INTERCONVERSION OF THE COMPONENTS OF RAT BRAIN MITOCHONDRIAL MALATE DEHYDROGENASE

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1. Introduction

The malate dehydrogenase (L-malate: NAD oxidoreductase EC 1.1.1.37) of many vertebrates exists as two main isoenzymic forms – one found in the cell cytoplasm (S-MDH) and the other in the mitochondria (M-MDH). The mitochondrial enzyme is often a family of catalytically active components, which can be separated by starch gel electrophoresis [1]. Kitto, Wassarman and Kaplan [2] have suggested, on the basis of reversible denaturation studies, that the components of M-MDH are 'conformers', differing only in conformation and being interconvertible under certain *in vitro* conditions. Schechter and Epstein [3] on the other hand were unable to observe interconversion of chicken heart M-MDH components, and believe that the differences between the components are not solely conformational. Mann and Vestling [4], in recent studies of rat liver M-MDH, have shown the enzyme to be a dimer. They suggest that there are two types of subunit, X and Y, and that the three major components of rat liver M-MDH are XX, XY and YY. The present paper presents evidence that the components of rat brain M-MDH are interconvertible under relatively mild conditions of storage, and that this interconversion is not readily compatible with the hypothesis of Mann and Vestling.

2. Experimental

Rat organs were homogenised for two minutes at 4° in a tight fitting glass-in-glass homogeniser, in a medium of 0.025 M sodium phosphate buffer pH 7.0 containing 0.5% (w/v) Triton X 100. After centrifugation at 30 000 rpm for 30 minutes at 4°, the supernatant was retained and the pellet subjected to a second homogenisation as before. The 30000 rpm supernatant from the second homogenisation was pooled with the first. By this method over 98% of total MDH could be extracted in soluble form [5].

Some of the extract was passed through a column of Sephadex G 25 (Pharmacia) equilibrated in 0.025 M sodium phosphate pH 7.0, and the first effluent retained. This effluent contained all the MDH applied to the Sephadex column.

M-MDH and S-MDH were separated by rapid chromatography on a small column of Amberlite CG 50 equilibrated with 0.025 M sodium phosphate pH 7.0. S-MDH emerged unretarded, M-MDH being subsequently eluted with 0.2 M sodium phosphate pH 7.0 [5]. Recoveries from the Amberlite chromatography were 96-100%.

The assay for MDH was carried out in a Beckman DK 2 spectrophotometer at 30°, in a medium containing 0.38 mM NAD, 33 mM sodium L-malate and 90 mM glycine buffer pH 10, by measuring the rate of increase of optical density at 340 mμ.

Starch gel electrophoresis, and the subsequent staining of the gel for MDH activity, were by the method previously described [1].

3. Results

Freshly prepared phosphate/Triton extracts of rat liver, kidney, heart and brain, on starch gel electrophoresis showed almost identical patterns. S-MDH

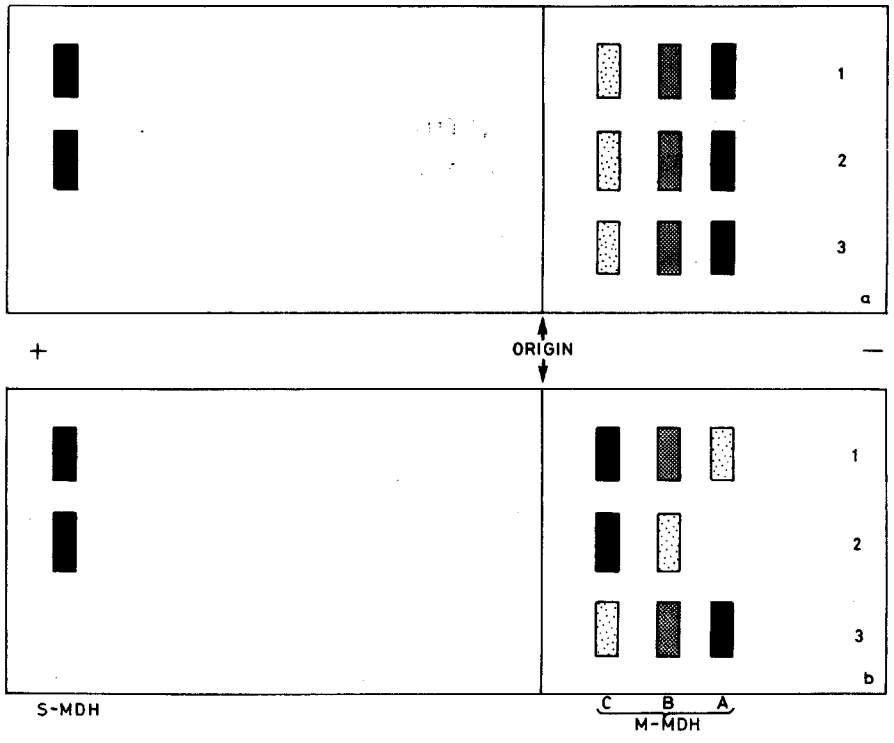

Fig. 1. Starch gel electrophoresis of rat brain malate dehydrogenase; (a) fresh extracts (less than 8 hours old) and (b) after storage at 4° for 10 days. (1) 0.025 M phosphate/0.5% Triton extract of brain. (2) 0.025 M phosphate/0.5% Triton extract after passage through Sephadex G25. (3) Retarded material from Amberlite CG 50 ('purified M-MDH'). The gels were stained for MDH activity (ref. [1]).

migrated to the anode, while M-MDH migrated towards the cathode and segregated into three components, designated as A, B and C beginning with the most cathodal [1].

