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## Note

# Thin-layer chromatography of cyclic 3′,5′-guanosine monophosphate on tetraborate-impregnated silica gel layers

UPTON[1] utilized the tendency of borate ions to form complexes with the 2′,3′-*cis*-diol grouping on simple sugars[2] for the development of a simple and efficient procedure for the separation of cyclic 3′,5′-adenosine monophosphate (cAMP) from other adenine derivatives. In the present study, tetraborate-impregnated silica gel layers were utilized for the separation of cyclic 3′,5′-guanosine monophosphate (cGMP) from other guanine derivatives.

*Method*

Thin-layer chromatographic plates were prepared by using a slurry made up of 30 g of Silica Gel $GF_{254}$ (Merck) and 60 ml of 5 % (w/v) aqueous sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). The film thickness was 250 or 500 $\mu$m and the plates were heated at 110° for 1 h. Substrates in water or in 50 % ethanol (50–100 $\mu$l of 0.05 % solution or radioactive sample) were applied with a capillary tube; care was taken to ensure that the spot was not more than 0.5 cm in diameter. Ascending chromatography was carried out with the solvent system *n*-butanol–methanol–ethyl acetate–ammonium hydroxide (1:1:1:1), and the development time was approximately 3 h for a plate 20 cm long. After development, the plates were dried at 22° and the spots were made visible by using UV light.

The assay of guanyl cyclase in rat lung preparation, except the cGMP separation, was identical with the procedure of WHITE AND AURBACH[3] involving the use of [$\alpha$-$^{32}$P]GTP.

TABLE I

TLC OF GUANINE DERIVATIVES ON TETRABORATE-IMPREGNATED LAYERS

Developing solvent: *n*-butanol–methanol–ethyl acetate– ammonium hydroxide (1:1:1:1).

| *Compound* | *$R_F$ value* |
|---|---|
| cGMP | 0.42 |
| 5′-GMP | <0.01 |
| 5′-GDP | <0.01 |
| 5′-GTP | <0.01 |
| Guanosine | 0.25 |
| Guanine | 0.53 |
| Inosine | 0.32 |
| Xanthine | 0.59 |
| Hypoxanthine | 0.68 |
| Theophylline | 0.76 |

*Results*

The separation of cGMP from other guanine derivatives on tetraborate-impregnated layers was very satisfactory. Table I shows the $R_F$ values of the main guanine derivatives and some of its metabolites. The spot of cGMP did not overlap with the spots of any of the other drugs used in these experiments.

TABLE II

EFFECT OF $MnCl_2$ ON GUANYL CYCLASE ACTIVITY IN RAT LUNG SUPERNATANT

Guanyl cyclase activity in 120 $\mu$g of supernatant protein was determined after incubation for 15 min at 37° by measuring the production of [$^{32}$P]cGMP under the conditions described by WHITE AND AURBACH[3]. cGMP was separated on tetraborate-impregnated silica gel layers, as described in the text. Each figure is a mean value of duplicate determinations after the subtraction of the reaction blank value.

| *Treatment with $MnCl_2$ (concn. × $10^{-3}M$)* | *Guanyl cyclase activity: cGMP (pmole/mg protein/15 min)* |
|---|---|
| 0 | 5 |
| 1 | 316 |
| 3 | 517 |
| 10 | 450 |

Some radioactive impurity in commercial [$\alpha$-$^{32}$P]GTP moves with cGMP on tetraborate-impregnated layers. However, the blank was less than 0.04% of added radioactivity in [$\alpha$-$^{32}$P]GTP. When high concentrations of guanyl cyclase are present, the reaction rate becomes great enough to make the blank acceptable, and a single chromatographic procedure on tetraborate-impregnated layers can be used for the determination of guanyl cyclase activity (Table II). The results of experiments in which guanyl cyclase was stimulated with $MnCl_2$ were similar to those obtained in other guanyl cyclase assays, *e.g.*, that of WHITE AND AURBACH[3], or our own yet unpublished method[4] in which cGMP is separated on Dowex 50 and subsequently purified by precipitation with $ZnSO_4$ and $Ba(OH)_2$ at pH 6.2–6.4.

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