

Case 20

NAD⁺-dependent Glyceraldehyde-3-phosphate Dehydrogenase from *Thermoproteus tenax*

Last modified 1 Dec 2004

Focus concept

Glycolytic enzymes from *Thermoproteus tenax* are regulated in an unusual manner.

Prerequisites

- The glycolytic pathway.
- Enzyme kinetics and inhibition.
- The cooperative nature of regulated enzymes.

Background

Carbohydrate metabolism in the thermophilic archaeal bacterium *Thermoproteus tenax* is rather peculiar compared to the types of organisms usually studied in introductory biochemistry. For example, the phosphofructokinase reaction in *T. tenax* is reversible, and is dependent upon pyrophosphate rather than ATP. In addition, *T. tenax* has two different glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoenzymes. One is well known and, although it requires NADP⁺ as a cofactor instead of NAD⁺, resembles the GAPDH enzyme we studied in class and is referred to as the “phosphorylating GAPDH”. In contrast, the second isoenzyme is irreversible and requires NAD⁺ as a cofactor and is referred to as the “nonphosphorylating GAPDH”. In this case, we will consider the properties of the latter enzyme. The balanced equation of the reaction catalyzed by the nonphosphorylating NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase is shown below.

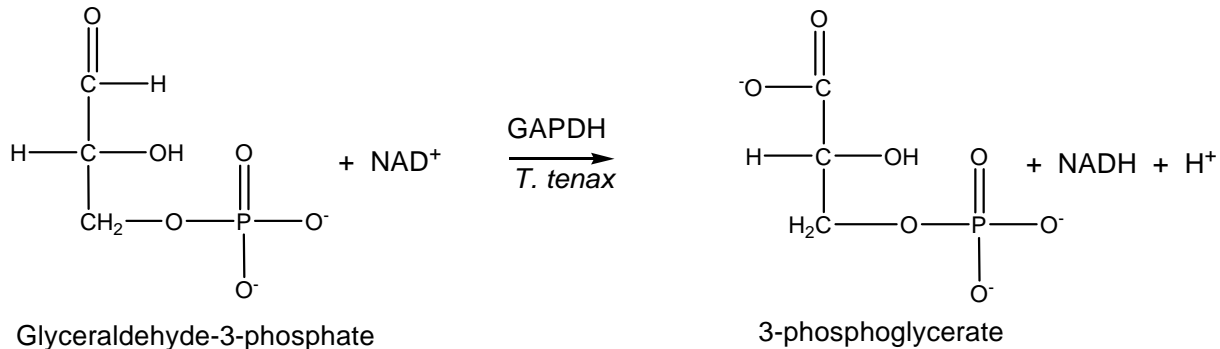


Figure 20.1: Non-phosphorylating NAD⁺-dependent GAPDH in *T. tenax*.

T. tenax stores energy in the form of glycogen, which is degraded to glucose-1-phosphate. The glucose-1-phosphate is then converted to glucose-6-phosphate and then enters the glycolytic pathway. The two GAPDH enzymes are probably differently regulated in *T. tenax*. The authors of this study propose that “phosphorylating, NADP⁺-dependent” GAPDH is involved in efficient ATP production whereas the “non-phosphorylating, NAD⁺-dependent” GAPDH is somewhat involved in ATP produc-

tion but is also involved in providing intermediates for cellular biosynthetic reactions.

The gene for the non-phosphorylating, NAD^+ -dependent GAPDH was cloned and sequenced and its kinetic characteristics were studied. Summary information is presented in Table 1.1.

Table 20.1: Kinetic properties of NAD^+ -dependent GAPDH isolated from *T. tenax*.

