THE ROLE OF DEOXYRIBOSE 5-PHOSPHATE ALDOLASE IN THE SYNTHESIS OF DEOXYRIBONUCLEOTIDE IN MAMMALIAN CELLS *

D. P. Groth† and N. Jiang

Departments of Biochemistry and Pharmacology
Emory University, Atlanta, Georgia

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The deoxyribose 5-phosphate aldolase reaction (I) has generally been considered to function largely as a step in the degradative pathway for deoxyribonucleotides.

I Deoxyribose 5-phosphate $\rightleftharpoons$ Glyceraldehyde 3-phosphate + Acetaldehyde

Pricer and Horecker (1960) and Groth (1965) have shown that the enzymes from L. plantarum and rat liver, respectively, are activated by polycarboxylic acids and are able to synthesize deoxyribose 5-phosphate at low concentrations of acetaldehyde. The possibility that deoxyribose 5-phosphate aldolase might have an important function in deoxynucleotide synthesis in mammalian tissues was therefore investigated.

Meso a, β diphenylsuccinate(DPS) has been shown to be a specific competitive inhibitor of deoxyribose 5-phosphate aldolase. In intact cells this inhibitor selectively interferes with deoxyribonucleotide synthesis by blocking the formation of deoxyribose 5-phosphate via the aldolase reaction I. Other enzymatic steps involved in nucleic acid and protein synthesis were unaffected by diphenylsuccinate. The selective inhibition of deoxyribonucleotide synthesis by DPS establishes an important synthetic role for deoxyribose 5-phosphate aldolase in the mammalian cell.

MATERIALS AND METHODS

Deoxyribose 5-phosphate aldolase was purified from rat liver as pre-

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viously described (Groth, 1965). Ehrlich ascites tumor cells were obtained from Swiss mice innoculated 7-10 days earlier with 10^8 cells from donor mice. The cells were harvested by centrifugation and washed three times with 10 volumes of cold isotonic saline. Slices of regenerating rat liver were prepared and incubated as described by Hecht and Potter (1958). After incubation with a radioactive precursor, the mixed nucleic acids were prepared, RNA separated from DNA, and the free bases of DNA obtained by paper chromatography using the isopropanol HCL system. (Hecht and Potter 1958). The RNA nucleotides were also chromatographed with the isopropanol HCl system. After elution from the paper, the specific activity of each base and nucleotide was determined spectrophotometically (Wyatt 1951) and by counting in a liquid scintillation counter. (Peckham and Knobil 1962). Protein samples were heated at 100° in 0.8M perchloric acid, washed with 0.2M perchloric acid, dissolved twice in 98% formic acid, reprecipitated with 0.8M perchloric acid, washed with water, and dissolved in 1M Hyamine for radioactivity determinations by liquid scintillation. Protein concentration was determined with the biuret reagent.

Orotic acid-6^{14}C, glycine-2^{14}C, threonine-^{14}C UL, aspartate-^{14}C UL, uridine-6^{3}H, thymidine-CH_3-^{3}H, and deoxyuridine-^{3}H were obtained from New England Nuclear Corp. Meso, a, β diphenylsuccinic acid was obtained from Aldrich Chemicals.

RESULTS

Inhibition of Deoxyribose 5-Phosphate Aldolase. - DPS competitively inhibits highly purified preparations of rat liver deoxyribose 5-phosphate aldolase as is illustrated in Fig. 1. The K_i for DPS was determined to be 3×10^{-5}M. DPS was strictly competitive with either citrate or succinate at concentrations of DPS below 10^{-4}M.

Other carboxylic acids which were capable of inhibiting the deoxyribose 5-phosphate aldolase are compared in Table I. DPS was the most potent carboxylic acid tested with either purified or crude enzyme preparations.

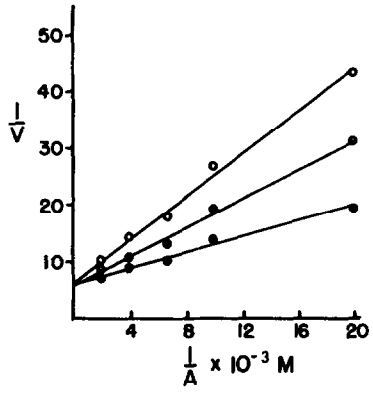

Fig. 1 The inhibition of purified deoxyribose 5-phosphate aldolase by DPS. Citrate (activator) concentration was varied over the range of 0.05 mM to 0.5 mM at 3 different concentrations of DPS: ●——● -no DPS, ●——○ -0.03 mM DPS, and ○——○ -0.06 mM DPS. Incubation mixtures contained 0.5 mM deoxyribose 5-phosphate, 5 mM Tris, pH 7.5, 1 mM EDTA, 0.2 mM DPNH, 50 μg crystalline alcohol dehydrogenase, 10 μg bovine serum albumin, and 1 μg of deoxyribose 5-phosphate aldolase in a final volume of 03 ml. Absorbancy readings at 340 mμ were taken at 30 second intervals for 5 minutes at 22°.

