# THE POSSIBLE IDENTITY OF THYMIDINE PHOSPHORYLASE AND PYRIMIDINE DEOXYRIBOSYL TRANSFERASE OF RAT LIVER

Morris Zimmerman

Merck Institute for Therapeutic Research, Rahway, New Jersey

Received May 11, 1962

Pyrimidine deoxyribosyl transferase activity, found earlier in bacterial extracts (MacNutt 1952, Roush and Betz 1958) was reported by deVerdier and Potter (deVerdier and Potter 1960) to be present in rat liver. A number of other rat tissues, including the Novikoff hepatoma, had no measurable transfer activity. This was surprising since the hepatoma possesses thymidine phosphorylase activity which, in the presence of base, nucleoside and phosphate should catalyze deoxyribose transfer (1) by reversal of phosphorolysis (2) where B is thymine or uracil, and dR

$$B_1 + B_2dR \rightleftarrows B_2 + B_1dR \qquad (1)$$

$$BdR + \text{Phosphate} \rightleftarrows B + \text{dR-1-phosphate} \qquad (2)$$

is deoxyribose.

We have confirmed the inability of the Novikoff hepatoma to catalyze the exchange reaction in spite of the fact that it has thymidine phosphorylase activity measurable in both directions. However, with both liver and hepatoma supernatant fractions, the amount of deoxyribose phosphate formed by phosphorolysis of thymidine under our assay conditions is insufficient to drive the reaction from labeled base to nucleoside. In addition, rat liver supernatant catalyzes the transfer reaction under conditions preventing the formation of deoxyriboside from base and deoxyribose phosphate such as in arsenate (deVerdier and Potter 1960) or with high concentrations of phosphate (Table I). Thus, deoxyribosyl

transfer would appear to be independent of nucleoside phosphorylase activity.

TABLE I

Effect of Phosphate on Formation of Thymidine from Labeled Thymine[1]

| Buffer | μmoles/hr./g. wet weight | | | |
|---|---|---|---|---|
| | Deoxyribose Source | | | |
| | TdR | UdR | CdR | dR-1-P |
| Tris-HCl (.04M) | 26 | 26 | 0 | 11 |
| Phosphate (.1M) | 26 | 26 | 0 | 0 |

[1]Assay system: 1mM Thymine-2-$C^{14}$, 2mM deoxyribose donor, 25 mg. tissue equivalent of 30,000 g. rat liver supn. in .15M KCl-$10^{-3}$M versene and buffer (pH 7.5) in a final vol. of 0.5 ml. After incubation at 37°C. the reaction was terminated with 2 vols. isopropanol. Thymidine formation was determined essentially by the method described by deVerdier and Potter (deVerdier and Potter 1960).

N-Pentosyl transfer reactions have been reported with DPNase (Zatman et al., 1953), a hydrolytic enzyme, and nicotinamide riboside phosphorylase (Grossman and Kaplan 1958). Roush and Betz (Roush and Betz 1958) found that bacterial deoxyribosyl transferase appeared to be associated with nucleoside hydrolase activity. Since there is no demonstrable pyrimidine deoxyriboside hydrolase activity in mammalian tissue, the possibility that deoxyribosyl transfer was actually carried out by the thymidine phosphorylase was examined further.

A number of normal and tumor tissues of both rodents and man having thymidine phosphorylase activity were assayed for pyrimidine deoxyribosyl transferase activity, determined as described above using phosphate buffer. Thymidine phosphorylase was assayed by measuring the formation of deoxyribose phosphate (Boxer and Shonk 1958). Representative results are shown in Table II. All the rodent tumors tested, including the Morris 5123 hepatoma, had no transfer activity. All tissues of man examined possessed both activities in approximately the same ratio, al-

though specific activity and tissue of origin varied widely. It was noted that deoxyribosyl transferase activity was either absent or in relatively constant ratio to phosphorylase activity. Purification of rat liver thymidine phosphorylase was then attempted to determine whether the two activities could be separated.

TABLE II

Thymidine Phosphorylase and Deoxyribosyl Transferase in Normal and Tumor Tissues of Rodents and Man

| Animal | Tissue[1] | Thymidine Phosphorylase μmoles/hr./g. wet wt. | Deoxyribosyl Transferase / Phosphorylase |
|---|---|---|---|
| Rat | Liver | 14 | 1.8 |
| | Intestinal mucosa | 25 | 0 |
| | Novikoff hepatoma | 4.5 | 0 |
| | Morris 5123 hepatoma | 7.6 | 0 |
| Rabbit | Liver | 18 | 1.9 |
| | Intestinal mucosa | 11 | 1.7 |
| Man | Liver | 45.5 | 0.8 |
| | Kidney | 11.5 | 1.0 |
| | Spleen | 113.0 | 0.8 |
| | Prostate tumor | 8.2 | 1.3 |
| | Lung tumor | 50.7 | 1.0 |
| | Kidney tumor | 130.0 | 1.2 |

[1]A 30,000 g. supernatant fraction was used for all determinations.

