Volume 214, number 1, 192–194 FEB 04586 April 1987

Differentiation of creatine kinase MB and IgA-linked BB isoenzymes on electrophoresis

Ann Chen and Shan S. Wong*

Department of Pathology, Tri-Serive General Hospital, National Defense Medical Center, Taiwan, Republic of China and **The Biochemistry Program, Department of Chemistry, University of Lowell, Lowell, MA 01854, USA*

Received 10 February 1987

IgA-linked creatine kinase (CK, EC 2.7.3.2) is a macro CK type 1 isoenzyme that has an identical electrophoretic mobility to CK-MB. Its presence has the potential of causing misdiagnosis of myocardial infarction. Mixing anti-CK-B antiserum with the sample prior to electrophoresis did not unequivocally distinguish between the two isoenzymes. Similarly, anti-human IgG and IgM antibodies were also ineffective. However, the IgA-linked isoenzyme band was removed by anti-human IgA antiserum. While anti-CK-M antibodies did not affect the electrophoretic mobility of IgA-linked CK-BB, the antibody eliminated both the CK-MB and CK-MM bands. Thus, specific anti-IgA and anti-CK-M antibodies may be used to establish the presence of the myocardial isoenzyme.

Creatine kinase; Immunoglobulin-linked enzyme; Cardiac enzyme; Electrophoresis; Myocardial infarction

1. INTRODUCTION

Several recent articles have reported the presence of various atypical variants of creatine kinase in sera of patients [1–4]. The macro CK type 1 variant consists of immunoglobulin linked CK-BB. The IgA-bound species migrates slightly more anodically than the IgG-linked isoenzyme and has an identical electrophoretic mobility to the myocardial isoenzyme, CK-MB. Thus, patients with such atypical IgA-linked CK-BB isoenzyme may be misdiagnosed for myocardial injury, if electrophoresis is used as the sole basis of analysis. Although several methods have been proposed for the differentiation and characterization of these isoenzymes, the procedures involve the use of either column chromatography and immunoprecipitation [2] or immunofixation [3], which are quite involved and tedious. We describe here a modified procedure that can be used readily to differentiate CK-MB from its IgA-linked CK-BB isoenzyme.

Correspondence address: S.S. Wong, The Biochemistry Program, Department of Chemistry, University of Lowell, Lowell, MA 01854, USA

Abbreviations: CK-BB, -MB and -MM, creatine kinase isoenzyme BB, MB and MM, respectively

2. MATERIALS AND METHODS

2.1. *Materials*

Goat anti-human IgA antibodies were obtained from Sigma. Other anti-human immunoglobulins were obtained from Meloy. Anti-human CK-M antiserum was from Roche Diagnostics. Anti-human CK-B was from Hybritech. Serum containing macro CK type 1 isoenzymes was obtained from a hospitalized patient. Beckman I.D. zone CK isoenzyme was used as a control specimen.

2.2. *Electrophoresis*

The electrophoresis of CK isoenzyme was carried out on cellulose acetate according to the Helena CPK-US isoenzyme electrophoresis pro-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

cedure. When various antibodies were used, the specimen was mixed with a 1:1 (v/v) ratio of the commercially obtained antibody (1 mg/ml) prior to electrophoresis.

3. RESULTS AND DISCUSSION

The electrophoretic pattern of a specimen containing macro CK type 1 is shown in fig.1 (lane 2). The band that corresponded to the CK-MB isoenzyme contained IgA-linked CK-BB and that between CK-MB and CK-MM bands contained IgG-linked CK-BB [1,3]. The sample contained only a minor fraction of CK-MM. The fact that the macro CK type 1 bands contained isoenzymes of the *CK*-BB origin can be established by preincubating the serum with anti-CK-B antibody prior to electrophoresis as depicted in fig.1 (lane 4). The bands disappear as a result of complex formation between the antibody and isoenzyme. Since CK-MB also binds anti-CK-B antibody through the B subunit, the presence of the antibody slightly retarded its electrophoretic mobility (fig.2, lane 3). Fig.2 (lane 3) also shows the disappearance of the CK-BB band in the presence of the antibody. Because the electrophoresis of both CK-MB and macro CK type 1 is influenced by anti-CK-B antibody, there remains ambiguity in interpretation of the results.

