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The feedback inhibition of thymidine kinase

During the course of a study to determine whether TDP is an intermediate in the conversion of [^{3}H]thymidine to TTP by the 105 000 × *g* supernatant fraction from regenerating rat liver, unlabeled TDP was added to the reaction mixture in an attempt to trap any labeled TDP formed. It was observed, however, that there was a marked decrease in the amount of labeled TDP formed under these conditions. There was also a corresponding decrease in formation of labeled TMP which suggested that TDP was inhibiting thymidine kinase (ATP: thymidine 5'-phosphotransferase, EC 2.7.1.21). This report will present evidence that both TDP and TTP exert a feedback inhibition on thymidine kinase in both crude and partially purified enzyme preparations. After this portion of the work was completed the abstract of IVES *et al.*[1] was published in which TTP was reported to inhibit thymidine kinase in crude 105 000 × *g* supernatant fraction from regenerating rat liver.

Partial hepatectomies were performed by the method of HIGGINS AND ANDERSON[2] on rats weighing approx. 250 g. The livers were removed 18 h after the operation and homogenized in a sucrose–KCl–Tris medium[3]. The supernatant fraction used as enzyme source was obtained by centrifuging the homogenate for 60 min at 105 000 × *g*. TMP kinase activity was removed by treatment with Sephadex. For this purpose, 1 ml of the 105 000 × *g* supernatant fraction was applied to a 1 × 15 cm Sephadex-G-50 column, previously equilibrated with 0.05 M Tris–HCl (pH 8), and eluted with this buffer. 1-ml fractions were collected. Almost all the protein appeared in Fractions 4 and 5; nucleotides were completely separated. All operations from homogenization through the Sephadex treatment were conducted at 0–5°.

A partially purified thymidine kinase was prepared from the 0.2–0.25 satd. ammonium sulfate precipitate from the 105 000 × *g* supernatant fraction[1]. The precipitate was dissolved in the sucrose–KCl–Tris homogenizing medium and stored frozen until use.

The ability of the enzyme preparations to phosphorylate thymidine was measured in the reaction mixture described by BOLLUM AND POTTER[5]. Reactions were started by the addition of enzyme preparation to the reaction mixture which had been preincubated at 38°. Controls contained serum albumin instead of enzyme. At suitable intervals after the addition of enzyme, 25-μl aliquots were removed and pipetted onto numbered DEAE-cellulose paper discs (Whatman Diethylaminoethyl Cellulose Paper DE 20), 16 mm in diameter. The discs were immediately dropped into a beaker containing 20 ml of 0.001 N ammonium formate per disc to elute non-phosphorylated [^{3}H]thymidine. The discs were washed by gentle swirling and decantation, twice with 0.001 N ammonium formate, once with water, and once with absolute ethanol. The discs were air dried and each was placed in a standard 20-ml glass counting vial and 5 ml of toluene phosphor solution (containing 4 g 2,5-diphenyloxazole and 0.05 g 1,4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene) were added. Radioactivity measurements were made in a Packard Tri-Carb at 1200 V, discriminator setting 10–100. The washing procedure removed 98% of the [^{3}H]-thymidine while 100% of the phosphorylated derivatives of thymidine remained

Abbreviations: TMP, TDP, and TTP, thymidine 5'-mono-, di-, and triphosphates, respectively.

adsorbed to the ion-exchange paper discs. The counting efficiency was approx. 2%.

In Fig. 1 A are presented the effects of the addition of TDP and TTP on the initial rates of formation of TMP by the Sephadex-treated enzyme. This preparation retained less than 5% of the original TMP kinase activity. On a molar basis, TTP was slightly more inhibitory than was TDP. Similar experiments with TMP resulted in much less inhibition, indicating that the effects observed with TDP and TTP were not due to dephosphorylation to TMP (*i.e.*, product inhibition) or thymidine (dilution of [^{3}H]thymidine). Further support for this interpretation was obtained by use of the partially purified thymidine kinase preparations obtained by ammonium sulfate

