

# Lability of human creatine kinase isoenzymes at 37°C: a complication of electrophoretic separation

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**SYNOPSIS** The activity of the brain specific isoenzyme of creatine kinase is shown to fall off rapidly at 37°C, particularly in the presence of albumin. Dithiothreitol cannot reverse this lability. The implications of this finding suggest that electrophoretic techniques which use incubation methods to detect the brain specific isoenzyme of creatine kinase may underestimate the true activity.

We (Nealon and Henderson, 1975a) have shown that the use of DEAE-cellulose is superior to DEAE-Sephadex (Mercer, 1974) for the separation, in a mini-column, of the human creatine kinase (ATP: creatine phosphotransferase, EC 2.7.3.2, CK) isoenzymes. Subsequently we showed (Nealon and Henderson, 1975b) that CK<sub>1</sub><sup>1</sup> could be detected in the sera of patients who had undergone brain surgery. A survey of the literature dealing with the elevation of serum creatine kinase following the acute onset of serious neurological conditions such as head injury, cerebrovascular accidents, encephalitis, etc. shows that CK<sub>1</sub> has not been detected in the serum of these patients.

CK<sub>1</sub> has been found in the serum of persons suffering from the rare condition of malignant hyperpyrexia (Zsigmond *et al.*, 1972) although to date this finding has never been substantiated by other workers (Britt, 1974). Application of our technique to the sera of known cases of malignant hyperpyrexia established the presence, in appreciable quantities, of the brain specific isoenzyme (Nealon *et al.*, 1975). It occurred to us that the general failure to demonstrate the presence of CK<sub>1</sub> in sera of cases of malignant hyperpyrexia or acute neurological conditions could be due to the difference in technique between ourselves and other workers. Most workers had used an electrophoretic technique

<sup>1</sup>The CK isoenzymes are named on the basis of their electrophoretic mobility towards the anode. Thus the isoenzymes BB, MB, and MM of creatine kinase are named CK<sub>1</sub>, CK<sub>2</sub>, and CK<sub>3</sub> respectively (Commission on Biochemical Nomenclature, 1972).

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for separation of the isoenzymes with subsequent incubation at 37°C with the CK assay media for periods of up to two hours whereas we have used a chromatographic technique. We have therefore investigated the heat stability of the three CK isoenzymes at 37°C.

## Materials and Methods

### ENZYME ASSAY

Creatine kinase was determined at 37°C by the Rosalki-Oliver method (Rosalki, 1967) using the CPK Fast-Pak (Calbiochem, San Diego, Calif. 92112). Assays were performed on a Unicam SP 1700 spectrophotometer (Canadian Laboratory Supplies Ltd, Toronto, Ontario, Canada). Normal serum activity, in this laboratory, extends up to 135 U/litre.

### ISOENZYME PREPARATIONS

Human brain, heart, and skeletal muscle were used as sources of CK<sub>1</sub>, CK<sub>2</sub>, and CK<sub>3</sub> respectively. All tissues were homogenized in 100 mmol/litre KCl (1 g wet weight in 2 ml) and clarified by centrifugation at 1300 g for 30 min at 10°C.

The separation and partial purification of the isoenzymes was accomplished by the method previously described (Nealon and Henderson, 1975b). Solutions of each isoenzyme were prepared for analysis as follows:

(a) CK<sub>3</sub> (final activity 216 U/litre) and CK<sub>2</sub> (final activity 197 U/litre) were prepared in 50 mmol/litre Tris-HCl buffer, pH 7.0 (25°C), with and without 7.5 g/litre bovine serum albumin (BSA).

(b) CK<sub>1</sub> (final activity 223 U/litre) was prepared in the same buffer described above, with and without BSA, in the following concentrations—1.5, 2.5, 4.0, 5.0, 6.5, 7.5, and 15 g/litre.

**Results**

Figure 1 illustrates the activity of the CK isoenzymes as a function of incubation time at 37°C. In the case of CK<sub>3</sub> and CK<sub>2</sub>, albumin (BSA at a concentration of 7.5 g/litre) has very little effect on the activity of the isoenzymes over a two-hour period so that with or without albumin there is a loss of less than 20% of total activity. On the other hand, CK<sub>1</sub> is shown to be extremely unstable at 37°C, particularly in the presence of albumin. For clarity, only the results of the experiments with CK<sub>1</sub> in the

presence of 7.5 and 15 g/litre BSA are shown. Decreasing the protein concentration, as described in Materials and Methods, produced results which approached those obtained in the absence of protein.

Figure 2 illustrates that dithiothreitol in the presence, or absence, of BSA exerts a stabilizing effect on the isoenzymes. With each of these isoenzymes, however, there is an increase in lability in the presence of BSA. CK<sub>1</sub> is clearly stabilized by dithiothreitol (compare with fig 1) but the presence of BSA (at a concentration of 15 g/litre) makes the isoenzyme extremely heat labile. Again the extent of the heat lability is directly proportional to concentrations of BSA (compare with fig 1).

Experiments with purified human albumin produced exactly the same results, therefore the observed effects are albumin specific but not species specific.

