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Identification of Guanosine 5′-Triphosphate and Uridine 5′-Triphosphate in Subcellular Monoamine-Storage Organelles

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The subcellular organelles storing biogenic monoamines, e.g. the 5-hydroxytryptamine-storage organelles of blood platelets and the chromaffin granules of adrenal medulla, contain substantial amounts of ATP. This nucleotide has been shown to form high-molecular-weight aggregates with amines *in vitro* and *in vivo* (Berneis, Da Prada & Pletscher, 1969*a*; Berneis, Pletscher & Da Prada, 1969*b*, 1970). Aggregation with ATP seems to be a mechanism by which the amines are stored within the organelles. Thereby additional factors might be involved since the apparent molecular weights of the contents of 5-hydroxytryptamine-storage organelles of blood platelets are higher than those of corresponding artificial mixtures of 5-hydroxytryptamine plus ATP (Berneis *et al.* 1969*a*).

The present paper demonstrates the presence of other nucleotides, i.e. GTP and UTP, in amine-storage organelles of blood platelets and adrenal medulla.

Isolation of subcellular organelles storing 5-hydroxytryptamine or catecholamines was carried out by ultracentrifugation of homogenates of isolated platelets of rabbits (Da Prada & Pletscher, 1968) or of bovine adrenal medulla (M. Da Prada & A. Pletscher, unpublished work) in a continuous urografin–sucrose gradient. The organelles were then destroyed by addition of water (0.1ml to organelles obtained from 300ml of blood or from about 1g of adrenal medulla respectively). After removal of the membrane debris by centrifugation at 90000*g* for 15min, the supernatant was directly submitted to paper chromatography and to rechromatography (see below). U.v. spectroscopy was carried out with a DB-G spectrophotometer (Beckman) and mass spectroscopy with an MS9 instrument (AEI, Manchester, U.K.).

The following substances were used as references for chromatography, for u.v. spectroscopy and for radioassays: adenine, guanine, uracil, hypoxanthine, cytidine, thymine; adenosine, guanosine, uridine, inosine, cytosine, thymidine and their 5′-mono-, -di- and -tri-phosphates (all from Fluka A.G., Buchs, Switzerland, or Sigma Chemical Co., St Louis, Mo., U.S.A.); [8-^{14}C]guanine sulphate (sp. radioactivity 36.1mCi/mmol) and [U-^{14}C]-guanosine (sp. radioactivity 518mCi/mmol) (both from The Radiochemical Centre, Amersham, Bucks., U.K.).

On initial paper chromatography (Schleicher–Schuell no. 2043), solvent isobutyric acid–water–aq. NH_3 (sp.gr. 0.91) (66:33:1, by vol.) of the contents of the 5-hydroxytryptamine-storage organelles and of the adrenal chromaffin granules, several u.v.-absorbing (254nm) components were obtained. Besides the known constituents ATP and ADP, a new, distinct, spot not previously described in storage organelles appeared (Fig. 1). It showed R_F 0.08–0.12, i.e. similar to that of GTP (0.11), UTP (0.12) and ITP (0.11), but smaller than that of the 5′-mono- and -di-phosphates of the same nucleotides as well as of the 5′-mono-, -di- and -tri-phosphates of adenosine, cytosine and thymidine. The corresponding nucleosides and free bases migrated even more than the nucleotides. In addition to the distinct spot a faint spot with R_F about 0.17, which corresponds to that of GDP, was found (Fig. 1). The material forming the distinct spot showed two components when resubmitted to chromatography in two systems in which GTP as well as UTP can be separated from other nucleotide triphosphates [Schleicher–Schuell 2043 paper, solvent propan-2-ol–HCl–water (65:16.6:18.4, by vol.); or precoated cellulose F t.l.c. plates (0.1mm thick; E. Merck A.-G., Darmstadt, Germany), solvent 0.1M-sodium phosphate buffer, pH 6.8–satd. ammonium sulphate–propan-1-ol (50:30:1, by vol.)]. The major part of the material had R_F values corresponding to GTP (0.38 and 0.67 respectively), whereas a minor part migrated like UTP (R_F 0.71, 0.81) (Fig. 1). The R_F values of ITP are different (0.34, 0.74). The spot corresponding to GTP showed a violet fluorescence in u.v. light, which is typical of derivatives of guanine and xanthine in acidic medium. The material (GDP-like) constituting the faint spot in the initial paper chromatography migrated like authentic GDP (R_F 0.58) when rechromatographed on cellulose; UDP and IDP showed different R_F values. All the new spots obtained by chromatography reacted with Hanes' reagent (ammonium molybdate–

