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INHIBITION OF DEOXYTHYMIDINE KINASE BY BERYLLIUM*†

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Abstract—Deoxythymidine kinase, partially purified from 24 hr regenerating rat liver, is markedly inhibited by $BeSO_4$. The inhibition is competitive with the cofactor, magnesium. The 50 per cent inhibitory level of $BeSO_4$ was 2·2 mM when 2·5 mM $MgCl_2$ was included in the assay system. The K_m for magnesium–enzyme was $7{\cdot}5 \times 10^{-4}$ M; the K_i was $9{\cdot}5 \times 10^{-5}$ M.

IN 1951, CHEVRÈMONT and Firket[1] first reported the inhibition of DNA synthesis by beryllium.§ Since that time, additional reports have indicated that beryllium inhibits mitosis in cultured rat fibroblasts,[2] reduces the incorporation of labelled thymidine into the DNA of regenerating rat liver,[3] but has little effect upon the incorporation of leucine and orotic acid into protein and RNA, respectively, in the latter model. Witschi[3] was also able to demonstrate the correlation of the extent of inhibition of thymidine incorporation into DNA with the spectroscopically determined concentration of beryllium in liver.

The intravenous administration of beryllium is attended by a concentration of the ions in two organelles of the liver, lysosomes and nuclei.[4] Similar conclusions, based upon histochemical evidence using normal and regenerating rat liver, were reached by Truhaut *et al.*[5]

The adverse effects of beryllium could be partially neutralized by the administration of large amounts of magnesium ions.[1,6] In fact, the addition of magnesium to the diet was responsible for decreasing the inhibition of growth and the rate of mortality that was observed in beryllium-fed chickens.[7] Malendowicz[8] attempted to correlate the neutralization by magnesium of the toxicity and the inhibition of DNA synthesis observed in beryllium-treated animals. He suggested that beryllium displaced magnesium ions from the cell nucleus and thus stimulated a latent deoxyribonuclease activity.

We decided to investigate the effects of beryllium upon a magnesium-dependent enzyme which had been partially purified by modifications of a previously reported method.[9] The interactions of beryllium and magnesium upon deoxythymidine kinase are reported in this paper.

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§ Abbreviations employed included: DEAE-cellulose, diethylaminoethyl-cellulose; TdR, deoxythymidine; ATP, adenosine-5′-triphosphate; *d*-TMP, deoxythymidine-5′-monophosphate; PPO, 2,5-diphenyloxazole; dimethyl-POPOP; 1,4-*bis*[2(4-methyl-5-phenyloxazolyl)]benzene.

EXPERIMENTAL

Purification of deoxythymidine kinase. Adult male rats obtained from Cheek-Jones Company, Houston, Texas, were subjected to partial hepatectomy according to the procedure of Higgins and Anderson.[10] The rats were sacrificed 24 hr later by aortic transsection; the liver remnant was removed, washed in cold saline, and homogenized (1g/4·5 ml) in cold 0·25 M sucrose containing 3 mM β-mercaptoethanol. The homogenate was centrifuged at 105,000 *g* for 60 min. The fat layer was carefully aspirated from the top of the supernatant fraction and the remaining part of the supernatant was decanted from the precipitate. The volume of the supernatant was recorded and sufficient ammonium sulfate was added to yield 30 per cent saturation. The suspension was stirred magnetically in the cold for about 5 min and then centrifuged at 10,000 g for 10 min in a Sorvall refrigerated centrifuge. The supernatant fraction was discarded and the precipitate was dissolved in cold 0·05 M Tris buffer, pH 8·0, containing 6 mM β-mercaptoethanol and 1 mM deoxythymidine. The latter solution was immersed in a water bath set at 55° and shaken there for 5 min. The suspension containing denatured protein was then quickly immersed in an ice bath and cooled to 4°. After standing for 5 min at 4°, the denatured protein was removed by centrifugation at 10,000 *g* for 10 min. Calcium phosphate gel was added to the supernatant (2 mg/mg protein) and the suspension was stirred in the cold for 5 min. The gel was removed by centrifugation and discarded. To the supernatant was again added sufficient ammonium sulfate to yield 30 per cent saturation. The suspension was stirred in the cold for 10 min and the suspension was then centrifuged at 10,000 *g* for 10 min. The precipitate was redissolved in 0·05 M Tris buffer, pH 8·0, containing 50 % gylcerol. This procedure yielded a partially purified preparation of deoxythymidine kinase in about 30 per cent yield and purified by 100 to 200-fold. The preparation can be kept for at least a month at 4° without appreciable loss in activity.