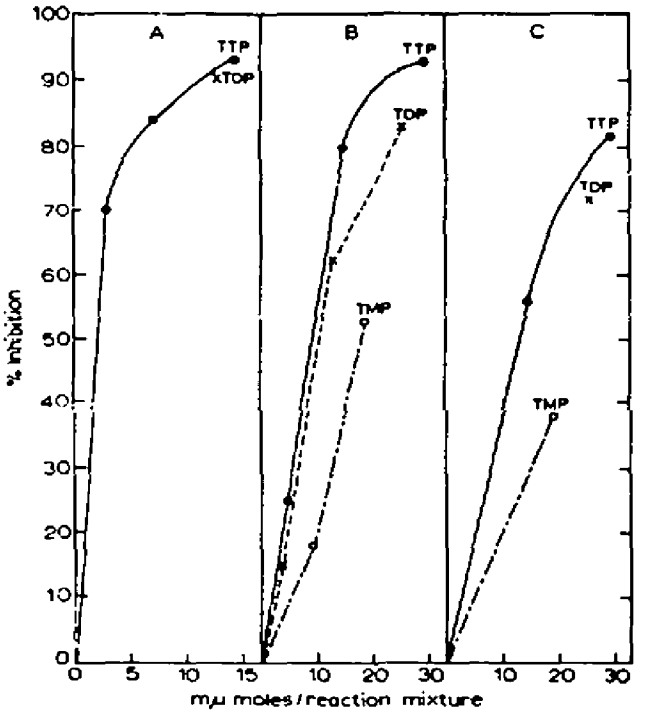

Fig. 1. A. Effect of TDP and TTP on the phosphorylation of thymidine by a Sephadex-treated 105 000 × *g* supernatant fraction from regenerating rat liver. The complete reaction mixture contained in a final volume of 0.25 ml: 10 μmoles Tris·HCl (pH 8), 1.5 μmoles 3-phosphoglycerate, 1.25 μmoles $MgSO_4$, 1.25 μmoles ATP, 0.7 mg protein of Sephadex-treated enzyme; 1.6 mμmoles [^{4}H]thymidine (specific activity, 3.15 C/mmole), 11.25 mμmoles thymidine and additions as indicated. B, and C. Effect of TMP, TDP, and TTP on the phosphorylation of thymidine by a partially purified thymidine kinase preparation obtained by ammonium sulfate fractionation. Conditions: B, same as A except that 0.117 mg of partially purified enzyme protein and 1.93 mμmoles [^{3}H]thymidine (specific activity 2.66 C/mmole) were added. C, same as B except that 0.065 mg of partially purified enzyme protein and 56.3 mμmoles thymidine were added.

fractionation (Fig. 1 B and C). With these enzyme preparations TTP was slightly more effective than TDP in inhibiting the formation of TMP. When the thymidine concentration was increased, higher concentrations of TDP and TTP were required for inhibition (Fig. 1 C). This is suggestive of competitive inhibition.

It can be calculated from these data that the inhibition observed as a result of

the addition of either TDP or TTP was greater than the dilution of [^{3}H]thymidine which would have occurred if these compounds had been completely dephosphorylated. On the other hand, the inhibition observed as a result of the addition of TMP was generally less than that which could be explained by complete conversion to thymidine. Therefore, the inhibition observed with TMP may in part be a result of [^{3}H]thymidine dilution.

The greater inhibitory effects of TDP with comparatively fresh liver extracts (Fig. 1 A) as compared to a preparation which had been stored frozen for a week (Fig. 1 B) may be explained by the inactivation of TDP kinase on storage. The extreme lability of TDP kinase[5] makes it appear unlikely that TDP inhibits in the partially purified enzyme preparations because of its conversion to TTP. Thus it appears that both TDP and TTP inhibit thymidine kinase; TTP is the more potent inhibitor.

No inhibition of thymidine kinase was observed when the nucleoside triphosphates of deoxyadenosine, deoxycytidine, deoxyguanosine, cytidine, guanosine and uridine were added to the reaction mixture at concentrations 10-fold greater than that concentration of TTP which inhibited the phosphorylation of thymidine more than 90%. Thus the observed inhibition by TDP and TTP appears to be specific for these nucleotides.

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