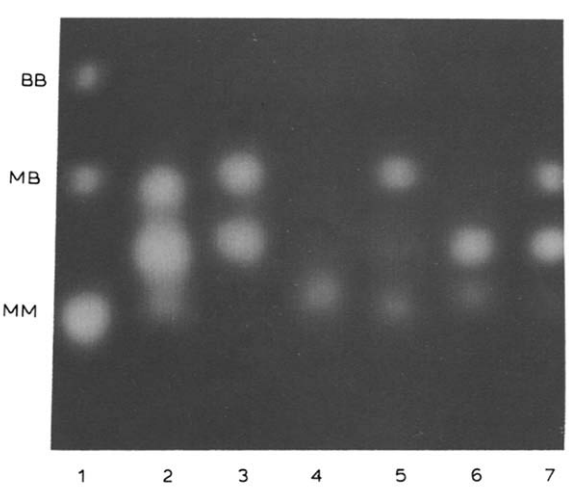

Fig.1. Electrophoretic pattern of a specimen containing macro CK type 1 isoenzyme. Lanes: 1, control; 2, patient specimen; 3–7, patient specimen mixed with various antibodies: anti-human CK-M (lane 3), anti-human CK-B (lane 4), anti-human IgG (lane 5), anti-human IgA (lane 6) and anti-human IgM (lane 7).

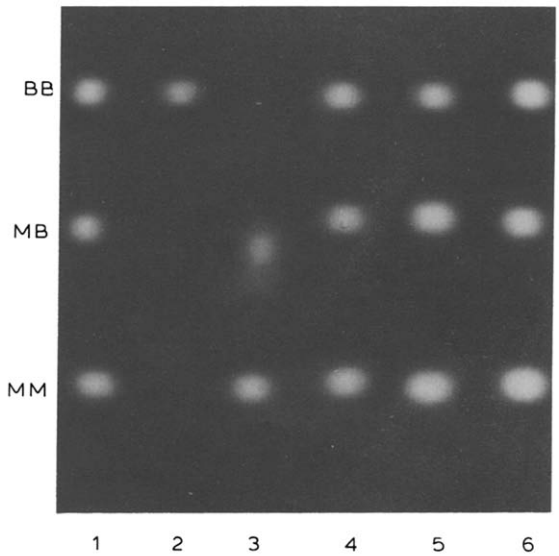

Fig.2. Typical electrophoretic pattern of CK isoenzymes. A control sample containing all three isoenzymes was mixed with various antibodies. Lanes: 1, none; 2, anti-CK-M; 3, anti-CK-B; 4, anti-human IgA; 5, anti-human IgG; 6, anti-human IgM.

To differentiate further the CK-MB and IgA-linked CK-BB macro type 1 isoenzymes, we mixed the specimen with anti-human IgG, IgA and IgM antisera prior to electrophoresis. These antibodies had no effect on the electrophoretic mobilities of CK-MM, MB or BB (fig.2, lanes 4–6). While anti-human IgM antibody also had no effect on macro CK type 1 isoenzymes (fig.1, lane 7), anti-IgG antibody greatly diminished the IgG-linked CK-BB band (fig.1, lane 5). The incomplete removal of the band is probably due to the presence of excess antigen. Anti-human IgA, on the other hand, specifically removes the IgA-linked CK-BB band (fig.1, lane 6). Thus anti-human IgA antibody can be used to differentiate CK-MB and IgA-linked CK-BB.

In addition, we have also used anti-CK-M subunit antibody for differentiation of the isoenzymes. As can be seen in fig.1 (lane 3) and fig.2 (lane 2) this antibody effectively removed the CK-MB and CK-MM bands but had no effect on the macro CK type 1 isoenzymes. Therefore the anti-CK-M antibody may also be used to distinguish CK-MB and IgA-linked CK-BB.

The use of specific antibodies provides an efficient means to distinguish between the electrophoretically identical isoenzymes of CK-MB and IgA-linked CK-BB. The complex formation of the antibody with the CK isoenzyme will change the electrophoretic mobility of the isoenzyme and in the case of anti-CK-M also inhibits the enzyme activity so that the enzyme is not detected. As long as the antibody is in excess of the antigen, the isoenzymes can be unequivocally separated and identified. The procedure involves only pre-mixing of the specimen with excess antisera prior to electrophoresis and does not require immunoprecipitation or immunofixation as previously suggested [2,3].

REFERENCES

[1] Jocker-Wretou, E. and Plessing, E. (1979) J. Clin. Chem. Clin. Biochem. 17, 731–737.
[2] Abbot, L.B. and Van Lente, F. (1985) Clin. Chem. 31, 445–447.
[3] Medeiros, L.J., Greco, F.A., Walsh, D. and Gerson, B. (1985) Clin. Chem. 31, 1393–1396.
[4] Mifflin, T.E. and Bruns, D.E. (1985) Clin. Chem. 31, 1743–1748.