SELECTIVE INHIBITION BY DEOXYGLUCOSYL THYMINE OF THYMIDINE PHOSPHORYLASES NOT CATALYZING DEOXYRIBOSYL TRANSFER

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It was recently reported (Langen and Etzold, 1963) that deoxyglucosyl thymine (1-/2-deoxy-D-arabino-hexopyranosyl/-thymine) inhibited thymidine phosphorylase (E.C.2.4.2.4) in an extract of ascites tumor cells. It was also stated that the nucleoside phosphorylase in an extract of a rat hepatoma was inhibited by the compound but that a human colon carcinoma enzyme preparation was not inhibited. This observation appeared to relate to a study we have made (Zimmerman, 1962; Zimmerman and Seidenberg, in press) on the ability of some thymidine phosphorylases to catalyze a nucleoside:pyrimidine deoxyribosyl transferase reaction (E.C.2.4.2.6). It had been found that mouse ascites tumor extracts, a number of rat hepatomas and other rodent tissues catalyzed the phosphorolysis of thymidine but not an exchange reaction between a pyrimidine and its deoxyriboside. All human tissues, including tumors, catalyzed both reactions. We have presented extensive evidence (Zimmerman and Seidenberg, in press; Zimmerman, 1963; Zimmerman, in press) that both activities are catalyzed by the same enzyme protein. Since deoxyglucosyl thymine appeared to inhibit those phosphorylases we had found to be incapable of catalyzing the exchange reaction, the possibility that such a correlation did exist was examined.

Homogenates of tissues in .154 M KCl containing .003 M EDTA were prepared using a Teflon pestle homogenizer. The 30,000 g (1 hour)

supernatant prepared using a refrigerated centrifuge was the enzyme source. Deoxyglucosyl thymine was made available to us through the kindness of Dr. William Zorbach of Georgetown University who originally prepared the compound (Zorbach and Durr, 1962). Thymidine phosphorylase activity was determined both by measurement of the deoxyribose phosphate formed using diphenylamine (Boxer and Shonk, 1958) and by the formation of thymine (Friedkin and Roberts, 1954). Deoxyribosyl transferase activity was determined using labeled thymine and unlabeled thymidine as described previously (Zimmerman, 1962).

The inhibition by deoxyglucosyl thymine of the thymidine phosphorylases of various tissues is shown in Table 1. The enzyme from

TABLE 1

EFFECT OF DEOXYGLUCOSYL THYMINE ON THYMIDINE PHOSPHORYLASES FROM VARIOUS SOURCES

Enzyme Source	Phosphorylase Units/g wet wt.	Deoxyribosyl Transferase Units/g wet wt.	Inhibition %
Novikoff Hepatoma	4.0	0	79
Dunning Hepatoma	4.7	0	84
Walker 256 Carcinoma	8.8	0	83
Ehrlich Ascites	8.2	0	84
Normal Rat Bone Marrow (Tibia)	2.1	0	79
Normal Rat Liver	19.2	31	0
Human Spleen (Purified Enzyme)	1100*	960*	0

*Units/mg protein.

The assays were performed using 10^{-3}M thymidine and 10^{-3}M inhibitor. The enzyme unit is μmoles nucleoside cleaved/hour.

human spleen is a highly purified preparation whose isolation has been described (Zimmerman, in press). All other enzyme preparations were supernatant fractions from whole tissue homogenates. From the table it can be seen that only in those tissues not capable of catalyzing the exchange reaction is the phosphorylase inhibited, whereas those enzymes which also catalyze the nucleoside deoxyribosyl transferase reaction are not inhibited under these conditions.

Two other hexopyranosyl thymines, also supplied through the kindness of Dr. Zorbach, had no inhibitory effect on the Dunning hepatoma enzyme. These compounds were 1-(2,6-dideoxy-D-ribo-hexopyranosyl)thymine (digitoxosyl thymine) Zorbach and Durr, 1962) and 2'-deoxyallosyl thymine (Zorbach and Saeki, in press), demonstrating a specificity for the deoxyglucosyl moiety. This is surprising since the substrate, e.g., thymidine, not only has the furanose structure in the glycosyl moiety but also differs from the inhibitor in the configuration of the 3'-hydroxyl.

None of the three hexopyranosyl thymines examined are substrates for the enzyme, nor do they inhibit the transfer activity of those enzymes catalyzing this reaction.

Our previous studies suggested that there were two different thymidine phosphorylases, one of which could also catalyze deoxyribosyl transfer. It is of interest that an inhibitor, deoxyglucosyl thymine, is able to differentiate between the two types of phosphorylase, suggesting that primary structural differences between the two phosphorylases may exist.

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