectric precipitation at pH 5.2. Resuspended in buffered salt-sucrose media; approx. 14 mg protein.

§§ Supernatant from isoelectric precipitation at pH 5.2 (§), adjusted to pH 7.4; approx. 3 mg protein.

§§§ pH 5 supernatant fraction dialyzed against 5 vol. 0.25 M sucrose for 18 h followed by 3 changes of 0.25 M sucrose (5 vol., 2 h each).

† Initial 0.25 M sucrose dialysate (§§§), concentrated by lyophilization.

†† Obtained from the pH 5 supernatant, following removal of the 20% (w/v) $(\text{NH}_4)_2\text{SO}_4$ fraction, with the further addition of solid $(\text{NH}_4)_2\text{SO}_4$ at 4° to a final concentration of 40% (w/v). Resuspended and dialyzed against 0.25 M sucrose overnight at 4° .

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