in the system before the transfer of the activated amino acid to the "soluble" RNA. This transfer is known to be reversed by free AMP (and pyrophosphate), suggesting that free AMP appears after the transfer has taken place (for discussion, see LIPMANN *et al.*[6]). The dilemma can be resolved if we assume that the amino-acyl thioester and amino-acyl ribose ester bonds are in equilibrium with each other, perhaps through an intermediary complex containing Mg^{++}. In the stable amino acid-activating systems this hypothetical complex may effect the "coupling" of thioester bond synthesis to the transfer of the activated amino acid to "soluble" RNA, the formation of thioester bonds being controlled through the response of the thioester-generating system to Mg^{++}. This controlling or "coupling" action may represent a highly interesting property of "soluble" RNA.

Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, Ducane Road, London, W. 12 (Great Britain) PRISCILLA HELE

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The enzymic synthesis of thymidine diphosphate glucose and thymidine diphosphate rhamnose

Recently a large variety of thymidine-linked sugar nucleotides have been isolated from microorganisms[1–4]. The sugars include rhamnose[1,4], mannose[4] and a variety of unidentified sugars[2,3]. We wish to report evidence for the enzymic formation of thymidine diphosphate glucose and its conversion to thymidine diphosphate rhamnose in cell-free extracts of *Pseudomonas aeruginosa* (ATCC 7700). This organism has been shown to secrete a rhamnose-containing lipid into the culture medium[5,6].

Cells were grown in a 3% glycerol–mineral salts medium[6] for 100 h at 32°. The cells were harvested by centrifugation and washed with 0.05 *M* Tris–0.001 *M* EDTA, pH 8. The washed cells were suspended in 0.05 *M* Tris–0.01 *M* $MgCl_2$–0.001 *M* EDTA, pH 8, and ruptured by 18-min treatment in a 10 kc magnetostriction oscillator. Intact cells and large particles were removed by centrifugation at 20,000 × *g* for 20 min.

When the sonic extract was incubated with TTP and α-glucose 1-phosphate, followed by isolation of the nucleotides by adsorption on charcoal and chromatography in the neutral ethanol–ammonium acetate solvent[7], a new nucleotide spot

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; TMP, thymidine 5′-phosphate; TDP, thymidine 5′-diphosphate; TTP, thymidine 5′-triphosphate; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; EDTA, ethylenediaminetetraacetate.

with R_{TMP} of 1.26 was observed. This compound was not formed when α-mannose 1-phosphate or α-galactose 1-phosphate were used instead of α-glucose 1-phosphate.

When ^{14}C-labelled α-glucose 1-phosphate was used, the isolated compound contained 1 μmole sugar/μmole nucleotide. After mild-acid hydrolysis (pH 2, 100°, 20 min) only part of the sugar could be accounted for as glucose either by chromatography or by enzymic assay with glucose 6-phosphate dehydrogenase and hexokinase. Depending on the time of incubation preparations containing 0.3–0.8 μmole glucose/μmole nucleotide were found. The remainder of the nucleotide was linked to sugars which have not been positively identified.

A somewhat purified enzyme was prepared by removal of nucleic acid with protamine, followed by ammonium sulfate fractionation between 55 and 75 % satn.

A typical reaction mixture contained 75 μmoles Tris, 15 μmoles $MgCl_2$, 1.5 μmoles EDTA, 5 μmoles cysteine, 0.1 μmole adenosine 5′-triphosphate, 10 μmoles phosphoenolpyruvate, 5 μmoles TTP, 4 μmoles α-glucose 1-phosphate, 0.2 mg lactic dehydrogenase containing pyruvic kinase, and 13 mg of the ammonium sulfate fraction in a final volume of 3.3 ml, pH 8.0. The pyruvic kinase and catalytic amounts of adenosine 5′-triphosphate were added to regenerate any TTP which was hydrolyzed by contaminating enzymes. After 1-h incubation at 37° protein was removed by precipitation with $HClO_4$ and the nucleotides isolated by adsorption on charcoal, followed by paper chromatography in the neutral ethanol–ammonium acetate solvent. 1.7 μmoles of thymidine diphosphate glucose were obtained.

