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Note

Sensitive spray reagent for guanine nucleotides

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The major ribonucleoside mono-, di-, and triphosphates can be conveniently separated by thin-layer chromatographic systems such as the polyethyleneimine (PEI)-impregnated cellulose method developed by Randerath and Randerath[1]. The simplest method of identifying these nucleotides on thin-layer plates with a sensitivity of 0.5 to 1 nmole using the commercially available UV fluorescent indicator plates is by their UV absorbance. There are, however, very few nucleoside-specific detection methods available. The adenine-containing nucleotides can be specifically detected after reaction with chloroacetaldehyde. This leads to the formation of the highly fluorescent 1,N^6-etheno adduct of adenine[2]. We demonstrate here a simple, sensitive, and specific method for the detection of guanine nucleotides utilizing their ability to enhance specifically the Tb^{3+} fluorescence.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials

Guanine nucleotides were obtained from P-L Biochemicals (Milwaukee, WI, U.S.A.). ATP, UTP, and CTP were obtained from Sigma (St. Louis, MO, U.S.A.). $Tb(NO_3)_3$ was a product of Alfa Products (Danvers, MA, U.S.A.). Polyethyleneimine-impregnated cellulose thin-layer plates were obtained from Brinkman Instruments (Westbury, NY, U.S.A.).

Methods and results

Nucleotides were separated on PEI-cellulose thin-layer sheets without fluorescent indicator. GTP, dGTP and ATP were developed with 2 *M* HCOOH–2 *M* LiCl (1:1); GDP, CTP, UTP with 2 *M* HCOONa, pH 3.45; and 5′-GMP, 2′-GMP, 3′-GMP with 1 *M* HCOONa, pH 3.45, cGMP with 0.25 *N* LiCl. The developed plates were dried and soaked in absolute methanol for 10 min to remove salt and acid from the plates[1]. The plates were again air dried and were sprayed with a freshly made solution of 10 m*M* $Tb(NO_3)_3$. The plates were dried in a stream of hot air and were then viewed under a short UV light source. Nucleosides were developed with water and dried before spraying.

Nucleoside tri- and diphosphates were also separated by developing with 0.85 *M* or 0.5 *M* KH_2PO_4, pH 3.45 (ref. 3). The high concentration of phosphate on these plates were removed by washing the plates for 15 min in 10% citric acid following by

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2 washes in water[4] before spraying with $Tb(NO_3)_3$. Chromatograms containing nucleoside di- and triphosphates can be soaked in 10 m*M* $Tb(NO_3)_3$, 10 m*M* Tris-OAc, pH 8 for 3 min with occasional shaking instead of spraying.

After treatment with $Tb(NO_3)_3$, the guanine nucleotides appeared as greenish-yellow fluorescent spots under short UV light. Fig. 1 shows a typical comparison between unsprayed and sprayed chromatograms. A visual detection limit of about 0.1 to 1 nmole was obtained depending on spot size. This limit is approximate to that obtained for visualizing under UV with a fluorescent indicator plate. Neither ATP, ITP, UTP, nor CTP fluoresce under this condition even when applied at 100 nmoles per spot.

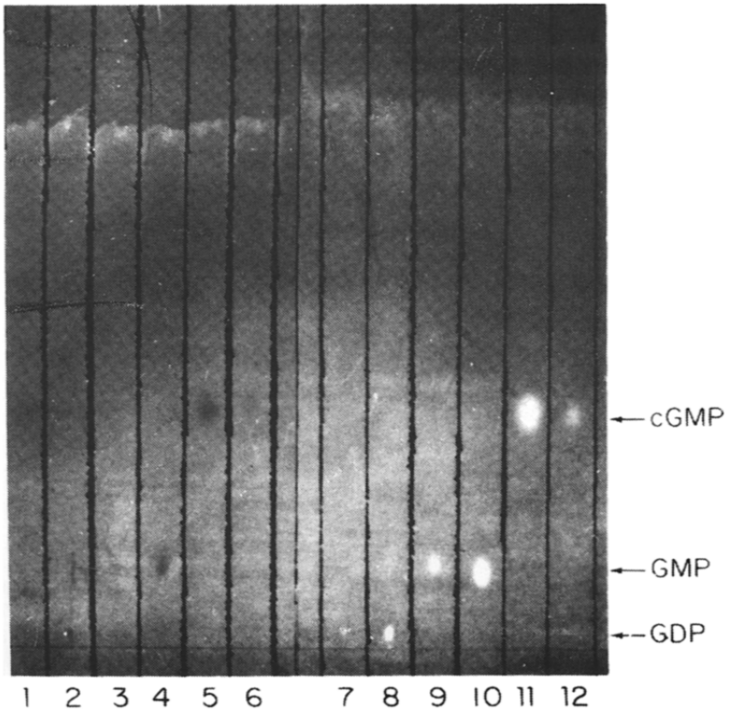

Fig. 1. Tb^{3+} fluorescence enhancement by guanine nucleotides on a thin-layer plate. The following amounts of nucleotides were applied to PEI-cellulose plates: GDP, 0.1 nmole (1, 7) and 1 nmole (2, 8); GMP, 1 nmole (3, 9) and 5 nmoles (4, 10); 3′,5′-cyclic GMP, 5 nmoles (5, 11) and 1 nmole (6, 12). After developing with 0.5 *M* LiCl and washing with absolute methanol, one half of the chromatogram (lanes 7 to 12) was sprayed with $TbNO_3$ as described in *Methods and results*. After drying, the chromatograms were photographed under short-wave UV light.

The following nucleotides were found to fluoresce after spraying with Tb^{3+}: GTP, dGTP, GDP, dGDP, 5′-GMP, 2′-GMP, 3′-GMP, 2′,3′-cyclic GMP, 3′,5′-cyclic GMP, GDP-glucose, GDP-mannose, and xanthosine triphosphate. Guanosine but not adenosine also fluoresced.

DISCUSSION

The fluorescence emission of Tb^{3+} at 490 and 545 nm is greatly increased on binding of Tb^{3+} to nucleic acids[5–8]. This enhancement has been attributed to the complex formation between Tb^{3+} and guanine residues[5–8]. Studies on mononucleotide enhancement of Tb^{3+} emission indicate that of the commonly occurring nucleotides only the ribo- and deoxyriboguanosine phosphates as well as xanthosine triphosphate are effective[5,8]. GTP is reported to enhance substantially better than GDP[9]. 2′,3′-Cyclic GMP and 3′,5′-cyclic GMP and guanosine were reported not to enhance Tb^{3+} fluorescence[5,9]. However, when used as a spraying reagent, Tb^{3+} fluorescence was enhanced by all guanosine-containing compounds and there were no significant differences in the detection limit of these compounds. These results suggest that charge interaction between the phosphate residues of the guanosine compounds and Tb^{3+}, which is essential for fluorescence enhancement in solution, is not required. This is possibly due to the close proximity of guanosine residue and Tb^{3+} in the "dry" stage of a thin-layer chromatogram.

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