BERYLLIUM-INDUCED MISINCORPORATION BY A DNA POLYMERASE: A possible factor in beryllium toxicity.

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Received December 2,1974

SUMMARY: Divalent beryllium increases misincorporation during polymerization of poly-d(A-T) by Micrococcus luteus DNA polymerase. The effect is associated with a strong inhibition of the 3'→5' exonuclease (editing) activity of the enzyme by beryllium relative to the polymerase activity. Possible connections between these results and the toxicity of beryllium are discussed.

The toxic effects of beryllium (Be) are poorly understood in biochemical terms and may involve more than one mechanism. Be is reported to be concentrated in nuclei (1) and there is evidence to suggest it may interfere with DNA metabolism in liver (2-4). In addition, Be is reported to induce chromosomal and mitotic abnormalities in cell cultures (5) and to be carcinogenic in animals (6,7) and possibly in man (8,9). These effects could reflect in part an interference with enzymes associated with DNA polymerization and repair. We consider here the *in vitro* affect of Be on a DNA polymerase isolated from Micrococcus luteus (10-12). This enzyme contains as an integral part of the molecule two exonuclease activities, one active in the 5'→3' direction against double stranded DNA and stimulated by the four deoxynucleotide triphosphates (dNTPs), and another active in the 3'→5' direction against single stranded DNA. The latter activity in a similar enzyme, Escherichia coli DNA polymerase I, can remove nucleotides from single stranded 3'-OH ends of double stranded DNA (13) and is thought to perform an "editing" function to remove non-complementary (incorrect) nucleotides during polymerization.

We report here that Be strongly inhibits the 3'→5' exonuclease activity of M. luteus DNA polymerase relative to the polymerase activity. This is accompanied by an increased incorporation of the non-complementary deoxyribonucleotide, dCTP, into a synthetic template, poly-d(A-T) (misincorporation).

MATERIALS AND METHODS: DNA polymerase from M. luteus was purified and assayed for exonuclease and polymerase activities by the methods of Hamilton et al. (12). DNA, both unlabeled and labeled with [^{3}H]-thymidine, was extracted from E. coli by the method of Grossman (14). Calf thymus DNA 'activated' by pancreatic DNase I was prepared as described by Richardson (15). Single stranded DNA was prepared immediately before use by heat denaturation (100°, 15 min) in a solution of low ionic strength. Poly-d(A-T) was obtained from Collaborative Research, Waltham.

Assays: Exonuclease assays contained 0.1-0.28 unit of DNA polymerase (12), 8.75 μM [^{3}H]DNA (double stranded for 5'→3' activity and single stranded for 3'→5' activity), 50 mM Tris-HCl (pH 8.0), 0.5 mM 2-mercaptoethanol, and

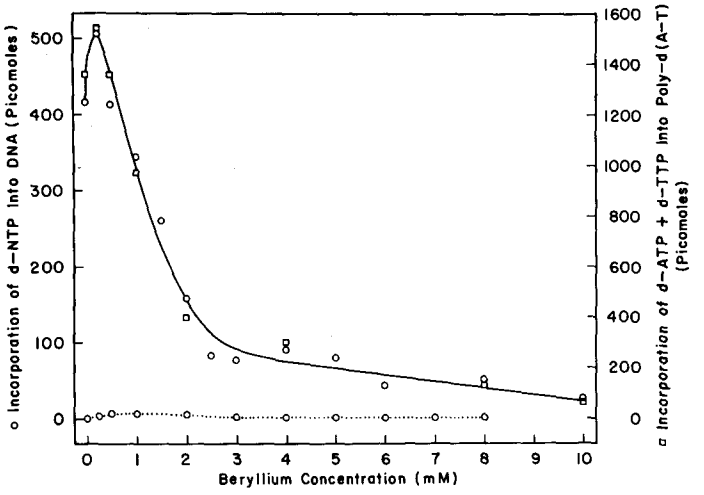

Fig. 1. The effect of Be on the polymerase activity of M. Luteus DNA polymerase. Circles: incorporation of [^{3}H]dATP into 'activated' DNA from calf thymus, 0.1 unit enzyme. Squares: incorporation of [^{3}H]dATP into poly-d(A-T), 0.33 unit enzyme. Dashed line: without Mg. Solid line: 4 mM Mg. Reaction mixtures as described under METHODS were incubated 30 min ('activated' DNA) or 45 min (poly-d(A-T)) at 37°.

