Differentiation and Resolution of Erythrocyte and Muscle Adenylate Kinase Activities in Serum by Electrophoresis

Vadiraja V. Murthy,* Fahd Ali, and Edward R. Burns

Department of Pathology, Albert Einstein College of Medicine, The Bronx, New York

Adenylate kinase activity originating from erythrocytes has been shown to be distinct from muscle adenylate kinase or myokinase activity, until now considered to be identical enzyme activities. The two activities

can be differentiated by electrophoretic fractionation, thus making it possible to quantify the erythrocyte adenylate kinase activity present in serum. J. Clin. Lab. Anal. 11:235–237, 1997. © 1997 Wiley-Liss, Inc.

Key words: erythrocyte adenylate kinase; myokinase; hemolysis

INTRODUCTION

The laboratory diagnosis of red cell hemolysis is non-specific and relies on the multivariate assessment of serum levels of lactate dehydrogenase (LD), bilirubin, and haptoglobin, as well as the reticulocyte index and red cell morphology (1). A better red cell specific enzyme marker would enhance the accuracy of diagnosing hemolysis. Adenylate kinase (ATP:AMP phosphotransferase; AK) activity in serum is comprised primarily of myokinase or muscle adenylate kinase isoenzyme with a very minor component of AK isoenzyme derived from erythrocytes, if any. The erythrocyte fraction is released following *in vivo* or *in vitro* damage to red blood cells. The isoenzyme originating from red blood cells is the predominant AK isoenzyme fraction present in hemolyzed serum. Other investigators had considered erythrocyte AK to be identical to myokinase or muscle AK (2). We have recently shown that erythrocyte AK (EAK) can be fractionated by electrophoresis and that it migrates on agarose gel in manner similar to the MM isoenzyme of creatine kinase (CK) in serum (3). We now report that serum erythrocyte and muscle AK isoenzyme activities can be resolved by this procedure to quantify the relative proportion of EAK activity in serum. Such a procedure would be useful in assessing *in vivo* hemolysis.

MATERIALS AND METHODS

We used the Beckman Paragon CK electrophoresis instrument (Brea, CA) to fractionate the erythrocyte AK isoenzyme, using agarose gels used for fractionating CK isoenzymes. All other reagents were purchased from Sigma, St. Louis, MO.

Total AK activity in serum was assayed in a Cobas-fara centrifugal analyzer (Roche Diagnostics Systems, Somerville, NJ), using a 10 fold diluted visualization reagent described

below. The diluted reagent is comparable to that employed by Brolin (4).

Electrophoresis in the Beckman Paragon CK instrument, employing agarose gels used for CK fractionation was used to resolve the AK isoenzymes and visualized with the AK visualization reagent, containing 12 mMol/L adenosine diphosphate, 60 mMol/L each of glucose and magnesium acetate, 6 mMol/L nicotinamide adenine nucleotide (NAD), 9000 U/L hexokinase, 7,800 U/L of glucose-6-phosphate dehydrogenase, and 100 mMol/L tris, pH 7.6. After the samples were applied on the gel, they were allowed to penetrate the gel for 5 min before performing the electrophoresis at 200 volts for 25 min at 25°C. After electrophoresis, the gel was incubated with the visualization reagent for 20 min at 37°C and dried for 10 min at 60°C. The AK activity on the gel was visualized as a 360 nm fluorescence spot of the NADH product formed at the site of isoenzyme activity using the coupled enzyme reagent described below:

*Correspondence to: Dr. V.V. Murthy, Special Chemistry Laboratories, Room 2S-11, Nurses Residence Building, Jacobi Medical Center, The Bronx, NY 10461.

Received 13 January 1997; Accepted 7 March 1997

The relative percentage of AK isoenzyme fraction(s) present was computed by scanning the NADH fluorescence associated with the isoenzyme activity spot(s) on the gel. The erythrocyte and muscle AK activities were quantified by multiplying % AK isoenzyme fraction by the total AK activity of the sample in U/L. Freshly collected blood from a volunteer donor was used to obtain red blood cell hemolysate as described previously (3). Human skeletal gastrocnemius muscle tissue was obtained from a surgical specimen immediately after amputation and was placed in cold RPMI 1640 medium (Life Technologies, Inc., Grand Island, NY) for transportation and storage at 4°C until processing. The muscle tissue was cleansed free of excess blood by tearing apart the muscle fibers and washing repeatedly with an ice-cold solution of 9 g/L NaCl (saline) till the supernatant was free of any red color. In a typical experiment, 5 g of the cleaned muscle tissue was cut into small pieces and placed in a disposable sterile Potter-Elvehjem tissue homogenizer assembly for disruption in the presence of 5 mL of 100 mMol/L tris buffer, pH 7.8 and subjected to homogenization. The resulting homogenate was centrifuged at $3,500 \times g$ to remove cell debris and the cell-free homogenate was used as the source of muscle adenylate kinase (myokinase). The hemolysate sample, the myokinase preparation, and the two samples mixed in equal volume were subjected to AK isoenzyme fractionation as described above.