A 30,000 g. supernatant fraction from a 30% Waring Blendor homogenate of rat liver in .154M KCl - .01M versene was prepared. The pH was lowered to 4.5 with glacial acetic acid, brought back to 5.5 after 2 minutes, and the precipitate removed by centrifugation and discarded. Fractionation with solid ammonium sulfate at pH 6.5 precipitated the activity between 25 and 45% saturation. The precipitate was dissolved in .02M phosphate buffer, pH 7.0, to a protein concentration of approximately 10 mg./ml., and one-third volume of cold 95% ethanol added, dropping

the temperature to -10°C. during the addition. The precipitate which formed was removed by centrifugation, and the supernatant fraction was acidified to approximately pH 5 at -10°C. The precipitate from this step contained the phosphorylase activity. A solution of the precipitate was dialyzed against .02M phosphate buffer, pH 7.0, containing 5mM β-mercaptoethanol. A portion of this material was applied to a DEAE-Sephadex (A25-Medium) column equilibrated with the same buffer. After washing with .02M and .04M phosphate buffers at the same pH, a linear gradient from .04M - .2M phosphate was applied. Considerable spreading of the activity was observed; however, stepwise elution or use of other gradients did not improve appreciably the sharpness of the elution pattern. The most active fractions, representing approximately 40% of the activity applied were pooled. Activities of both phosphorylase and transferase at various stages of purification are noted in Table III. One column fraction, representing a 210X purification from the liver supernatant had the same ratio of the two activities as the pooled active fractions and fractions from other portions of the effluent curve. Further purification was impeded by the lability of the enzyme in dilute solution and large losses of activity during attempts to concentrate the column effluent by various means.

Both activities have optimum stability at pH 6.5 when heated at 50°C., the curves being virtually superimposable. Both activities also have the same substrate specificity, 4-keto-pyrimidines and their deoxyribosides including the 5-halogenated uracil deoxyribosides being substrates but cytosine and its deoxyriboside are neither substrates nor inhibitors.

Although the rat liver enzyme is not sufficiently pure to state unequivocally that both the transfer and phosphorylase activities are present on the same protein molecule, the evidence is highly suggestive that this is the case. This raises a problem as to the absence of deoxyribosyl transfer in tissues which have thymidine phosphorylase activity. A precedent for such a case is the demonstration (Grossman and Kaplan 1958)

TABLE III

Purification of Rat Liver Thymidine Phosphorylase

| Fraction | ml. | Protein mg./ml. | Phosphorylase (P) units[1]/ml. | Deoxyribosyl Transferase (T) units[2]/ml. | Ratio T/P | Specific Activity[3] units/mg. |
|---|---|---|---|---|---|---|
| Supn. | 1300 | 44 | 4.4 | 5.7 | 1.3 | .10 |
| pH 5 Supn. | 1120 | 28 | 3.9 | 4.2 | 1.2 | .14 |
| 45% Sat. Amm. Sulf. ppt. | 845 | 10 | 3.8 | 7.4 | 1.9 | .38 |
| Acid Alcohol ppt. | 200 | 13.6 | 12.2 | 17.4 | 1.4 | .90 |
| DEAE-Sephadex combined peak fractions | | .14 | 2.5 | 2.7 | 1.1 | 17.8 |

[1]μmoles thymidine split/hour.

[2]μmoles of labeled thymidine formed/hour.

[3]Specific activity of thymidine phosphorylase.

that erythrocyte nicotinamide riboside phosphorylase, when purified, lost its ability to catalyze an exchange reaction between nicotinamide or other pyridine derivatives and nicotinamide riboside. In this case, activity was restored by a soluble extract of erythrocytes or by ergothioneine. Attempts to reactivate transfer activity in the Novikoff hepatoma by addition of alcohol or TCA extracts of rat liver or by adding a large excess of purified liver thymidine phosphorylase, inactivated by warming at pH 5 or pH 8.5, have been unsuccessful. Further evidence against a dissociable co-factor is the relative stability of rat liver transfer activity during a variety of purification steps. The possibility that alteration of the secondary or tertiary structure of the enzyme could dissociate transfer from phosphorylase activity is suggested by the complete loss in 5.5M

urea of deoxyribosyl transferase activity, whereas 50% of the phosphorylase activity remains. This possibility is currently under investigation.

Complete details of this work will be reported elsewhere.

This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, under the National Institutes of Health, Contract Number SA-43-ph-1886.

## REFERENCES

MacNutt, W. S., Biochem. J., 50, 384 (1952).

Roush, A. H., and Betz, R. F., J. Biol. Chem., 233, 261 (1958).

deVerdier, C. H., and Potter, V. R., J. Nat'l. Cancer Inst., 24, 13 (1960).

Zatman, L. J., Kaplan, N. O., and Colowick, S. P., J. Biol. Chem., 200, 197 (1953).

Grossman, L., and Kaplan, N. O., J. Biol. Chem. 231, 727 (1958).

Boxer, G. E., and Shonk, C. E., J. Biol. Chem., 233, 535 (1958).