THE PYRIDINE NUCLEOTIDE SPECIFICITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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Studies of the highly active glucose-6-phosphate dehydrogenase (G6PD) of lactating rat mammary glands have revealed that DPN and certain DPN analogues may replace TPN in the enzymatic oxidation of glucose-6-phosphate to 6-phosphogluconate. Under suitable conditions, the rate of reaction with DPN (D-G6PD) approached that with TPN (T-G6PD), contrary to the widespread view that G6PD is strictly TPN-specific in yeast (Warburg and Christian, 1936; see Kornberg and Horecker, 1955) and animal tissues (Dickens and Glock, 1952). No evidence has been obtained so far, that the reaction of DPN with G6PD is the consequence of a coupling between T-G6PD and a pyridine nucleotide transhydrogenase.

Mammary glands of lactating (14-19 days postpartum) Sprague-Dawley rats were minced and homogenized for 30 seconds in a Waring Blendor with a medium of pH 7.4 containing 0.004 M $MgCl_2$, 0.03 M nicotinamide and 0.1 M potassium phosphate. Removal of fat and cellular debris by low speed centrifugation was followed by centrifuging for 30 minutes at 20,000 x g. The proteins precipitating between 0.3 and 0.6 saturation of ammonium sulfate were collected and dialyzed against a solution of pH 7.4 containing 0.001 M EDTA, 0.007 M β-mercaptoethanol and 0.005 M potassium phosphate. This material was applied to a DEAE-cellulose column and eluted with a gradient of phosphate of pH 7.4 (limit = 0.5 M). G6PD emerged as a sharp peak, cleanly separated from 6-phosphogluconic dehydrogenase. The over-all purification was 20-fold. The enzyme was concentrated with the aid of a second DEAE-cellulose column and stored at -20°; it was dialyzed against a solution of pH 7.4 containing 0.02 M Tris hydrochloride, 0.001 M EDTA and 0.007 M β-mercaptoethanol. Repeated thawing and freezing of the enzyme solution led to a progressive and parallel loss of both T-G6PD and D-G6PD activities.

The products of the enzymatic oxidation of glucose-6-phosphate by DPN were identified as equimolar amounts of DPNH and 6-phosphogluconate. Thus, in one experiment 0.111 μmole of DPN was added to a cuvette containing Tris hydrochloride and excess glucose-6-phosphate and enzyme (pH = 8.4). Under these conditions of limiting DPN concentration the reaction proceeded slowly. After 115 minutes 0.105 μmole of DPN (i.e. 95%) had been reduced, as determined from the change in absorbancy at 340 mμ. The addition of excess yeast alcohol dehydrogenase and acetaldehyde then led to the rapid and complete reoxidation of the DPNH. In a similar experiment with excess DPN, 0.0499 μmole of glucose-6-phosphate yielded 0.0515 μmole of 6-phosphogluconate (103%) which was identified by its oxidation with excess TPN and 6-phosphogluconic dehydrogenase. The rate of the D-G6PD reaction was directly proportional to enzyme concentration over a fifty-fold range. The Michaelis constant for glucose-6-phosphate in the D-G6PD reaction was found to be 3.9×10^{-4} M, which is about 7 times the value reported by McLean (1958) for mammary T-G6PD. The affinity for DPN was very low (K_M = ca. 0.014 M) and all experiments were performed with concentrations below the Michaelis constant.

Efforts to ascertain whether the D-G6PD and T-G6PD activities of mammary gland were catalyzed by the same protein have included purification and studies on the effects of various reagents (Table 1). $MgCl_2$, which is routinely employed in assaying G6PD (See Kornberg and Horecker, 1955) was shown to inhibit D-G6PD activity. Addition of low concentrations of TPN to a mixture in which DPN was undergoing reduction by glucose-6-phosphate resulted in its rapid and quantitative reduction. The further reduction of DPN was then strongly inhibited by the TPNH thus formed. The D-G6PD was also strongly inhibited by 2'-AMP and to a lesser extent by 5'-AMP. Neither of these partial analogues of TPN affected the T-G6PD reaction; 3'-AMP was without effect upon either D- or T-G6PD activities. The inhibitory effect of 2'-AMP upon TPN-linked dehydrogenases, including yeast G6PD, was studied by Neufeld et al., (1955).