TABLE I

Comparison of Several Carboxylic Acids as Inhibitors of Deoxyribose 5-Phosphate Aldolase

Carboxylic acid	% Inhibition	
	A*	B*
Diphenyl Succinate	92	70
Phenyl Succinate	65	35
3-Phenyl, 3-propyl Glutarate	50	31
3-Phenyl, 3-ethyl Glutarate	60	34

* Inhibition of purified deoxyribose 5-phosphate aldolase (A). Incubation mixtures contained 0.5 mM deoxyribose 5-phosphate; 3 mM citrate, 5 mM Tris, pH 7.5; enzyme; 1 mM EDTA; 0.2 mM DPNH; 50 μg crystalline alcohol dehydrogenase; and 10 μg bovine serum albumin. The inhibitor was added at a concentration of 3 mM. Final volume 0.3 ml. Absorbancy readings at 340 m were taken at 30 second intervals for 5 minutes at 22°.
Inhibition of crude deoxyribose 5-phosphate aldolase (B). A 25% homogenate of mouse liver in 0.05 M Tris, pH 7.5 was centrifuged for 15 minutes at 10,000 r.p.m. The supernate was assayed for activity in the presence and absence of 1 mM inhibitor. Incubation mixtures contained 0.5 mM deoxyribose 5-phosphate, 3 mM citrate, 5 mM Tris, pH 7.5, 1 mM EDTA, and enzyme in a volume of 0.3 ml. Mixtures were incubated at 22° for 30 minutes. The reaction was stopped by the addition of 0.1 ml of 3 M perchloric acid. Diphenylamine reagent was used to measure residual deoxyribose 5-phosphate (Jiang and Groth 1962).

In vivo Inhibition Studies - Eight 25 g Swiss mice were injected intraperitoneally with 0.5 ml of 0.2 M DPS. Two mice were sacrificed at 15, 30, 60, and 120 minutes after injection, the liver was quickly removed and homogenized in 3 volumes of cold 0.05 M Tris buffer, pH 7.5. The homogenates were assayed for deoxyribose 5-phosphate aldolase activity as in Table I (B). Control animals consisted of mice injected with 0.5 ml of 0.2 M disodium succinate and mice injected with DPS 24 hours previously. It was apparent that DPS inhibited the liver enzyme in vivo for at least 2 hours. After 24 hours the inhibition was completely reversed. (Table II).

TABLE II

In vivo Inhibition of Deoxyribose 5-phosphate Aldolase by DPS

Time After DPS Injection min.	mμM Dr-5-P Metabolized*	% Inhibition
Controls	260 ± 30	-
15	105 ± 10	60
30	97 ± 15	63
60	108 ± 10	58
120	112 ± 10	57
24 hours	250 ± 10	-

* Assays were carried out as in the legend of Table I (B).

Inhibition of DNA Synthesis in Regenerating Rat Liver Slices - The effects of DPS upon the synthesis of DNA, RNA, and protein were studied with the appropriate labeled precursor. The conditions of incubation employed allowed for an approximately linear incorporation of label over the period of incubation. Table III presents results on the incorporation of various precursors in regenerating rat liver at varying times following partial hepatectomy. It was evident that DPS specifically inhibited the incorporation of orotate and uracil (from uridine) into DNA pyrimidines. The incorporations of the same precursors into RNA pyrimidines, thymidine into DNA, and glycine into RNA and protein were not inhibited by DPS.

TABLE III.

Effect of DPS on the Incorporation of Various Precursors into DNA, RNA, and Protein of Slices from Regenerating Rat Liver*

Time of Regeneration	Precursors	DPS	DNA				RNA				Protein	
			THY Cpm/ μM	%I	CYT Cpm/ μM	%I	URA Cpm/ μM	%I	CYT Cpm/ μM	%I	Cpm/mg.	%I
24 hrs.	Orotic	-	3000	-			16150	-				
		++	2300	23			16120	0				
36	Orotic	-	1801	-	1109	-	12900	-	8040	-		
		++	979	46	733	34	15000	0	8490	0		
36	Glycine	-	-		-		(817)†	-			5050	-
		++	-		-		(757)†	7			5485	0
36	Uridine	-	773	-	290	-	2460	-	1030	-		
		++	334	57	172	41	3320	0	1030	0		
36	Thymidine	-	1172	-	-							
		++	1140	2	-							
48	Orotic	-	194	-	86	-	7140	-	2780	-		
		++	79	59	55	36	6920	3	3355	0		

