Structural Specificity of the Inhibitors of a Microbial Glucose-6-Phosphate Dehydrogenase[1]

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The mechanisms by which hormones and functionally related compounds control or regulate metabolic reactions have presented some very intriguing areas of study. Investigations in this laboratory have shown that diethylstilbestrol, a synthetic estrogen, inhibits the oxidation of cer-

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tain substrates by resting cell suspensions of *Aerobacter aerogenes* (Durham and Perry, 1959). Additional studies indicated that this synthetic estrogen also inhibited the reduction of triphosphopyridine nucleotide by a glucose-6-phosphate dehydrogenase demonstrated to be present in fractionated extracts of this organism and it was suggested that diethylstilbestrol influenced the enzymatic reaction by interacting with the dehydrogenase enzyme (Durham, 1960; Durham and Leach, 1961). Structurally and functionally related compounds, such as dienestrol and hexestrol, also inhibited glucose-6-phosphate dehydrogenase activity but other functionally related compounds such as estrone, 17 *beta*-estradiol, and 17 *alpha*-estradiol did not suppress reduction of the nucleotide.

Since the mechanism of inhibition appeared to be an interaction between the synthetic estrogen and the enzyme thereby producing a rate-limiting component in the enzymatic reaction, a study was undertaken to elucidate the structural specificity that a compound must possess to function as an inhibitor.

An ammonium sulfate fractionated extract containing the glucose-6-phosphate dehydrogenase was prepared from *Aerobacter aerogenes* as previously described (Durham and Leach, 1961). The enzymatic reaction was followed by measuring the reduction of triphosphopyridine nucleotide (TPN) at 340 $m\mu$ in the Beckman DU spectrophotometer at room temperature.

The reaction system was prepared by adding the following components to the cuvette: 0.1 ml fractionated extract; 20 μmoles glucose-6-phosphate; 0.2 μmole TPN; 200 μmoles Tris buffer, pH 7.8; 1.0 μmole $MnCl_2$; 0.1 ml of 1:1 propylene glycol:water (containing test compound in desired concentration); and water to volume of 2.8 ml. All components except the nucleotide were added directly to the cuvette, mixed, and the reaction was started by the addition of TPN. Protein was determined with a modified Folin reagent (Sutherland *et al.*, 1949).

The effect of several different additives on TPN reduction by the glucose-6-phosphate dehydrogenase enzyme was investigated and the results are presented in Table I. These findings show that diethylstilbestrol, dienestrol, and hexestrol suppress the reduction of the nucleotide by glucose-6-phosphate dehydrogenase while several other compounds, exhibiting varying degrees of structural similarity, did not influence the enzymatic reaction. The results suggest that the phenolic hydroxyl group may be one of the essential binding or associating sites since the removal of this group (*trans*-stilbene or mandelic acid) or substitution of propionate for the hydroxyl group in a compound which otherwise structurally resembled the synthetic estrogen (diethylstilbestrol dipropionate) resulted in the loss of inhibitory capacity. However, since compounds possessing the phenolic hydroxyl group (phenol, *para*-aminophenol, and *para*-butylphenol) were equally ineffective as inhibitors, the findings suggest that a second binding group may also be essential in order that the inhibitor may attach or associate with the enzyme molecule. The specificity and spacial configuration of the ethyl group, which is characteristic of the three snythetic estrogens which function so efficiently as inhibitors, may fulfill this requirement. Removal of the ethyl groups from the diphenolic nucleus (*trans*-stilbene) or the presence of an NH_2 (*p*-aminophenol) or CH_2 -CH_2 -CH_2 -CH_3 (*p*-butylphenol) *para* to the hydroxyl group did not permit the compound to function efficiently as an inhibitor thus illustrating the structural specificity and spacial configuration are extremely important in this inhibition.

TABLE I. EFFECT OF VARIOUS ADDITIVES ON TRIPHOSPHOPYRIDINE NUCLEOTIDE (TPN) REDUCTION BY GLUCOSE-6-PHOSPHATE DEHYDROGENASE.

Additive	Concentration 10^{-7} moles/cuvette	Rate of TPN reduction △ O. D. 340/2 minutes
Solvent control	-	.123
Diethylstilbestrol	1	.079
	2	.057
	4	.029
Dienestrol	1	.090
	2	.074
	4	.048
Hexestrol	1	.105
	2	.089
	4	.060
trans-Stilbene	1	.128
	4	.121
Diethylstilbestrol dipropionate	1	.120
	4	.124
Progesterone	4	.129
Phenol	4	.127
para-Aminophenol	4	.118
Mandelic acid	4	.121
para-Butylphenol	4	.117

All additives were dissolved in the desired concentration in a 1:1 propylene glycol:water solvent system. The reaction rate was linear during the time interval in which the calculations were made. Extract contained 2.85 mg protein/ml.

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