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A METHOD DESCRIPTION FOR NAD-DEPENDENT ISOCITRATE DEHYDROGENASE: A SIMPLE SPETROPHOTOMETRIC METHOD

Dr. Nuri BAKAN x
Dr. Ebubekir BAKAN xx
Dr. M. Ramazan YİĞİTOĞLU x
Dr. Bahattin ADAM x

ÖZET

Bu çalışmada, vucut sıvılarında NAD ye bağımlı izositrat dehidrogenaz aktivitesini tayin etmek için basit bir spektrofotometrik metod tarif edildi Bu amaçla, reaksiyon ortamı tampon, pH, koenzim (NAD) substrat (DL-izositrat) ve bazı iyonların etkisi yönünden incelendi. NAD ye bağımlı izositrat dehidrogenaz tayini için en uygun durumlar olarak şu hususlar tesbit edildi: trietanolamin tamponu (100 mM, pH 7,5), DL-izositrat (4,0 mM), NAD (0,4 mM), Mn (II) (5,0mM) ve NaCI (50 mM). Vucut sıvılarında NAD ya bağımlı izositrat dehidrogenaz mitokondriyal enziminin tayini için bu, spektrofotometrik ve basit bir metoddur.

INTRODUCTION

Isocitrat dehydrogenase (ICD) catalyzes the oxidative decarboxylation of isocitrate to α-oxoglutarate. This enzyme has two distinct forms: NADP-dependent (EC 1.1.1.42; ICD-P) and NAD-dependent (EC 1.1.1.41; ICD-H). The former that is found in serum is primarily of liver origin, although it is found to some extent in all cells (1). The latter participates in the Krebs tricarboxylic acid cycle. and a distinct enzyme with quite different properties. While ICD-P has two isoenzymes:one cytoplasmic (soluble) and the other mitochondrial, ICD-H is located only in mitochondria (2). In this study, ICD-H actvity is determined by a spectrophotometric method in serum pool having high levels of glutamate dehydrogenase (GLDH), another, unlocalized mitochondrial enzyme, in order to describe the reaction parameters of IDC-H.

x) A.Ü. Tıp Fak. Biyokimya Anabilim Dalı, Araş. Gör.
xx) A.Ü. Tıp Fak. Biyokimya Anabilim Dalı, Doç. Dr.

MATERIALS AND METHODS

After GLDH activity was determined, the sera high GLDH levels were pooled and this pool was used to definite the optimal conditions. GLDH reagent kit, triethanolamine, DL-isocitrate, and NAD were obtained from Boehringer, Mannheim, F.G.R. The enzyme activity was determined with Clinical Photometer, Hitachi 4020 (Boehringer, Mannheim, F.G.R.). The measurements were made as duplicates, and the results different from each other up to 5 % were excluded.

The different concentrations of triethanolamine (60-140 mM), DL- isocitrate (2.0-6.0 mM), NAD (0.2-0.8 mM), Mn^{+2} (1-10 mM), Mg^{+2} (1-10 mM), and NaCl (20-100 mM) and pH (ranging 7.0-8.0) were tried in order to define optimal reaction conditions. The reaction temperature was selected as 37°C, commonly used in measurement of clinically interest enzyme.

Measurement of ICD-H Activity

Reagents

Solution 1 (buffered substrate) contains triethanolamine buffer, DL-isocitrate, and sodium chloride. Solution 2 is composed of NAD and $MnSO_4$. The concentrations in solutions were selected within different ranges as mentioned above.

The principle of the test reaction is

$$\text{DL-isocitrate} + NAD^+ \xleftrightarrow[Mn^{+2}]{\text{ICD—H}} \alpha\text{-oxoglutarate} + CO_2 + NADH + H^+$$

where the increase in NADH is measured at 340 nm.

