Spectrophotometric assay for measuring polyol dehydrogenase activity

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Abstract

Polyol dehydrogenases are enzymes that convert polyalcohols into sugars, using NAD^+ as hydrogen acceptor. Sorbitol, mannitol or other polyalcohols can be used as substrates, and the activity of these enzymes is assessed by monitoring the production of NADH. This protocol was based on previously published methods (Maret and Auld, 1988; Fernández et al., 1993), and was used to evaluate polyol dehydrogenase activities in the digestive gland of gastropods. With the proper adaptations this procedure can be applied to other dehydrogenases and other organisms.

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Before start

Prepare the following solutions:

Homogenization medium for marine gastropods

(adapted from Stewart et al. 1994 and Moyers et al. 1991)

Sucrose - 500 mM

Potassium chloride - 150 mM

Ethylenediamine tetraacetic acid (EDTA), sodium salt - 1 mM

Tris-HCl 50 mM pH 7.4

This medium, without phenylmethylsulfonyl fluoride (PMSF), can be stored at -20º C in aliquots. To avoid the inactivation of PMSF in *aqueous solution,* add 5 µl of 200 mM PMSF (in absolute ethanol) per ml of medium just before use (final PMSF concentration 1 mM).

Good results were also obtained with freshwater and terrestrial gastropods using this high osmolarity medium.

Substrate solutions

A. 50 mM NAD^+solution in water (can be stored frozen in aliquots)

B. 57 mM polyalcohol (sorbitol, mannitol or other) in 50 mM glycine/NaOH buffer pH 10.0

Materials

- Potter-Elvehjem homogenizer by Contributed by users
- Bath sonicator by Contributed by users
- Centrifuge by Contributed by users
- Double-bean UV-visible spectrophotometer, with temperature control in the cuvette compartment by Contributed by users

Protocol

Sample preparation

Step 1.

1. Homogenize approximately 0.05-0.06 g of digestive gland tissue per ml of cold homogenization medium using a Potter-Elvehjem homogenizer at 1,000 rpm, keeping the tube in ice.
2. Register the exact weight of digestive gland tissue used and the final homogenate volume.
3. Sonicate the homogenate during 3 x 15 s using a bath sonicator, keeping the tube in an ice-water bath.
4. Centrifuge the homogenate at 1,000 g for 5 min. at 4º C, and use the supernatant to assess polyol dehydrogenase activities. Keep samples on ice and use immediately after preparation.

Assessment of polyol dehydrogenase activity

Step 2.

1. In a tube, mix 100 µl of substrate solution **A** with 880 µl of substrate solution **B** and 20 µl of sample, agitate with a vortex and pour into the spectrophotometer. Final concentrations in the assay: 5 mM NAD^+, 50 mM polyalcohol. Sample dilution factor in the assay: 50.
2. Measure the absorbance at 340 nm, at 25º C, during 3-5 min (or longer for samples with very low enzyme activity). Make sure that activity is linear in time and proportional to sample concentration. If not, adjust sample dilution.
3. For control, monitor non-specific increases of absorbance at 340 nm using medium without polyalcohol, and subtract if necessary.

Determination of enzyme activity.

Step 3.

Activity (nmol . ml^{-1}. min^{-1}) = $\frac{\Delta \text{ Abs340 } min^{-1}}{0.00622\ \mu M^{-1}}$ **x** 50*

NADH $\varepsilon_{340\ nm}$ = 6.22 mM^{-1}. cm^{-1}

* sample dilution factor in the assay

Calculate enzyme activity per g of tissue (nmol . g^{-1}. min^{-1}) or per mg of protein (nmol . mg^{-1}. min^{-1}).

References

Step 4.

Fernández M.R., Jörnvall H., Moreno A., Kaiser R. and Parés X., 1993. Cephalopod alcohol dehydrogenase: purification and enzymatic characterization. FEBS Lett. **328**: 235−238.

Maret W. and Auld, D.S., 1988. Purification and characterization of human liver sorbitol dehydrogenase. Biochemistry **27**: 1622−1628.

Moyes C.D., Suarez R.K., Brown G.S. and Hochachka P.W., 1991. Peroxisomal β-oxidation: insights from comparative biochemistry. J. Exp. Zool. **260**: 267–273.

Stewart J.M., Carlin R.C., Macdonald J.A. and Van Iderstine S., 1994. Fatty acid binding proteins and fatty acid catabolism in marine invertebrates: Peroxisomal β-oxidation. Inv. Rep. Dev. **25**: 73–82.