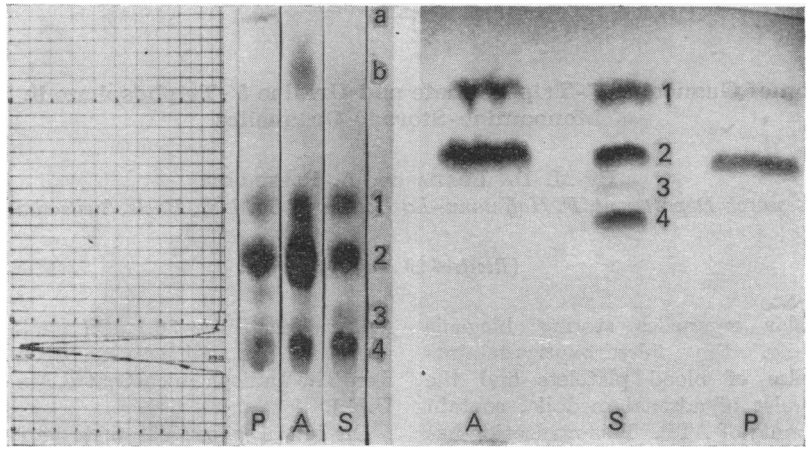

Fig. 1. Chromatograms of isolated 5-hydroxytryptamine-storage organelles of rabbit blood platelets (P) and isolated bovine adrenomedullary granules (A); S, standards. (*a*) Paper chromatogram (Schleicher–Schuell no. 2043; isobutyric acid system) and radiochromatogram of isolated 5-hydroxytryptamine-storage organelles of platelets preincubated with [^{14}C]guanosine. The paper chromatogram was developed with Hanes' reagent; spots 1–4 show the blue colour typical for phosphate esters. 1, ADP; 2, ATP; 3, GDP; 4, GTP; a, 5-hydroxytryptamine; b, adrenaline+noradrenaline. (*b*) Re-chromatography (on cellulose F t.l.c. plates; 0.1M-phosphate buffer system) of the material corresponding to spots 4P and 4A on paper chromatogram (*a*). Detection was by u.v. light. 1, UTP; 2, GTP; 3, GDP; 4, GMP.

perchloric acid) (Fig. 1), indicating the presence of phosphate esters.

The u.v. spectra at pH 1.0, 7.0 and 11.0 of the GTP-like material eluted from the cellulose were identical with those of authentic GTP and different from those of ATP with regard to $\lambda_{max.}$ and shape. The spectra of the UTP-like material corresponded to those of authentic UTP and differed in their $\lambda_{max.}$ from ATP and GTP.

The GTP- and UTP-like material was eluted from the cellulose plates and hydrolysed with 2M-HCl at 100°C for 20min (Thomson, 1969). Rechromatography on paper (isobutyric acid system, as described above) showed two spots with R_F values corresponding to guanine and uracil respectively. The mass spectra of the eluted material representing these spots were identical with those of authentic guanine and uracil respectively. From our preliminary results, the amount of GTP is 15–20% and of UTP 3–6% of that of the ATP in the organelles of both platelets and adrenal medulla.

Experiments with labelled precursors of GTP indicate that blood platelets of rabbits are able to synthesize GTP and that the newly synthesized nucleotide is accumulated in the 5-hydroxytryptamine-storage organelles. Thus, after incubation of isolated platelets with [^{14}C]guanine (5.2μg/ml) or [^{14}C]guanosine (0.5μg/ml) for 2h at 37°C in Tyrode solution (Bartholini, Pletscher & Gey, 1961; Bartholini & Pletscher, 1964), a labelled compound was found in the storage organelles that behaved like [^{14}C]GTP on paper chromatography (isobutyric acid system, as described above) (Fig. 1) and on rechromatography on cellulose plates. The concentration of the labelled nucleotide (relative to the protein content) in the organelles was 10–50 times that in the other subcellular particulate fractions. Contamination of the endogenous GTP with [^{14}C]guanine or [^{14}C]guanosine has been excluded, since the labelled substances added to the contents of the isolated 5-hydroxytryptamine-storage organelles or to authentic unlabelled GTP were completely separated from the GTP (endogenous and authentic) on paper chromatography.

It may therefore be concluded that the amine-storage organelles of blood platelets and of adrenal medulla contain nucleotides other than ATP, especially GTP and in a smaller quantity also UTP. Whether the ADP and the small amounts of GDP found represent degradation products of the corresponding triphosphates occurring during the isolation procedure remains to be investigated. GTP might also be involved in the storage of biogeni camines. Thus preliminary results indicate that this nucleotide has a considerably greater tendency than ATP to form high-molecular-weight aggregates with 5-hydroxytryptamine and nor-

adrenaline. UTP, on the contrary, seems to aggregate little with these amines.

Mass spectroscopy was carried out by Dr W. Vetter, Department of Physics, F. Hoffmann–La Roche and Co. Ltd., Basle.

Bartholini, G. & Pletscher, A. (1964). *Experientia*, **20**, 376.

Bartholini, G., Pletscher, A. & Gey, K. F. (1961). *Experientia*, **17**, 541.

Berneis, K. H., Da Prada, M. & Pletscher, A. (1969*a*). *Science, N.Y.*, **165**, 913.

Berneis, K. H., Pletscher, A. & Da Prada, M. (1969*b*). *Nature, Lond.*, **224**, 281.

Berneis, K. H., Pletscher, A. & Da Prada, M. (1970). *Br. J. Pharmac. Chemother.* (in the Press).

Da Prada, M. & Pletscher, A. (1968). *Br. J. Pharmac. Chemother.* **34**, 591.

Thomson, R. Y. (1969). In *Chromatographic and Electrophoretic Techniques*, 3rd ed., vol. 1, p. 295. Ed. by Smith., I. London: W. Heinemann Medical Books Ltd.