Enzyme assay. Deoxythymidine kinase activity was measured essentially as described in a previous publication,[9] using the DEAE-cellulose[4] disc method adapted from the report of Breitman.[11] The dried discs were immersed in a toluene phosphor containing 0·5% PPO and 0·03% dimethyl-POPOP. The reaction mixture included: the enzyme; deoxythymidine-2-^{14}C, 3·3 mμmoles (0·05 μc); ATP, 5 mM; magnesium chloride, 2·5 mM, unless indicated otherwise; $BeSO_4$, as indicated; 0·05 M Tris buffer, pH 8·0, in a total volume of 0·125 ml. The incubation mixture was conducted in test tubes of 37° for 15 min.

The protein concentration of the enzyme extracts was estimated by the method of Lowry *et al.*[12] with bovine serum albumin as the reference standard.

Materials. Deoxythymidine-2-^{14}C (30 μc/μmole) was obtained from the New England Nuclear Corp. $BeSO_4$ and DEAE-cellulose DE-81 paper sheets (Whatman) were obtained from the Fisher Scientific Company and the Curtin Company respectively.

RESULTS

The addition of beryllium to the reaction mixture resulted in a marked inhibition of deoxythymidine kinase activity (Fig. 1). Under the assay conditions, with 2·5 mM $MgCl_2$ included, the 50 per cent inhibitory level for beryllium was 2·2 mM.

The dependence of enzymic activity upon magnesium concentration is indicated in Figs. 2 and 3. The dissociation constant of the enzyme–magnesium complex,

K_m, was $7{\cdot}5 \times 10^{-4}$ M, calculated from the data of Fig. 3. This figure is in excellent agreement with the previously determined value using the enzyme partially purified from the Walker carcinosarcoma.[9]

The magnitude of the inhibition caused by beryllium is further indicated in Figs. 2 and 3. It is obvious that the inhibition was competitively reversed by magnesium. The dissociation constant of the enzyme–inhibitor complex, K_I, was calculated from the data of Fig. 3 to be $9{\cdot}5 \times 10^{-5}$ M. Beryllium apparently binds ten times more avidly to deoxythymidine kinase than the natural cofactor, magnesium.

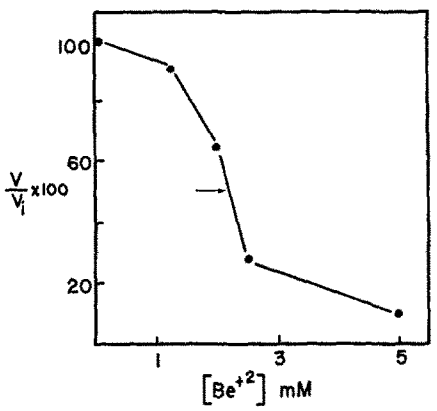

FIG. 1. Inhibition of deoxythymidine kinase by beryllium. Deoxythymidine kinase activity was assayed as described in the text. The number of millimicromoles of *d*-TMP formed in the absence of Be^{++} (V) was 2·3. V_i represents the number of millimicromoles of *d*-TMP formed in the presence of inhibitor.

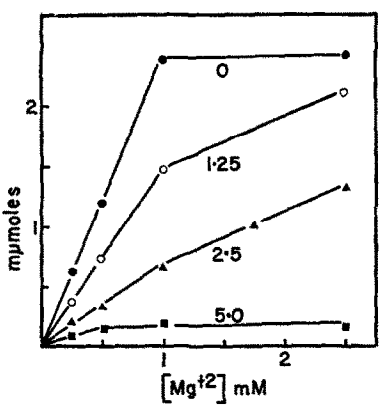

FIG. 2. TdR kinase activity as a function of $[Mg^{++}]$. TdR kinase activity was assayed as described in the text with the following exception: the $[Mg^{++}]$ was varied as indicated on the abscissa. Enzyme activity is expressed as millimicromoles of *d*-TMP produced under the standard assay conditions (ordinate) in the presence of varying amounts of $BeSO_4$ (mμmoles indicated in the figure).

