Studies on the Glucose-6-Phosphate and Isocitrate Dehydrogenase Systems in Aerobacter aerogenes[1]

NORMAN N. DURHAM, Department of Bacteriology
Oklahoma State University, Stillwater

Transhydrogenation reactions have received a great deal of attention since reports indicate that the transfer of hydrogen may be mediated by various steroids. Talalay and Williams-Ashman (1958) first reported that estradiol or testosterone may function as coenzymes for transporting hydrogen between the oxidized and reduced form of the nucleotides. Since these conversions play a very important role in energy-yielding reactions, we have investigated the influence of these compounds on the glucose-6-phosphate and isocitrate dehydrogenase systems of *Aerobacter aerogenes*.

Fractionation procedures were employed to obtain the enzymes in a cell-free system. Cells were harvested from nutrient agar, washed, and ruptured by forcing through a French Pressure Cell at 20,000 psi. This

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extract was centrifuged at 20,000 x g for 40 minutes and the supernatant collected for protamine sulfate treatment and ammonium sulfate fractionation. These fractions were reconstituted in M/100 tris buffer of pH 7.0 and dialyzed against distilled water for six hours prior to use.

Enzymic activity was followed by measuring the reduction of nucleotides at 340 mμ in the Beckman DU Spectrophotometer. The experimental system consisted of extract; tris buffer, 100 μM; $MnCl_2$, 0.001 mM; substrate, 10 μM; triphosphopyridine nucleotide (TPN), 0.05 μM; and water to a total volume of 3.0 ml. Diphosphopyridine nucleotide (DPN) was employed in a concentration of 2 μM and the steroids were dissolved in dioxane and employed in a final concentration of 0.004M. A control vessel also contained dioxane.

Results indicated that both of the dehydrogenases employed in this study were TPN dependent and that this coenzyme was limiting in the reaction system. The addition of TPN at various time intervals was followed by a rapid increase in optical density which plateaued when the oxidized form of the nucleotide became limiting. The addition of DPN did not increase the optical density and the simultaneous addition of DPN and estradiol during the reduction of TPN did not influence the reaction. Progesterone and testosterone were also added to the reaction vessel but exerted little, if any, effect on the reduction of the nucleotide.

These findings indicate that the steroids employed in this study did not influence the reduction of TPN by either glucose-6-phosphate or isocitric dehydrogenase and the apparent lack of activity when DPN and estradiol were added suggests that the cellular fractions employed in this investigation did not possess steroid mediated transhydrogenase activity.

Literature Cited

Talalay, P. and Williams-Ashman, H. G. 1958. Activation of hydrogen transfer between pyridine nucleotides by steroid hormones. Proc. Natl. Acad. Sci., 44: 15-26.