*Slices of rat regenerating liver were prepared and incubated in O_2 in Krebs-Ringer, buffered with glycylglycine, pH 7.8, for 3 hours at 37°. DPS was added at a final concentration of 10 mM. Addition of radioactive precursors was as follows: orotate-6 ^{14}C, 1 micro c. 0.5 mM; glycine-2 ^{14}C, 2 micro c. 0.2 mM; uridine-6 ^{3}H, 4 micro c., 0.2 mM; thymidine-CH_3 ^{3}H, 2.5 micro c., 0.2 mM. Final incubation volume- 2 ml, 300 mg slices added per flask.

†Expressed as Cpm per mg total unfractionated RNA.

Inhibition of DNA Synthesis in Ehrlich Ascites Tumor Cells by DPS - DPS specifically inhibited the incorporation of labelled orotate and adenine into DNA pyrimidines and purines. The incorporation of labeled deoxyuridine into DNA pyrimidines was stimulated by DPS, presumably due to the increased specific activity of the deoxynucleotide pool in DPS inhibited cells. The incorporations of labeled threonine and aspartate into deoxyribonucleotide presumably occurred via ribonucleotide reduction (Reichard 1962), e.g., from the complete de novo pathway of nucleotide synthesis. Deoxyribose 5-phosphate does not appear to be an intermediate in the de novo pathway.

TABLE IV.

Effect of DPS on the Incorporation of Various Precursors into DNA, RNA, and Protein of Ehrlich Ascites Tumor Cells*

		DNA				RNA				
Precursor	DPS	THY Cpm/ μM	%I	CYT Cpm/ μM	%I	URA Cpm/ μM	%I	CYT Cpm/ μM	%I	Protein %I Cpm/mg
Orotic	-	1040	-	176	-	14150	-	3890	-	
	+	312	70	94	47	14600	0	4120	0	
	++	88	92	44	75	13100	7	4170	0	
Deoxy-uridine	-	344	-	128	-	13250	-	1120	-	
	+	349	0	249	0	14500	0	1610	0	
	++	387	0	318	0	16750	0	2225	0	

		DNA				RNA			
		AD Cpm/ μM	%I	GU Cpm/ μM	%I	AD Cpm/ μM	%I	GU Cpm/ μM	%I
Adenine	-	1833	-	34	-	7930	-	1440	-
	++	985	46	8	76	7830	1	1303	9

		DNA								RNA*			
		AD Cpm/ μM	%I	GU Cpm/ μM	%I	CYT Cpm/ μM	%I	THY Cpm/ μM	%I	Cpm/mg.	%I		
Threonine	-	68	-	86	-	89	-	454	-	11200	-	6125	-
	++	66	3	78	9	98	0	413	9	11170	0	6307	0
Aspartate	-	13	-	8	-	21	-	37	-	478	-	4310	-
	++	13	0	19	0	20	5	41	0	483	0	4295	0

*Conditions of incubation were identical to Table III, except that 2 hours of incubation were used. DPS was added at two different concentrations: + 5 mM, and ++ 10 mM. The labeled precursors were added as follows: orotate-6 ^{14}C, 1 micro c., 0.5 mM; deoxyuridine-^{3}H, 2.5 micro c., 0.1 mM; aspartate-^{14}C UL, 2.5 micro c., 0.5 mM; adenine-^{3}H, 5 micro c., 0.5 mM; threonine-^{14}C UL, 2.5 micro c., 0.5 mM.

Discussion - Inhibition of deoxyribose 5-phosphate aldolase in mammalian cells produces a specific inhibition of the synthesis of deoxyribonucleotide for DNA. The incorporation of labeled purines and pyrimidines into DNA was strongly inhibited by DPS, under conditions where the synthesis of RNA ribonucleotide and protein was not inhibited. Possible other sites of DPS inhibition which could explain the observed results, i.e., DNA polymerase and ribonucleotide reductase were shown not to be inhibited by the use of labeled thymidine and (deoxyuridine) and threonine (and

aspartate), respectively. The use of DPS as an inhibitor has provided strong support for the involvement of deoxyribose 5-phosphate aldolase in the synthesis of deoxyribonucleotides in mammalian cells. The mechanism by which deoxyribose 5-phosphate is involved as an intermediate in nucleotide synthesis and the possible use of DPS as a chemotherapeutic agent, either alone or in combination with other inhibitors of DNA synthesis, are presently under investigation.

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