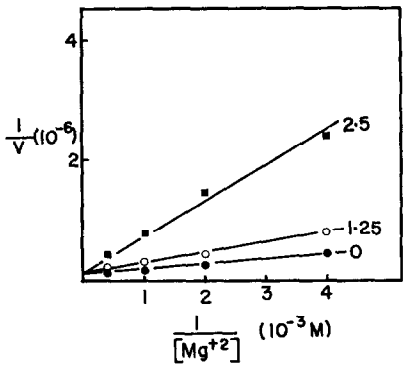

FIG. 3. Reciprocal plot of velocity vs. [Mg^{++}]. TdR kinase was assayed as described in the text except that [Mg^{++}] was varied. The velocity (V) represents the millimicromoles of *d*-TMP produced per liter of incubation mixture per 15-min assay in the presence and absence of $BeSO_4$ (mμmoles indicated in the figure).

DISCUSSION

Previous studies have indicated that the effects of beryllium upon enzymes are highly specific. The metal reacts with proteins containing a specific molecular configuration.[13,14] Beryllium exerted profound inhibition upon alkaline phosphatase,[15] phosphoglucomutase,[15] 5′-nucleotidase,[16] phosphohexokinase[17] and yeast carboxylase.[18] However, not all magnesium-activated enzymes were inhibited by beryllium. Potato apyrase was insensitive to the action of the latter metal.[13]

The data presented here indicate that deoxythymidine kinase, an enzyme peripherally related to the synthesis of DNA, is markedly sensitive to the action of beryllium. The ratio of the K_m/K_i was approximately 10, indicating a more avid binding of beryllium than of magnesium to deoxythymidine kinase. Aldridge and Thomas[14] have examined in detail the effects of beryllium upon phosphoglucomutase. Their data suggest the initial attachment of magnesium to the enzyme to yield an enzyme-magnesium complex. The data presented in this manuscript also suggest the initial attachment of magnesium to deoxythymidine kinase to yield the enzyme–magnesium complex. In an analogous manner, beryllium may form an enzyme–beryllium complex which, unlike the magnesium–enzyme derivative, is unable to chelate ATP. Consequently, no phosphorylation of substrate can occur. It is quite likely that beryllium interacts with the other enzymes responsible for the phosphorylation of deoxyribonucleoside mono- and diphosphates in an analogous manner. It is this action which is responsible for the inhibition of DNA synthesis.

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REFERENCES

1. M. CHEVREMONT and H. FIRKET, *C. r. Séanc. Soc. Biol.* **145**, 938 (1951).
2. R. BASSLEER, *C. r. Séanc. Soc. Biol.* **159**, 1620 (1965).
3. H. P. WITSCHI, *Lab. Invest.* **19**, 67 (1968).
4. H. P. WITSCHI and W. N. ALDRIDGE, *Biochem. J.* **106**, 811 (1968).
5. R. TRUHAUT, C. BOUDÈNE and J. Y. LETALAER, *Annls. Biol. clin.* **23**, 45 (1965).
6. P. MANIL and Z. STRASZEWSKA, *C. r. Séanc. Soc. Biol.* **147**, 525 (1951).

7. K. N. Chetty and C. H. Hill, *Fedn. Proc.* **26**, 634 (1967).
8. L. Malendowicz, *Folia Histochem. Cytochem.* **4**, 279 (1966).
9. E. Bresnick and U. B. Thompson, *J. biol. Chem.* **240**, 3967 (1965).
10. G. M. Higgins and R. M. Anderson, *Archs. Path.* **12**, 186 (1931).
11. T. R. Breitman, *Biochim. biophys. Acta* **67**, 153 (1963).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
13. N. Thomas and W. N. Aldridge, *Biochem. J.* **98**, 94 (1966).
14. W. N. Aldridge and N. Thomas, *Biochem. J.* **98**, 100 (1966).
15. H. P. Witschi and W. N. Aldridge, *Biochem. Pharmac.* **16**, 263 (1967).
16. R. O. Hurst, J. A. Little and G. C. Butler, *J. biol. Chem.* **188**, 705 (1951).
17. B. Axelrod, R. Saltman, R. S. Bandurski and R. S. Baker, *J. biol. Chem.* **197**, 89 (1952).
18. F. W. Klemperer, *J. biol. Chem.* **187**, 189 (1950).