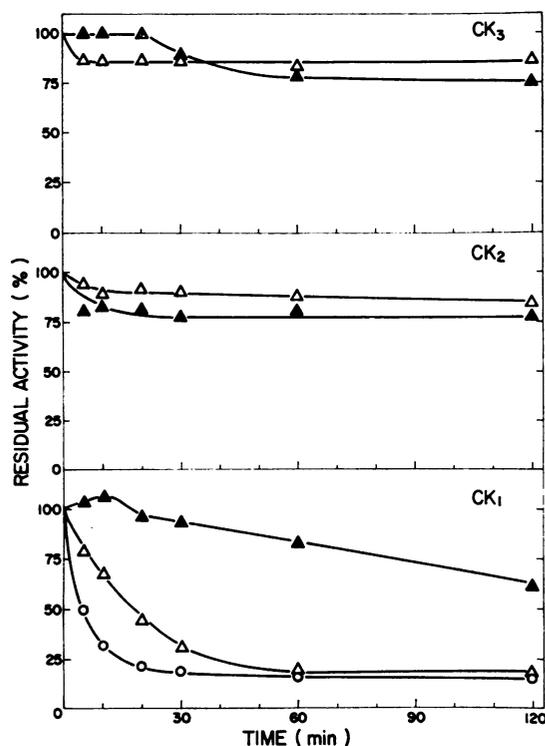


Fig 1 Effect of incubation at 37°C on human creatine kinase isoenzymes with or without added bovine serum albumin.

Each isoenzyme was incubated at 37°C and sampled, for enzyme assay, at the time shown. The isoenzymes were either incubated without BSA (▲—▲) or with BSA at a concentration of 7.5 g/litre (△—△) or 15 g/litre (○—○). The other BSA concentrations used in the experiment are omitted for the sake of clarity.

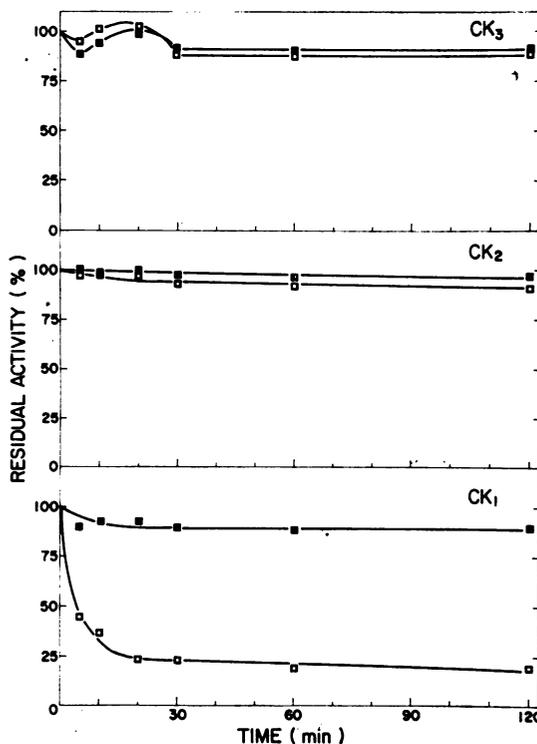


Fig 2 Effect of incubation at 37°C on human creatine kinase isoenzymes in the presence of dithiothreitol with or without added bovine serum albumin.

Each isoenzyme was incubated at 37°C and sampled, for enzyme assay, at the times shown. The isoenzymes were either incubated with dithiothreitol alone (■—■) or with added BSA at a concentration of 15 g/litre (□—□). The other BSA concentrations used in the experiment are omitted for the sake of clarity.

## Discussion

The results clearly show that CK<sub>1</sub>, in the presence of albumin, in concentrations much less than those present in normal serum, is inactivated to such an extent that within 15 min at 37°C only about 20% of its original activity remains (fig 1). Even the presence of dithiothreitol, which is a known activator of creatine kinase (Bishop *et al*, 1971; Warren, 1972), has little influence on this heat lability (fig 2).

The lability of CK<sub>1</sub> is clearly related to the presence of albumin and it is known that serum albumin migrates with, or close to, CK<sub>1</sub> on electrophoresis (Wood, 1963; Trainer and Gruenig, 1968; Smith, 1972). It therefore seems very likely that unless the isoenzymes are eluted from the electrophoretic media immediately after separation for reaction rate assay (Roberts *et al*, 1974) any incubation of the electrophoresis medium is likely to destroy CK<sub>1</sub>. Examination of the many current electrophoresis techniques indicates that incubation of the separated CK isoenzymes with the CK assay medium ranges over the following: 25 min at 37°C (Klein *et al*, 1973), 30 min at 37°C (Wolf *et al*, 1974; Goto *et al*, 1969), 1 h at 30°C (Roe *et al*, 1972), 1 h at 37°C (Zsigmond *et al*, 1972), 30 min-2 h at 37°C (Smith, 1972), and 2 h at 37°C (Sommer and Kontinen, 1972b; Goto, 1974).

Therefore low levels of serum CK<sub>1</sub> could very well be missed by an electrophoretic technique, especially as it has been suggested that this technique will not detect less than about 2 U/litre (at 37°C) of CK activity (Yasmineh and Hanson, 1975). We suggest that the only satisfactory technique for detecting CK<sub>1</sub> is the column chromatographic method already referred to.

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