Preliminary Notes

Intermediate reactions in protein biosynthesis*, **

Previous studies in this laboratory furnished evidence that L-amino acids are activated as amino acyl-adenylate compounds bound to specific enzymes derived from the soluble protein of rat liver[1]. Further substance has been given this hypothesis by the finding that synthetic amino acyl-adenylate compounds, when incubated with activating enzymes and pyrophosphate (PP), are able to form ATP***[2]. This paper presents evidence for another step in the reaction sequence between amino acid activation and peptide bond condensation.

The rat liver activating enzyme preparation[1] contains ribonucleic acid (RNA): about 5 mg

TABLE I

TRANSFER OF LEUCINE-^{14}C FROM PRELABELED ACTIVATING ENZYME FRACTION TO MICROSOMAL PROTEIN

Microsomes and pH 5 enzymes (activating enzymes) were prepared from rat liver as previously described[5]. Labeled pH 5 enzymes were prepared by incubating pH 5 enzymes (approximately 100 mg protein) for 10 min at 37° C with 0.01 *M* $MgNa_2$ ATP (Sigma), 0.1 m*M* ^{14}C-leucine ($1.8 \cdot 10^6$ c.p.m./μmole) and the medium[5] at pH 7.5 in a total volume of 20 ml. The reaction mixture was then diluted to 60 ml with cold water and the pH brought to 5.2 with *M* acetic acid to precipitate the enzymes. This dilution and precipitation was repeated after redissolving at pH 7.5. The final precipitate was dissolved in 4 ml of medium. The microsomes were suspended in 4 ml of the same medium. RNA was determined on an aliquot of each. 0.6 ml of microsome suspension (14 mg of protein; 2.3 mg RNA) and 0.4 ml of pH 5 enzyme fraction containing the labeled S-RNA (6 mg protein, 0.3 mg RNA) were then incubated in a volume of 2 ml for 15 min at 37° C with 0.5 m*M* nucleoside triphosphates as indicated, plus the triphosphate generating system (0.01 *M* phosphoenol pyruvate and 0.04 mg pyruvate kinase[6]). ATP, not shown here, is also inert. The reaction was stopped by the addition of $HClO_4$ (final concentration, 0.4 *M*), the precipitate washed 4 times with cold 0.2 *M* $HClO_4$, once each with 5:1 ethanol: 0.2 *M* $HClO_4$, ethanol (at 25° C) and 3:1 ethanol-ether at 50° C[6]. RNA was extracted with 10% NaCl at 100° for 30 min and then precipitated twice with 60% ethanol at —10° C. The final alcohol suspension was filtered onto paper discs. The dried RNA was counted using a Nuclear "Micromil" window gas flow counter. The RNA was then eluted from the paper with dilute alkali, and the 260/280 mμ absorption ratio of the extract determined in a Beckman spectrophotometer. Protein was washed, weighed and counted as previously described[5]. The total counts in RNA were multiplied by the ratio of the amount of RNA initially added to the amount recovered.

	Total counts in	
	RNA	*Protein*
Complete system (before incubation)	489	30
Complete system (after incubation)	180	374
Complete system, *minus* GTP	111	40
Complete system, *minus* generating system	72	155
Complete system, *minus* both GTP and generating system	23	30
Complete system, *minus* generating system but with 5 × GTP	145	129
Complete system, CTP replacing GTP	96	44
Complete system, UTP replacing GTP	101	53
Complete system, *plus* 0.005 *M* ^{12}C-leucine	183	314

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*** ATP, GTP, CTP, UTP are the triphosphates of adenosine, guanosine, cytidine, and uridine respectively.

per 100 mg protein. This is apparently a low molecular weight RNA (S-RNA) with different metabolic properties from the high molecular weight RNA of the ribonucleoprotein of the microsomes. When the amino acid activating enzyme preparation is incubated with ATP and ^{14}C-carboxyl labeled leucine, at pH 7.5, the S-RNA subsequently isolated from this fraction is found to be labeled (0.02 to 0.05 μmoles leucine per mg RNA). The time curve of labeling is linear for 2 min, maximal at 10 min, and thereafter the ^{14}C-leucine label is rapidly lost. Microsomal RNA is labeled at approximately a tenth this rate. 0.005 M leucine and 0.01 M ATP saturate the system, and ribonuclease is completely inhibitory. Labeling is additive when 0.005 M ^{14}C-valine and 0.005 M ^{14}C-glycine are also incubated in the same preparation. Yeast RNA, microsomal RNA, and degraded microsomal RNA (prepared by mild alkaline hydrolysis of microsomes) do not give increased labeling when added to the system.

Leucine-labeled S-RNA so obtained from the activating enzymes preparation is non-dialysable and is charcoal and Dowex-1 adsorbable. The ^{14}C-leucine-RNA bond is acid stable and alkali labile, and does not exchange with free ^{12}C-leucine. The ninhydrin-CO_2 method indicates no free ^{14}C-leucine. When labeled S-RNA is incubated with anhydrous hydroxylamine and the products are chromatographed on paper a spot corresponding to leucine hydroxamic acid contains all the radioactivity.

The activating enzyme preparation labeled with ^{14}C-leucine (and reprecipitated twice at pH 5.2 from dilute solution to remove free ^{14}C-leucine and ATP) will transfer the bound ^{14}C-leucine to microsome protein upon subsequent incubation with microsomes and GTP (Table I). The GTP effect is apparently specific and is potentiated by addition of a nucleotide triphosphate generating system. With this complete system a high percentage of the S-RNA bound ^{14}C-leucine is transferred to protein, the rest remaining in S-RNA.

^{14}C-Leucine-S-RNA prepared by a phenol method[3] has been found to transfer ^{14}C-leucine to microsome protein in the absence of added activating enzymes, provided GTP is present.

Preliminary results, using an ascites tumor *in vivo* incorporation system[4], reveal that S-RNA becomes labeled with ^{14}C-leucine more rapidly than does the protein of the ribonucleoprotein particles of the microsomes, the most rapidly labeled protein fraction in this system.

These experiments suggest that incorporation of labeled amino acids into protein is indeed dependent upon the amino acid activation system. The initial formation of an enzyme-bound amino acyl-AMP compound, as originally suggested, accounts for hydroxamic acid formation and PP–ATP exchange[1]. It is now further postulated that this initial activation of amino acids is followed by a transfer of activated amino acid to S-RNA. This latter reaction is ribonuclease sensitive, while the former is not. GTP mediates the transfer of this activated amino acid to peptide linkage via the microsome by a mechanism as yet unknown.

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N-Succinyl-L-diaminopimelic acid, an intermediate in the biosynthesis of diaminopimelic acid*

α,ε-Diaminopimelic acid (DAP) has been shown to occur quite generally in bacteria and blue green algae[1]. In these organisms it appears to serve not only as a cell constituent but also as a precursor of lysine[2,3]. However, little is known concerning the intermediates which participate in DAP biosynthesis. In an effort to gain such information, several mutants of *E. coli* were obtained

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