Short Communication

Guanosine-3'-diphosphate Synthesis by Nucleotide Pyrophosphokinase-Ribonuclease Reaction

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Since guanosine-3'-diphosphate was recently shown to be involved as a natural glucocorticoid amplification principle in the rat liver system, its ready availability is now urgently desired. This nucleotide was assumed to be excreted in extremely low concentrations from several enteric bacteria, Proteus and Lactobacillus species, into the intestinal tract and to markedly enhance the pleiotropic hormone actions in the host animal. We now report a method for enzymic synthesis of the compound. Streptomyces adephospholyticus nucleotide pyrophosphokinase (E.C.2.7.6.4) transfers the 5'-β, γ-pyrophosphoryl group of ATP or dATP to UpG at the 3'-position to give AMP or dAMP and UpGpp3) which in turn is split by pancreas ribonuclease into Up and Gpp. dATP, 6 mm, and UpG (Sigma Chem. Co.), 5 mm, were reacted with 0.7 units of the kinase in 50 μl of 0.08 M Tris-HCl, pH 9.0-7.5 mm MgCl2. The reaction was found completed in 2 hours at 37°C as judged by the disappearance of UpG when 2 μg ribonuclease was added and incubation continued for another 1 hour. Avicel thin layer chromatographic analysis of the reaction with an isobutyric acid-0.5 M NH₄OH (pH 3.6) solvent system showed that two new ultraviolet absorbing spots appeared in the kinase reaction (Fig. 1). One is dAMP. The other, the slowest, disappeared on ribonuclease treat-ment, and instead yielded another new spot migrating a little faster than the former and also very close to marker 5'-GDP, well separated from all the other spots, namely dAMP, dATP which remained unreacted and Up which is formed from UpGpp with ribonuclease treatment and almost overlaps dATP. This new spot was isolated by thin layer separation followed by extraction with 0.05 M NH₄HCO₃, evaporation under reduced pressure and freeze-drying. The product was structurally characterized by various degradation methods (Fig. 2). Alkaline hydrolysis gave guanosine-3'(2')-monophosphate and Pi; E. coli phosphatase,3) guanosine and Pi; Penicillium nuclease P1 (Yamasa Shoyu Co.), guanosine and Pi without any intermediary formation of Gp4); Zn²⁺-activated yeast inorganic pyrophosphatase (Sigma Chem. Co.),5) Gp and Pi; rye-grass 3'-nucleotidase (Sigma Chem. Co.),5,6) inert; the pyrophosphatase followed by the 3'-nucleotidase,5) guanosine and Pi; T₁ and pancreas ribonucleases, inert. Pi and PPI were detected on the thin layer plate with a molybdate—ascorbic acid spray.7) Colorimetric determination of PPI5) indicated equimolar formation of guanosine (24.1 nmole) and PPI (23.6 nmole) on 2 hr incubation with

Fig. 1. dATP-UpG Reaction Analysis.
Thin layer chromatograms were recorded with a Shimadzu TLC scanner CS 900 at 270/320 nm. 1 & 2, before and after ribonuclease digestion; 3-9, UpG, dATP, dAMP, Up, guanosine, 5'-GDP and Gp, respectively, as markers.
Fig. 2. Structural Characterization of Guanosine-3'-diphosphate.

1, Control, ca. 1 m\textmu mole product; 2, 0.25 N KOH, 37°C, overnight; 3, 0.1 \textmu g \textit{E. coli} alkaline phosphatase, pH 8.9, 37°C, 50 min; 4, 0.25 \textmu g nuclease P1, pH 6, 37°C, 50 min; 5, 5 \textmu g pyrophosphatase, 2 mm ZnCl2, pH 7.0, 37°C, 50 min; 6, 0.15 \textmu g rye-grass 3'-nucleotidase, pH 7.0, 37°C, 50 min; 7, 5 followed by 6; 8, 0.5 \textmu g ribonuclease T1, pH 8.0, 37°C, 50 min; 9, 0.1 \textmu g pancreas ribonuclease, pH 8.0, 37°C, 50 min; 10-13, guanosine, Gp, pG, and 5'-GDP, respectively, as markers; \textrightarrow, Pi; \textrightarrow, PPi.

nuclease P1 under the same conditions. These data together allowed us to conclude that the compound obtained is guanosine-3'-diphosphate. The present method is applicable to the preparation of any 3'-diphosphates by the use of a suitable short oligonucleotide acceptor with the desired nucleotide at the 3'-hydroxy terminus, nucleotide pyrophosphokinase and an enzyme capable of splitting off the penultimate phosphodiester bond.

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\textbf{REFERENCES}