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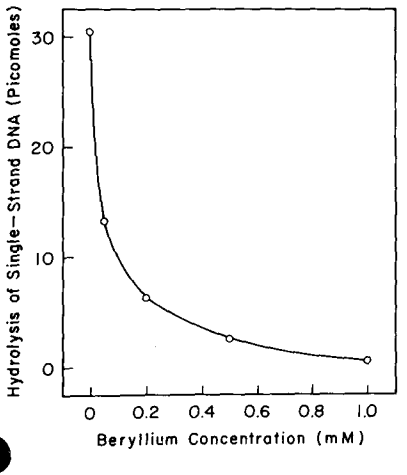
50 mM KCl in a final volume of 0.2 ml. For 5'→3' exonuclease activity, 6.25 µM of each of the four dNTPs was included in the reaction. DNA polymerase assays were carried out under identical conditions with reactant concentrations as follows: For 'activated' DNA, 190 µM DNA, 25 µM each of dTTP, dCTP, dGTP, and [^{3}H]dATP, and 0.1 unit of enzyme; for poly-d(A-T) as template, 35 µM poly-d(A-T), 5-10 µM each of dTTP and [^{3}H]dATP, and 0.33 unit of enzyme.

The incorporation of an incorrect nucleotide, dCTP, into poly-d(A-T) in the presence of dATP and dTTP, carried out in parallel with the usual polymerase assay, was taken as an index of misincorporation. For this purpose [^{3}H]dCTP (26 Ci x mmole^{-1}) at 1 µM was used. Bovine serum albumin (0.1 mg) plus 25 nmole dCTP replaced calf thymus DNA during precipitation by TCA-pyrophosphate. The [^{3}H]dCTP appeared pure chromatographically, except for a trace of dCMP. The absence of terminal transferase activity was confirmed using the assay of Bollum (16).

Be was added as $BeCl_2$.

RESULTS: As can be seen in Fig. 1, Be alone does not serve to activate polymerase. This is different from the effect of Mn and Mg which activate the enzyme maximally at about 0.1 mM and 4 mM, respectively. In the presence of 4 mM Mg, however, Be affects incorporation in a complex manner, first enhancing activity and then inhibiting. Note that 50% inhibition occurs at 1-2 mM Be and that the effect is the same both for 'activated' DNA and poly-d(A-T).

The 3'→5' exonuclease activity of the enzyme, however, is strongly inhibited by Be (also in the presence of 4 mM Mg). As shown in Fig. 2, 50% inhibition occurs at about 0.05 mM Be. Activity above 1 mM Be is indistinguishable from zero. This strong inhibition is not seen in the case of 5'→3'



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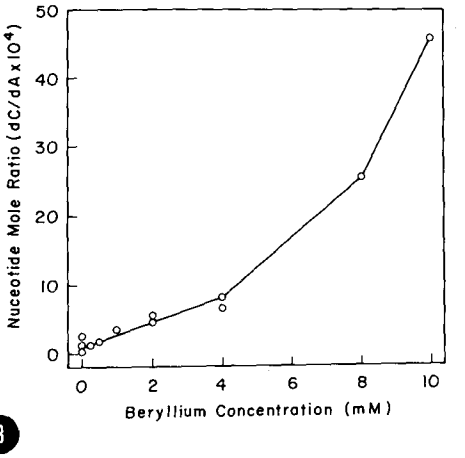

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Fig. 2. The effect of Be on the single-strand specific (3'→5') exonuclease activity of M. luteus DNA polymerase. The reaction mixtures contained 4 mM Mg, heat denatured [^{3}H]DNA from E. coli, and 0.28 unit enzyme and were incubated 45 min at 37°. The extent of hydrolysis above 1 mM Be was indistinguishable from zero. Reaction mixtures were as described under METHODS.