The isolated material pooled from several preparations had a typical thymidine spectrum both in neutral and alkaline solution. The ratio of thymidine to acid labile phosphate to total phosphate was 1:0.92:2.1.

After mild acid hydrolysis (pH 2, 100°, 20 min) all the nucleotide was converted to a compound with the mobility of TDP. Mild-acid hydrolysis released 0.8 μmole glucose/μmole of thymidine (determined enzymically with hexokinase and glucose 6-phosphate dehydrogenase).

When treated with snake-venom nucleotide pyrophosphatase 0.8 μmole α-glucose-1-phosphate was formed/μmole of nucleotide (determined with crystalline muscle phosphoglucomutase and glucose 6-phosphate dehydrogenase), indicating that the glucose is linked in the α-configuration. These data show that the new nucleotide has the structure of thymidine diphosphate glucose.

When [^{14}C]glucose-labelled thymidine diphosphate glucose was incubated with the sonic extract which had been centrifuged at 100,000 × g for 1 h to remove particulate material, in the presence of a DPNH-generating system (DPN, ethanol and crystalline yeast alcohol dehydrogenase) a sugar could be isolated from the nucleotide fraction which had the mobility of authentic rhamnose when chromatographed using either butanol–pyridine–water (6:4:3) or butanol–acetic acid–water (52:13:35) as the solvent. After addition of rhamnose carrier, rhamnose phenylosazone was prepared and crystallized to constant specific activity. No rhamnose was formed in the absence of a DPNH-generating system. When uridine diphosphate glucose was used as the substrate, no rhamnose was formed.

The enzymic synthesis of thymidine diphosphate rhamnose is formally similar to the enzymic synthesis of guanosine diphosphate fucose from guanosine diphosphate mannose, described by GINSBURG[8]. Attempts are in progress to determine the intermediates in the enzymic synthesis of thymidine diphosphate rhamnose.

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Department of Biological Chemistry,
Washington University School of Medicine,
St. Louis, Mo. (U.S.A.)

STUART KORNFELD
LUIS GLASER

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Hydrogen–deuterium exchange of small peptides in aqueous solution

Measurements of the rate of hydrogen–deuterium exchange in non-intramolecularly hydrogen-bonded peptides and peptide analogs in aqueous solution will greatly facilitate the quantitative interpretation[1,2] of H–D exchange in proteins and polypeptides as measured with the exchange technique of LINDERSTRØM-LANG[3]. A recent communication[4] has reported some preliminary infrared measurements of the H–D exchange in N-methylacetamide. Such studies have now been extended to some simple peptides.

To measure the rate of the exchange reaction of the peptide group

$$-CO-NH- + D_2O \rightarrow -CO-ND- + HDO$$

125 μl of a 2 % solution of the peptide in H_2O was lyophilized in a small test tube over conc. H_2SO_4. Subsequently a 1-ml syringe with a spring-loaded piston was filled with approximately 230 μl 99.8 % D_2O containing either small amounts of buffer acids and salts or of HCl. The lyophilized peptide was quickly dissolved by emptying the syringe into the test tube via a special two-way valve with a 0.3-mm bore and pumping the solution up and down a few times. Immediately following this operation the valve was turned, allowing the reaction mixture to be forced directly into a 0.10-mm optical cell with fluorite windows placed in the beam of a Perkin-Elmer Model 13U Infrared Spectrometer arranged for single-beam operation with a fluorite prism. In order to keep the cell temperature constant, thermospacers at 22° were used. The exchange reaction was followed by recording the increasing transmission at 1580 cm^{-1} (essentially the disappearance of the amide II band of the protonated peptide group). In the majority of cases it was possible to start following the course of the exchange reaction approximately 10 sec after its inception. "pD" was determined in the combined reaction mixtures from duplicate runs as described elsewhere[5].

With dipeptides (Gly–Gly, Gly–Ala, Gly–Leu, Ala-Gly, Ala–Ala) the exchange reaction of the peptide group was found to follow first-order kinetics up to at least 75 % conversion. (Amine and carboxyl hydrogen atoms exchange at a rate too fast