Component A is stained deeply, B less so, while component C seems to be present in very small amounts. Fig. 1a, line 1, shows the pattern obtained on electrophoresis of a freshly prepared extract of rat brain. Gel electrophoresis of the phosphate/Triton extract after passage through Sephadex G25 revealed that the staining pattern remained unchanged (fig. 1a, line 2). Electrophoresis of the material retarded by Amberlite CG 50 showed that the S-MDH had been removed, but that the pattern and distribution of components A, B and C of M-MDH remained unchanged (fig. 1a, line 3).

Storage at 4°, for up to 20 days, had no effect on the electrophoretic pattern shown by extracts of rat liver, kidney or heart. This was true in the case of phosphate/Triton extracts both before and after passage through Sephadex G25 and of material retarded by Amberlite CG50. Spectrophotometric assays confirmed that more than 90% of the MDH activity was retained in these extracts during the storage period. Storage of rat brain extracts, however, caused an apparent redistribution of the activity amongst the M-MDH components. The electrophoretic pattern of a phosphate/Triton extract of rat brain with or without passage through Sephadex G25, after storage for 10 days is shown in fig. 1b, lines 1 and 2. Component C of M-MDH is now most deeply stained, component B less so, and component A is very weakly stained. Spectrophotometric assays showed that 100% of the MDH activity of the phosphate/Triton extract was retained after 10 days of storage, as was 96% of the MDH activity of the phosphate/Triton extract after passage through Sephadex G25. In contrast to these

findings, the electrophoretic pattern of 'purified M-MDH' of rat brain (material retarded by Amberlite CG 50) remained largely unchanged on storage for 10 days (fig. 1b, line 3). Spectrophotometric assays of this material confirmed that 100% of its MDH activity was retained during the storage.

Electrophoresis of a phosphate/Triton extract of rat brain which had been stored for 40 days at 4° showed that only component C of M-MDH remained, components A and B having disappeared. In this case, spectrophotometric assays showed that 94% of the total MDH activity of the extract remained.

Storage of brain extracts at −20°, instead of 4°, for periods of up to 20 days, prevented the apparent redistribution of M-MDH activity amongst its constituent components. After such storage, the staining of the electrophoretically separated M-MDH components was identical to that seen in fresh extracts (A > B > C).

4. Discussion

Fresh extracts of four rat organs show almost identical patterns on starch gel electrophoresis, after specific staining for malate dehydrogenase by the tetrazolium method. It seems therefore that the S-MDH and M-MDH and the components of the latter isoenzyme, are similar, if not identical, in the different organs. Storage of extracts of liver, kidney and heart has no effect on their MDH electrophoretic pattern, but storage of brain extracts at 4° permits an apparent interconversion of the M-MDH components; the two most cathodal, originally staining most heavily, eventually disappear, while the least cathodal (M-MDH component C) increases in amount. Storage at −20° prevents the interconversion.

Since the total MDH activity of the brain extracts falls by no more than 4% during storage for 10 days at 4° and since the M-MDH: S-MDH ratio (as judged by Amberlite chromatography of fresh and aged material) remains essentially unchanged, it would seem that the altered staining pattern is due to genuine interconversion and not to any differential loss in activity of some of the electrophoretic components.

The composition of the extracting medium is unlikely to be of importance in the interconversion phenomenon, since it was common to all organs investigated. Only in the case of the brain extracts was any interconversion noted.

The interconversion of the brain M-MDH components is seen in both crude extracts and in extracts which have passed through Sephadex G 25; but 'purified M-MDH' changes little in electrophoretic pattern after storage. It may be that some high molecular weight substance, found only in brain extracts, is responsible for the interconversion, and that the Amberlite chromatography removes this from the M-MDH. Investigation of this possibility is under way.

The observations reported in the present communication are not sufficient to enable a decision to be reached on the nature of the differences between the M-MDH components. These could be of a conformational type as suggested by Kitto, Wassarman and Kaplan [2] or of a more complex type, as suggested by Schechter and Epstein [3]. The fact that M-MDH components A and B may apparently eventually disappear, whilst at the same time component C, originally staining very weakly, becomes dominant in amount renders the hypothesis of Mann and Vestling [4] less attractive. However, it may be that the differences between Mann and Vestling's proposed subunits X and Y are not so great as to eliminate the possibility of these subunits being interconverted (X → Y) during storage.

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