<u>NAD^+ saturation</u>	
<i>Without AMP</i>	
V_{max} , units/mg	36.5
K_M , mM	3.3
<i>With AMP</i>	
V_{max} , units/mg	37.0
K_M , mM	1.4
<u>Molecular Mass</u>	
Subunit (kD)	55,000
Native (kD)	220,000

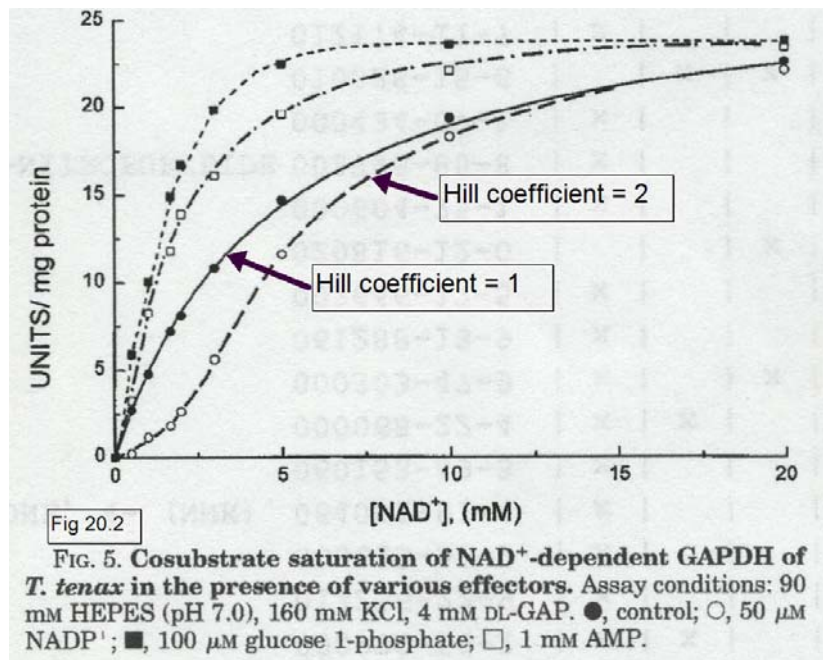
Questions

1. Name the three enzymes that catalyze irreversible, regulated reactions in glycolysis as studied in class.
2. What is the significance of the GAPDH reaction (in *E. coli*, the enzyme discussed in class) to glycolysis?
3. How does the reaction catalyzed by GAPDH from *T. tenax* presented here differ from the reaction carried out in *E. coli* (ie, the reaction discussed in class)?
4. The activity of the GAPDH enzyme was assayed in the presence of a constant amount of glyceraldehyde-3-phosphate and an increasing amount of NAD^+ . The activity of the control was compared to the activity in the presence of various metabolites. The results are shown in Figure 20.2. Additional data are given in Table 20.2.
 - a. Use the data in Figure 20.2 to estimate a K_M value for the enzyme in the presence of these metabolites. Classify the metabolites listed in Table 20.2 as inhibitors or activators. Explain how you decided whether these metabolites are inhibitors or activators, based on the graph.
 - b. How would you classify NADH, ADP and ATP? (These data are not presented in the graph). Are they inhibitors or activators? Add this information to Table 20.2.
 - c. Explain the physiological significance of your answers to questions 4a and 4b.

Table 20.2: Effect of various metabolites on the activity of NAD^+ -dependent GAPDH isolated from *T. tenax*. (Based on Brunner, et al., 1998.)

Metabolite	Apparent K_M , mM	Inhibitor or activator?
None		
$NADP^+$		
Glucose-1-phosphate		
AMP		
NADH		
ATP		
ADP		

- The Hill coefficients for NAD^+ binding to the *T. tenax* GAPDH in the presence and absence of $NADP^+$ were measured and are shown on the graph in Figure 20.2. What is the significance of the change in the value of the Hill coefficient? Is this consistent with (a) the shape of the curve and (b) the information given in the background on the enzyme's structure?
- What is the ATP yield for one mole of glucose oxidized by the pathway that uses the nonphosphorylating GAPDH enzyme?



Reference

Brunner, N. A., Brinkmann, H., Siebers, B., and Hensel, R. (1998) *J. Biol. Chem.* **273**, pp. 6149-6156.