RESULTS

A typical scan of a freshly prepared erythrocyte hemolysate sample containing 450 U/L AK activity after electrophoretic fractionation is shown in scan A of Figure 1. The hemolysate sample contained a single band of AK activity migrating near the cathode indicating that all the AK activity in the cell-free hemolysate from erythrocytes is due to a single AK isoenzyme activity (EAK). Similarly a band of muscle AK (myokinase) activity migrating more anodically to the erythrocyte isoenzyme along with a band migrating like the typical EAK fraction representing contaminating EAK activity were seen, when a cell-free supernatant of the muscle tissue (total AK activity 147 U/L) was similarly fractionated by electrophoresis. This cell-free preparation contained 46% EAK and 54% myokinase activities respectively (scan B in figure). When a mixture of the two samples was subjected to electrophoretic fractionation it was estimated to contain 73% EAK and 27% myokinase activities as shown in scan C in the figure. It is clear from these results that both the muscle and erythrocyte adenylate kinase exhibit different mobilities when subjected to electrophoresis.

DISCUSSION

Both muscle and erythrocyte adenylate kinases are encoded by the same kinase gene, and the two isoenzymes have very similar molecular weights as shown by sodium dodecylsulfate-polyacrylamide electrophoresis (5). The ability of our electro-

Fig. 1. Electrophoretic fractionation of erythrocyte and muscle adenylate kinase (AK) activities. Representative scans shown are: A) erythrocyte AK, B) a cell-free myokinase preparation containing 147 U/L of total AK activity and C) a mixture of an equal volume of A and B above. Anode is to the right and the migration is from left to right.

phoresis method to resolve the two forms of the AK enzyme may be attributed to a possible post-translational modification of the two forms. Similar to the case with alkaline phosphatase isoforms (6), differential glycosylation of the transcription product in erythrocytes and muscle tissues could account for the observed difference in the overall net charges of the muscle and the erythrocyte forms of AK. This makes it possible to resolve them by electrophoresis. The two enzyme activities should therefore be considered as isoforms rather than isoenzymes. Previous studies have suggested that AK activity in serum, thought to have originated from erythrocytes was actually myokinase (4, 5). The present study clearly demonstrates the unique nature and electrophoretic mobility of erythrocyte AK as being distinct from that of myokinase.

The ability to detect and quantify erythrocyte specific AK isoenzyme activity accurately provides an opportunity to use it as a sensitive and specific marker for identifying patients undergoing clinical hemolysis. We are currently conducting parallel clinical studies to verify this approach.

REFERENCES

1. Lee RC. The Hemolytic Disorders: General Considerations. In: Wintrobe's Clinical Hematology, Lee RC, Bithell TC, Foerster J, Athens JW, Lukens JN (Eds) Lea and Feibiger, Philadelphia, 1993 pp 944–64.
2. Mainzer K, Morsches B, Holzmann H. Serum myokinase (adenylate kinase) activity in intravascular hemolysis. *Aersth Forsch* 1972; 26:426–31.
3. Murthy VV. Adenylate kinase mimics creatine kinase-MM isoenzyme in a CK isoenzyme electrophoresis assay. *J Clin Lab Anal* 1994; 8:140–3.
4. Brolin SE. Adenylate kinase (myokinase –ATP:AMP phosphotransferase, EC 2.7.4.3). In *Methods of enzymatic Analysis* Vol III, Hans U. Bergmeyer ed, Verlag-Chemie, Weinheim, Germany 1983, pp 540–545.
5. Nealon DA. Rapid purification of adenylate kinase from human erythrocytes and skeletal muscle. *Arch Biochem Biophys* 1986;250:19–22.
6. Moss DW. Perspectives in alkaline phosphatase research. *Clin Chem* 1992;38:2486–92.