Demonstration of a Deoxycytidine Kinase Activity in Extracts of Bacillus megaterium KM

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Extracts of Bacillus megaterium KM have been shown to possess a deoxycytidine kinase activity which functions optimally with guanosine triphosphate as the phosphoryl donor.

Although mammalian cells have been reported to possess deoxycytidine (dC) kinase activity (5, 9, 10), this activity has not been detected in a variety of bacteria including Salmonella typhimurium (11), Diplococcus pneumoniae (7), and Escherichia coli B (8). In addition, E. coli B was also found to lack kinases for the phosphorylation of both deoxyadenosine (dA) and deoxyguanosine (dG) (8). In contrast to these findings, dC kinase activity was recently demonstrated in extracts of both Lactobacillus acidophilus (6) and L. leichmannii (J. W. Powell and J. T. Wachman, unpublished data).

We recently reported that extracts of Bacillus megaterium KM catalyze the phosphorylation of thymidine, dA, and dG (14). Incubation of B. megaterium extracts with radioactive dC as the phosphoryl acceptor and deoxyadenosine triphosphate (dATP) as the donor resulted in the accumulation of deoxyuridylic acid (dUMP), with no detectable formation of deoxycytidylic acid (dCMP). We concluded that, although extracts of B. megaterium lack detectable dC kinase activity, they do catalyze the deamination of dC to deoxyuridine (dU) and the phosphorylation of dU to dUMP (14).

A continuation of these experiments has shown that dC kinase activity can be demonstrated in B. megaterium extracts, if guanosine triphosphate (GTP) is supplied as the phosphoryl donor. When GTP-containing reaction mixtures (14) were subjected to two-dimensional chromatography on Whatman 3MM paper (n-butanol-water [86:14, vol/vol] in the first dimension and isopropanol-concentrated hydrochloride-water [65:16:7:18.3, vol/vol/vol] in the second dimension), dCMP formation was found to be linear for 30 to 60 min at 37 C.

In addition, the presence of tetrahydouridine (THU) (final concentration of 50 μg per 0.10 ml of reaction mixture) resulted in an 80 to 90% decrease in the rate of dUMP formation. THU, therefore, effectively increased the sensitivity of the dC kinase assay by decreasing the amount of radioactivity in the dUMP region of the two-dimensional chromatogram, thereby minimizing trailing into the dCMP region.

THU has been reported to inhibit a variety of different pyrimidine nucleoside deaminases (3), including those that use dC as a substrate (2). The deoxycytidine deaminase activity of B. megaterium extracts was found to be inhibited by about 95%, with THU concentrations ranging from 3 to 100 μg per 0.10 ml of reaction mixture. In these experiments, the conversion of 3H-dC to 3H-dU was monitored by chromatography of reaction mixtures on thin-layer cellulose sheets (Brinkmann MN-Polygram Cel 300), by using n-butanol-water (86:14, vol/vol) as the developing solvent.

Table 1 shows the effect of THU and different phosphoryl donors on the formation of both dCMP and dUMP from 3H-dC by extracts of B. megaterium. Reactions were carried out in 0.10 M phosphate buffer, pH 7.5, and activity was monitored by chromatography on sheets of thin-layer PEI cellulose, by using 0.12 N formic acid as the developing solvent. It is apparent that in the presence of THU, dCMP formation is optimal with GTP as the phosphoryl donor, followed by deoxyguanosine triphosphate, deoxythymidine triphosphate, and cytidine triphosphate, etc., in order of decreasing effectiveness. With most phosphoryl donors, the presence of THU resulted in a 4- to 10-fold decrease in the rate of dUMP formation. Curiously, the effect of THU on dCMP formation was found to vary with the nature of the phosphoryl donor. With GTP as the donor, the