Activity Determination

To 2.5 ml of buffered substrate was added 0.5 ml of serum and this was preincubated at 37°C for about 5 min. Then, 0.1 ml of solution 2 was added, the tube was mixed, and the absorbance change was recorded for three minutes with oneminute intervals at 340 nm. The change in absorbance per min ($\triangle$A/min) was determined and multiplied by 948, a factor derived from micromolar absorbtivity of NADH and from dilution factor as follows.

$$\text{ICD-H (U/L)} = \frac{\triangle A/\text{min}}{6.3 \times 10^{-3}} \times \frac{3100}{500} = (\triangle A/\text{min}) \times 948$$

Where;

$\triangle$ A/min : average absorbance change per minute

6.3×10^{-3} : micromolar absorbtion coefficient of NADH at 339 nm

3400 : total volume in cuvet in microliter

500 : sample volume in microliter

RESULTS AND DISCUSSION

The results obtained are shown Figures 1 and 2. When one parameters was studied, the others were kept constant. Consequently, the peak activity value was accepted as optimal. As can be seen from figures, the increase in ICD-H activity in the case of each parameter is only at one point or within rather limited ranges. Mn (II) and Mg (II) activate the enzyme, but the former is more suitable activator than the latter.

Although no method has been suggested for the determination of ICD-H in body fluids, ICD-P activity in human serum has been determined by spectrophotometric (3), two-point, colorimetric (4), and continuous-monitoring(5,6) methods.

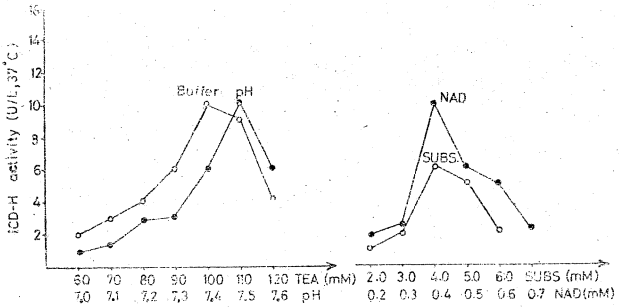

Fig. 1: The effects of buffer (TEA: triethanolamine), DL-isocitrate (SUBS: substrate), and NAD concentrations and pH on ICD-H activity.

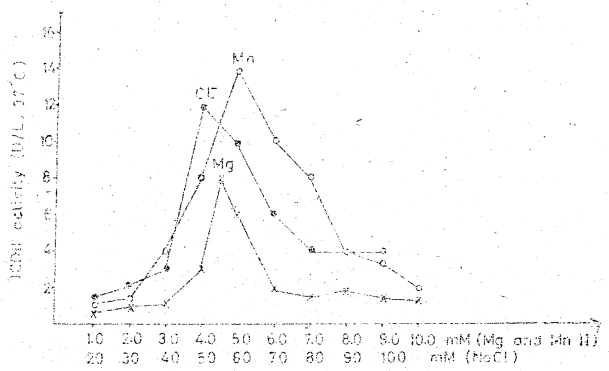

Fig. 2: The effect of some ions on ICD-H activity

Our results show that such optimal conditions as triethanolamine buffer (100 mM), pH (7.5), DL-isocitrate as substrate (4.0 mM), NAD as coenzyme (0.4 mM), Mn (II) as an activator (5.0 mM), chloride ions (50 mM). On the basis of the results, we suggest a spectrophotometric method, which is easy to perform

in routine laboratory and has only two steps. This is very important in selection of a test. On the other hand, ICD-H determination has a clinical, diagnostic value in hepatobiliyer disease as observed by us (unpublished data). However, further studies on this method introduced will show any more optimal conditions, since the present study is a preliminary one, and our detailed studies have been going on.

SUMMARY

In this study. a simple spectrophotometric method for determination of NAD-dependent isocitrate dehydrogenase activity in body fluidis was described. For this purpose, the reaction medium was investigated with respect to buffer, pH, coenzyme (NAD) substrate (DL-isocitrate), and the effect of some ions. The results showed that the optimal conditions for determination of NAD- dependent isocitrate dehydrogenase can be defined as: triethanolamine buffer (100 mM, pH 7.5), DL-isocitrate (4.0 mM), NAD (0.4 mM), Mn (II) (5.0 mM), and Nacl (50 mM). This is a simple, spectrophotometric method for the determination of mitochondrial enzyme NAD-dependent isocitrate dehydrogenase in body fluids.

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