Fig. 3. Increased misincorporation caused by Be. The incorporation of dCTP relative to dATP into poly-d(A-T) is used as the measure of misincorporation. The reaction mixtures contained 4 mM Mg, [^{3}H]-deoxynucleotide triphosphate, and 0.33 unit enzyme and were incubated 45 min at 37°. Reaction mixtures were as described under METHODS.

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exonuclease activity where 1-2 mM Be is required for 50% inhibition.

Profound inhibition of editing activity with respect to polymerase activity might be expected to increase misincorporation, as is the case with certain "mutator" polymerases (17,18). This expectation seems to be realized as shown in Fig. 3. Major increases in misincorporation occur when the 3'→5' exonuclease activity is strongly inhibited.

DISCUSSION: The 3'→5' exonuclease activity of M. luteus DNA polymerase is impaired by Be, and this could be important for the observed increase in misincorporation (18). It is possible, however, that Be may also increase the number of errors introduced during polymerization, especially at high concentrations of Be. This matter is under investigation. We are unaware of other inhibitors that discriminate strongly between the polymerase and 3'→5' exonuclease activities of DNA polymerase.

It is generally assumed that Be is toxic because it is an analog of Mg or some other divalent metal ion. Indeed several enzymes, such as alkaline phosphatase (19,20), phosphoglucomutase (21), and Na,K-activated ATPase (22) are inhibited by Be at μM concentrations. Most other enzymes either are not

inhibited or are inhibited at much higher concentrations (23). It would appear that the acute toxicity of Be is not due to the inhibition of the above sensitive enzymes (24,25). The synthesis of some inducible enzymes in liver was reported to be inhibited by Be (3,25), but overall protein and RNA synthesis seemed about normal as were other indicators of metabolism (2,3). DNA synthesis can be repressed by Be in regenerating liver (3,4) but the reason for this is unclear.

Chronic berylliosis in man and animals is a granulomatous interstitial lung disease which is thought to arise as the result of a delayed immune response (26) following the inhalation of Be. Other organs also may show degenerative changes. The precise role of Be in the onset and course of the disease remains uncertain. While Be may possibly form antigens by complexation with proteins (27-29), certain protein antigens found in the lungs of animals with berylliosis are reported not to contain Be (30). In any case, a specific antigen in berylliosis has not yet been demonstrated (26,31), and it is conceivable that Be may induce an immune response without necessarily being a constituent of the antigen.

Mn has been reported to increase misincorporation by a T_4 DNA polymerase (17,32) and to be mutagenic (33,34). If Be were to increase misincorporation into DNA *in vivo*, as suggested by our *in vitro* studies, it might particularly damage, kill, or mutagenize cells undergoing division or repair of DNA. Possible consequences, in addition to cancer induction (6-9) and chromosome damage (5) which have been reported, might be interference with repair of damage to DNA introduced by radiation or chemical agents, acceleration of senescence in fibroblast cultures (35), modification of gene expression during differentiation or development (as in virus replication or lymphocyte maturation), or production of abnormal enzymes (36) and antigenic proteins. The latter consequence could be a factor in berylliosis (30).

It is possible that certain metals, including Be and Mn, may act on polymerases to alter DNA. Zr may also belong to this group since Zr lactate is believed to have induced skin granulomas similar to those induced by Be (26).

ACKNOWLEDGEMENT: This work was supported by Rosenstiel Basic Medical Science Grant 2413, National Science Foundation Grants GB29337A1 and GB29172X, and American Cancer Society Grant NP-8D.

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