DEOXYRIBOSYL EXCHANGE ACTIVITY ASSOCIATED WITH NUCLEOSIDE PHOSPHORYLASE

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During an investigation of deoxyribotide synthesis in Ehrlich ascites cell extracts it was observed that incubation with a mixture of adenosine randomly labeled with C^{14} and unlabeled deoxyadenosine resulted in the formation of deoxyinosine labeled exclusively in the hypoxanthine moiety (Edmonds, 1958). A similar transfer of deoxyribose has been observed when unlabeled deoxyguanosine was incubated with C^{14}-guanosine or with C^{14}-guanine. While such an exchange reaction might be expected to be catalyzed by nucleoside phosphorylase in the presence of phosphate, this communication presents evidence that the exchange is, in fact, independent of the presence of inorganic phosphate, but is never-the-less associated with highly purified preparations of nucleoside phosphorylase.

METHODS

Ascites extract: Packed Ehrlich ascites cells were suspended in 2 volumes of 0.154 M KCl and ruptured by shaking with glass beads for 30 sec. in a Nossal vibrator. The homogenate was centrifuged for 30 min. at 105,000 x G and dialyzed for 40 hours against flowing deionized water at 4°.

Spleen nucleoside phosphorylase: Frozen calf spleen was thawed and homogenized with water in a Waring blender. The water extract was precipi-

tated at pH 5.2 and the supernatant solution adjusted to 20 per cent ethanol at -5° to precipitate the enzyme as described by Price, et al (1955). Ammonium sulfate fractionation, first between 0.47 and 0.67 saturation, and then between 0.45 and 0.53 saturation, was followed by chromatography on DEAE-cellulose, eluting with an exponential gradient from 0.01 M phosphate pH 7.2 to 0.10 M phosphate pH 6.0. The enzyme was adsorbed from the eluate by calcium phosphate gel and eluted with 0.3 saturated ammonium sulfate pH 7.5 followed by precipitation at 0.8 saturation. Finally, traces of phosphate were removed by exhaustive dialysis against deionized water and passage through a column of Dowex-1-chloride. The best preparations contained 3,600 units (μmoles hypoxanthine liberated per hour from inosine) per mg protein and were thus 3 times more active than the liver enzyme (1200 units per mg) which Korn and Buchanan (1955) estimated to be 33 per cent pure. Phosphorylase assay: A spectrophotometric assay was used with inosine as substrate in the presence of excess xanthine oxidase as described by Price, et al (1955). Deoxyribose exchange assay: Ascites extract or purified phosphorylase was incubated for 30 min. at 37° with 1 μmole of guanine-2-C^{14} and 5 μmoles of unlabeled deoxyguanosine in 4 ml of 0.05 M Tris buffer, pH 7.5. The reaction was stopped by heating at 100° for 2 min. Deoxyguanosine was re-isolated by chromatography on a column of Dowex-50-NH_4^+, 30 x 0.5 cm, using gradient elution with 0.125 M ammonium acetate pH 4.8 passed through a mixer flask containing 250 ml water. This procedure is capable of resolving a mixture of guanosine, deoxyguanosine, and guanine. The isolated deoxynucleoside was assayed for C^{14} content. The transfer of deoxyribose from guanine to guanine-2-C^{14} was found to be a linear function of time of incubation and enzyme concentration.

RESULTS

Attempts to purify the exchange enzyme from ascites extracts indicated a parallelism between transdeoxyribosylation in the absence of phosphate and nucleoside phosphorolysis in the presence of phosphate. As shown in Table I, this parallelism was reflected in the more extensive purification obtained with spleen nucleoside phosphorylase.

Table I

Deoxyribosyl Exchange Activity Observed During Purification of Nucleoside Phosphorylase

Source	Fraction	Nucleoside phosphorylase	Deoxyribosyl exchange	Ratio
		μmoles/hour/mg protein		
Ascites cells	Dialyzed extract	1.74	0.078	22
	0-0.47 sat. $(NH_4)_2SO_4$	0.21	0.011	19
	0.47-0.63 sat. $(NH_4)_2SO_4$	14.4	0.68	21
Beef liver	Crude fraction	21.1	1.57	13
Calf spleen	pH 5.2 soluble	12	1.1	11
	Alcohol ppt.	54	5.1	11
	0.45-0.53 sat. $(NH_4)_2SO_4$	245	24	10
	DEAE-cellulose	2060	130	16

Deoxyribosyl exchange was measured in Tris buffer as described in "Methods" with deoxyguanosine as the deoxyribosyl donor and guanine-2-C^{14} as the acceptor. The crude liver fraction was obtained from calf liver by applying the first two steps of the procedure of Korn and Buchanan (1955).

These results with preparations of varying degrees of purity suggest that the exchange reaction is a property of nucleoside phosphorylase. However, under the conditions used, not all substrates are suitable for demonstrating deoxyribosyl exchange. As indicated in Table II, the preferred re-

action involved transfer of deoxyribose from guanine to guanine. In the guanine-guanine transfer, replacement of deoxyribose by ribose reduced the rate by a factor of 10. Other purine nucleosides such as deoxyinosine were ineffective as deoxyribosyl donors even though they were perfectly adequate substrates for demonstrating phosphorylase activity in the presence of inorganic phosphate.

Table II

Deoxyribosyl Donor and Acceptor Specificity

Pentose donor	Acceptor	Deoxyribose transfer
		μmoles/hour/mg protein
Deoxyguanosine	Guanine	58.0
Deoxyadenosine	Guanine	0.3
Deoxyinosine	Guanine	0.8
Guanosine	Guanine	6.0
Adenosine	Guanine	0.3
Deoxyguanosine	Adenine	0
Deoxyadenosine	Adenine	0

The reaction mixture contained 4 μmoles of donor nucleoside, 2 μmoles of acceptor purine (C^{14} labeled), and 1 μg of purified spleen nucleoside phosphorylase in 4 ml of 0.05 M Tris buffer, pH 7.5.

In other experiments it has been observed that both the exchange reaction and phosphorolysis have pH maxima in the region of pH 6.5. At extremes of pH (5.5 or 8.5), exchange is repressed to less than 10 per cent of the maximal value while phosphorolysis continues at 80 to 90 per cent of the maximal rate.

DISCUSSION

Transdeoxyribosylation reactions were first observed in Lactobacilli by MacNutt (1952). Similar enzymes, devoid of phosphorolytic activity, have been observed in other bacteria but not in mammalian tissues. Kritskii

(1952), however, has presented evidence for a slow synthesis of inosine from hypoxanthine and guanosine by a crude rat liver phosphorylase preparation without added phosphate. The transfer was depressed by reprecipitation of the enzyme and greatly stimulated by inorganic phosphate. It was assumed that a transribosylation had occurred in which "enzyme-bound phosphate" played a catalytic role. In the experiments reported here, the close association of activities for phosphorolysis and transdeoxyribosylation suggests that both are properties of the same enzyme. If so, the lack of detectable exchange with some substrates and at some pH's may reflect variation in the ease with which the purine component dissociates from a deoxyribosyl-enzyme-purine complex.

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