J. Clin. Chem. Clin. Biochem.
Vol. 14, 1976, pp. 469–473

Determination of Serum Nucleotidase with Cytidine Monophosphate as Substrate

Part II: Improvement of the procedure

By *P. J. van der Kooij, A. Hout, J. P. Persijn* and *W. van der Slik*

From the Department of Clinical Chemistry (Head: Dr. W. van der Slik), University Hospital, Leiden; the Department of Biochemistry (Head: Dr. F. Berends), Medical Biological Laboratory, T.N.O., Delft; and the Department of Clinical Chemistry (Head: Dr. J. P. Persijn), Antoni van Leeuwenhoekziekenhuis, Amsterdam, The Netherlands

(Received September 17, 1975/June 27, 1976)

Summary: Serum nucleotidase activity (EC 3.1.3.5) is measured by the amount of ammonia liberated from cytidine after incubation with cytidine deaminase (EC 3.5.4.5). Purification of the cytidine deaminase results in the abolition of interfering reactions, so that routine application is possible.

Bestimmung von Nucleotidase im Serum mit Cytidinmonophosphat als Substrat. Teil II: Verbesserung der Methode

Zusammenfassung: Die Aktivität des Serums gegenüber Cytidin-5′-monophosphat wird bestimmt, indem das durch Inkubation mit Cytidindesaminase freigesetzte Ammoniak gemessen wird. Die Reinigung des Hilfsenzymes beseitigt Störreaktionen und ermöglicht dadurch die routinemäßige Anwendung dieses Prinzipes.

Introduction

Recently, we described a new coupled colorimetric assay for serum activity toward cytidine-5′-monophosphate (5′-nucleotidase activity, EC 3.1.3.5) based on measurement of the equivalent amount of ammonia released from cytidine by cytidine deaminase (EC 3.5.4.5) (1). In developing this assay we used a crude cytidine-deaminase preparation as the auxiliary enzyme, which resulted in rather high background extinction values and an overcorrection with respect to the reagent-control, probably due to an enzyme contaminating the cytidine deaminase. To circumvent this problem, we offered an indirect method. In this communication we prove that a simple and rapid purification of the cytidine deaminase is sufficient to give a reliable direct method, with none of the above disadvantages.

Materials and Methods

Reagents

1. Buffer solution:
   Dissolve 4.20 g sodium diethylbarbiturate and 6.30 g $MgSO_4 \cdot 7H_2O$ in about 800 ml of distilled water. Adjust the pH to 7.50 with HCl (1 mol/l) and dilute to 1,000 ml.
2. Cytidine deaminase solution:
   Dilute the stock cytidine deaminase (see below) with buffer solution to give an activity of about 225 U/l and dissolve 28 mg disodium phenylorthophosphate (British Drug Houses) per 10 ml of this solution (phenylphosphate concentration about 11 mmol/l). Remains stable for about one day at 4°C.
3. Cytidine-5′-monophosphate solution (38.5 mmol/l):
   Dissolve 458 mg cytidine-5′-monophosphate (Boehringer; disodium salt, 6 H_2O, free of cytidine) in 25 ml buffer solution. Prepare just before use.
4. EDTA solution:
   Dissolve 5.6 g EDTA (dipotassium salt, 2 H_2O) in distilled water and make up to 50 ml.
5. Concentrated phenol reagent:
   Dissolve 50 g phenol (A.R. grade) and 0.25 g disodium pentacyano-nitrosylferrate (A.R. grade) in distilled water and make up to 1,000 ml. Stable in an amber bottle at 4°C for at least two months.
6. Concentrated alkali-hypochlorite reagent:
   Dissolve 25 g sodium hydroxide in 60 ml distilled water. Add 72 ml 0.5 mol/l NaClO solution (e.g. from British Drug Houses, in 1 mol/l NaOH) and make up to 1,000 ml. Stable in an amber bottle at 4°C for at least two months.
7. Working phenol/EDTA colour reagent:
   Dilute 1 volume concentrated phenol reagent with 4 volumes distilled water. To 100 ml solution add 2 ml EDTA solution. Prepare fresh before use.
8. Working alkali-hypochlorite colour reagent:
   Dilute 1 volume concentrated alkali-hypochlorite reagent with 4 volumes distilled water. Prepare fresh before use.
9. Cytidine standard solution (3.74 mmol/l):
   Dissolve 92 mg cytidine (Boehringer, more than 99% pure) in saturated benzoic acid and dilute to 100 ml. Stable for months at 4°C.