Marks and Banks (1960) showed that G6PD from human erythrocytes and other mammalian tissues, but not yeast G6PD, were strongly inhibited by low concentrations of certain steroids, including dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one). Dehydroepiandrosterone is a

TABLE 1

Effects of Various Reagents on the Rates of the D-G6PD and T-G6PD Activities

Reagent	Molarity	D-G6PD*	T-G6PD*
Potassium phosphate	0.033	77	92
	0.067	58	76
$MgCl_2$	0.017	53	91
Raising pH from 7.0 to 8.5	-	320	100
$NaHCO_3$, pH = 9.0**	0.017	210	107
TPNH	4.1×10^{-6}	69	-
	4.1×10^{-5}	16	-
2'-AMP	8.3×10^{-4}	51	91
5'-AMP	8.3×10^{-4}	72	105
Dehydroepiandrosterone+	1×10^{-4}	120	11
	1×10^{-5}	90	40
	1×10^{-6}	102	77
	1×10^{-7}	104	89
	1×10^{-8}	99	98
Progesterone+	1×10^{-4}	121	54
	1×10^{-5}	105	73
	1×10^{-6}	102	83
	1×10^{-7}	105	70
	1×10^{-8}	96	69

Cuvettes contained: 40 μmoles of Tris hydrochloride; 3.0 μmoles of DPN or 0.05 μmole of TPN; the reagent indicated and enzyme (0.02 to 0.04 ml.); pH = 7.3 except where noted. Reactions were initiated with 2 μmoles of glucose-6-phosphate. Final volume = 0.60 ml. Controls contained all ingredients except nucleotide. Rates were obtained from initial linear portions of plots of absorbancy at 340 mμ against time.

* Per cent of control rate in absence of reagent. Control rates for best enzyme preparation were: 5.10 μmoles per minute per mg protein for T-G6PD and 0.455 μmole per minute per mg protein for D-G6PD, both measured at pH = 8.5.

+ Steroids were added in dioxane or methanol and controls received an equal volume of the solvent.

** Compared to control at pH = 9.0.

TABLE 2

The Properties of Glucose-6-phosphate Dehydrogenase from Various Sources

	Lactating Mammary Gland	Pregnant Mammary Gland	Adult Male Adrenal	Infant Liver[a]	Infant Brain[a]	Yeast[b]
T-G6PD μmoles/min per mg protein	1.45	0.0350	0.360	0.0345	0.0438	112
Relative rates[c]:						
TPN	100	100	100	100	100	100
DPN	7.16	7.50	8.76	5.90	7.12	0.0245
TN-*DPN[d]	15.7	18.6	22.4	16.2	21.0	0.0870
DA-*DPN[e]	0.792	1.02	1.30	0.777	0.945	0.00733
T-G6PD + 2'-AMP[f]	91	100	94	100	89	81
D-G6PD + 2'-AMP[f]	51	60	36	43	45	58
T-G6PD + DEA[g]	10	11	7	8	10	105
D-G6PD + DEA[g]	100	106	83	54	91	200
T-G6PD, pH 7.2[h]	75	93	92	97	90	83
D-G6PD, pH 6.9[h]	7	18	9	18	19	14

Conditions as for Table 1, except that measurements were made at pH = 8.5, except where noted. Cuvettes contained 0.05 μmole of TPN or 3.0 μmoles of DPN or DPN analogue. Reactions with TN-*DPN measured at 395 mμ; with DA-*DPN at 338 mμ.

[a] Prepared from suckling rats, 18-20 days old.

[b] Obtained from C. F. Boehringer.

[c] Relative rate, μmoles per minute per mg protein.

[d] TN-*DPN = thionicotinamide analogue of DPN.

[e] DA-*DPN = deamino analogue of DPN.

[f] Rate observed in presence of 0.5 μmole of 2'-AMP, per cent of control

[g] Rate observed in presence of 20 μg of dehydroepiandrosterone, per cent of control.

[h] Rate observed at the pH indicated, per cent of rate at pH = 8.5. For the yeast enzyme the measurements were made at pH's 7.2 and 9.0.

powerful inhibitor of mammary gland T-G6PD (Table 1). Progesterone also inhibited T-G6PD at very low concentrations. Estradiol was without effect. None of these steroids inhibited D-G6PD at the concentrations indicated.

Heat inactivation of both activities occurred at the same rate. Thus, after heating for 0.5, 1, 2 and 5 minutes at 47° the residual activity was: 63, 39, 20 and 4 per cent respectively for T-G6PD and 49, 38, 18 and 1 per cent respectively for D-G6PD. The addition of a large amount of purified yeast G6PD did not restore the partial loss of D-G6PD activity.

Reactivity with DPN is not a special feature of G6PD in the lactating mammary gland. G6PD was partially purified from several other rat tissues and its reactivity with DPN and other properties compared with the yeast enzyme (Table 2). The similarity between the enzymes of various rat tissues is evident. The relative reactivity of yeast G6PD with DPN and DPN analogues is two or three orders of magnitude lower than it is for the enzymes from rat tissues. Yeast T-G6PD activity is not inhibited by dehydroepiandrosterone (cf. Marks and Banks, 1960).

Although T-G6PD and D-G6PD can respond differentially to various reagents, the bulk of the evidence favors the view that one enzyme catalyzes both reactions.

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