Elution Profile of Adenylate Kinase, from Platelets, in Plasma

To the Editor:

In a recent Note (1), we reported that both muscle and erythrocytic adenylate kinase (AK; EC 2.7.4.3) were completely eluted from DEAE-Sephadex A-50 with buffered 100 mmol/L NaCl. This coincided with the elution of the MM isoenzyme of creatine kinase (CK; EC 2.7.3.2) and thus would not interfere in the chromatographic isolation of the serum CK-MB isoenzyme, when present (2). The practical importance of this observation was discussed.

After this study was completed, Szaaz et al. (3) reported that platelets can contribute 10–20% of their AK to the serum, resulting in an enhancement of apparent CK activity of 2–4 U/L (30 °C). To complete our investigation of possible interfering sources of AK in the Roche serum CK isoenzyme chromatographic procedure, we determined the elution profile of platelet AK.

The column, eluting buffers, and assay reagents were similar to those described previously (1). Platelet adenylate kinase was isolated from fresh human plasma as described by Szaaz et al. (3). The concentrated preparation had an apparent total CK activity of 47 U/L. An aliquot of the preparation (apparent CK activity, 12 U/L) was placed on the column and the enzyme was eluted as described (1). Aliquots of each fraction were assayed in a Gilford System 5 (Gilford Instrument Laboratories, Oberlin, OH 44074) set to detect 0.9 U/L, and the results were plotted.

Figure 1 shows a plot of the elution profile of human platelet AK with increasing buffered salt concentration. All the AK was promptly eluted with buffered 100 mmol/L NaCl. The pattern is similar to the profile for muscle and erythrocytic AK reported previously (1). No activity was found in the fractions obtained with more concentrated salt solutions. Analytical recovery of the apparent CK activity placed on the column was quantitative.

Electrophoresis on both agarose and cellulose acetate of an aliquot of the unfractionated AK preparation and the pooled and concentrated fractions eluted with 100 mmol/L NaCl demonstrated a single component with a mobility corresponding to the CK-MM isoenzyme of a myocardial extract in serum as a reference (2). No AK enzyme was found in the separate pooled and concentrated eluates obtained with 200 and 300 mmol/L NaCl, respectively.

We conclude that platelet AK does not significantly interfere with the chromatographic isolation and measurement of serum CK-MB isoenzyme.

References


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Constructing a Normal Range

To the Editor:

In your Information for Authors (1) it states: "Distributions must be gaussian if a ±2 SD range is to be considered the normal range." It is regrettable that you publish work that ignores this requirement. Haymond et al. (2) present normal values for urinary 3-methoxy-4-hydroxymandelic acid (VMA) in children. For each of five age groups (1–12 months, 1–2 years, 2–5 years, 5–10 years, 10–15 years) they present the range, mean, and SD for VMA, together with a normal range defined as the mean ±2 SD. In all five groups the mean is less than 2 SD so that the mean –2 SD is negative, but no comment is made about this and the lower limit of normal is given as zero. The data are obviously skewed and by no means gaussian, as the quoted ranges also show. Thus the use