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Table 1. Effect of tetrahydrouridine (THU) and different phosphoryl donors on the formation of dCMP and dUMP from dC

| Donor | Product formed | nmoles formed/60 min |
|-------|---------------|----------------------|
|       | +THU | -THU |
| GTP   | dCMP | 0.86 | 0.64 |
|       | dUMP | 0.21 | 0.29 |
| dGTP  | dCMP | 0.56 | 0.66 |
|       | dUMP | 0.20 | 0.88 |
| dTTP  | dCMP | 0.39 | 0.23 |
|       | dUMP | 0.10 | 0.07 |
| CTP   | dCMP | 0.35 | 0.13 |
|       | dUMP | 0.01 | 0.40 |
| ATP   | dCMP | 0.29 | 0.66 |
|       | dUMP | 0.37 | 1.95 |
| dCTP  | dCMP | 0.22 | 0.32 |
|       | dUMP | 0.13 | 0.63 |
| dATP  | dCMP | 0.14 | 0.30 |
|       | dUMP | 0.19 | 1.92 |

*Strain KM-T*; a previously described thymineless strain of *B. megaterium* KM (13), was grown at 37°C in a basal medium supplemented with 0.5 mM thymine (14). Exponentially growing cells were disrupted with lysozyme, the cell lysate was centrifuged at 0°C for 20 min at 27,000 × g, and the resulting supernatant fraction was dialyzed twice at 4°C against 100 vol of 0.01 M tris(hydroxymethyl)aminomethane (pH 7.4) containing 2 mM MgSO₄. Activity was determined in 0.10-ml reaction mixtures containing 0.10 M phosphate buffer, pH 7.5, 30 μg of bovine serum albumin, 2.5 mM dithiothreitol, 10 mM nucleoside triphosphate, 3 mM deoxycytidine G-3H (New England Nuclear, Boston, Mass.) of 1.4 × 10⁶ counts per min per μmol, and 270 μg of cell protein. THU was added at a final concentration of 50 μg/mL 0.10 ml of reaction mixture. To increase the effectiveness of THU (3), reaction mixtures were preincubated with THU for 15 min at 0°C in the absence of deoxycytidine; deoxycytidine was then added and the reaction was allowed to proceed at 37°C. As a function of time, 5-μliter samples were removed from the reaction mixtures and deposited on areas of water-washed sheets of thin-layer PEI cellulose (Brinkmann Polygram Cel 300 PEI) containing 25 nmol each of the appropriate carriers. The reaction was stopped by the addition of 5 μlitsers of an ice-cold acetic acid-containing solvent mixture (4), and chromatograms were developed by ascending chromatography for 2.5 h with 0.12 N formic acid. The approximate R₅ values observed for dUMP, dCMP, and a mixture of dC plus dU were 0.16, 0.39, and 0.73, respectively. Carrier compounds were located as ultraviolet-absorbing areas which were cut out, and the radioactivity was determined by standard scintillation counting. The coincidence between ultraviolet-absorbing area and radioactivity was periodically verified, by cutting out 0.50-cm strips in the direction of solvent flow and counting each strip. The nanomoles of dCMP and dUMP formed per 60 min represent average values of three to four samplings during a 30- to 60-min incubation.

The presence of THU resulted in a minimum of a 30% increase in the rate of dCMP formation, whereas with adenosine triphosphate (ATP) or dATP as donors, there was at least a 50% decrease in the rate of dCMP formation. In the absence of THU, both ATP and dATP are the best phosphoryl donors for dCMP formation, with dU presumably functioning as the direct phosphoryl acceptor. It is possible that these donors can also function in THU phosphorylation, thereby decreasing the available donor level for dCMP formation.

By using the assay conditions described in the legend of Table 1, dCMP formation was found to be linear for 30 to 60 min at 37°C, and linear with respect to protein concentration up to about 60 μg of supernatant protein per 0.10 ml of reaction mixture. dC kinase activity was found to be unaffected by the omission of either bovine serum albumin or dithiothreitol, or by the addition of 3 mM Mg²⁺ or 25 mM ethylenediaminetetraacetate.

Thus, *B. megaterium* extracts have the capacity to phosphorylate dC and the three other deoxynucleosides involved in DNA synthesis. It is possible, however, that one or more of these activities is due to the action of a nucleoside phosphorotransferase (1). Experiments on the purification of the dC phosphorylating system are now in progress. Recently, a dC kinase activity has been reported in extracts of *B. subtilis* (12).

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