Creatine kinase-1 is principally inactivated in serum by complexing with immunoglobulin-G

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Summary

1. The stability of enzyme activity of the creatine kinase-1-immunoglobulin-G complex has been determined at 37°C, 4°C and -20°C in heat-inactivated serum and buffer.

2. The complex was formed by incubating creatine kinase-1 and immunoglobulin G at 37°C for 30 min. It was isolated by Sephadex G-100 column chromatography.

3. At all temperatures the complex suspended in buffer was about twice as stable as it was in heat-inactivated serum. Stability decreased in the sequence of 4°C, 37°C and -20°C. Therefore all isolations of the complex were carried out at 4°C.

4. At 37°C the decay of enzyme activity of the complex was found to be biphasic first order. In serum the $K_d$ values were $-0.045$ min$^{-1}$ and $-0.0028$ min$^{-1}$, and in buffer $-0.023$ min$^{-1}$ and $-0.0016$ min$^{-1}$.

5. From column-chromatography experiments it was found that between 10 and 20% of the total creatine kinase-1 was involved in the complexing reaction after a 30-min incubation.

6. With this 10–20% proportion it can be calculated that the overall $t_{1/2}$ for the decay of total creatine kinase-1 activity must be in the range 95–201 min. This finding suggests, by comparison with other published data, that the enzyme–immunoglobulin complex is the main route of creatine kinase-1 catabolism in serum.

7. The difference between serum and buffer decay values for the complex is possibly due to the presence of cystine, urate and other substances in serum, which are additional potent creatine kinase inhibitors.

Key words: creatine kinase, immunoglobulins, isoenzymes.

Introduction

The processes by which serum enzymes disappear from the circulation are uncertain (Posen, 1970). In the rabbit it was shown that lactate dehydrogenase-5 (EC 1.1.1.27) activity was lost from the circulation at a faster rate than the radioactivity of $^{125}$I-labelled enzyme (Qureshi & Wilkinson, 1976). However, such findings give no information about the mechanism of enzyme catabolism except that the initial stages do occur in the intravascular compartment. A major advance occurred when Morin (1977) showed that the inactivation in vitro of human creatine kinase (EC 2.7.3.2) activity, in heat-inactivated serum, followed similar kinetics as inactivations in vivo. His data suggested that, for creatine kinase at least, inactivation was primarily a thermal process. We have shown that human creatine kinase-1 forms complexes in vitro with pure immunoglobulin-G and that this complex can be isolated, both from incubations of creatine kinase-1 in fresh serum in vitro and from patients with creatine kinase-1 activity in their serum (Prabhakaran, Nealon & Henderson, 1979). These patients had undergone craniotomy with neurosurgical manipulations—a procedure known to cause creatine kinase-1 to appear consistently in the serum postoperatively (Nealon & Henderson,
However, the contribution of this complexing reaction to the process by which creatine kinase-1 activity is lost in the intravascular compartment is not known. The present paper suggests that this complexing reaction is probably the major route of creatine kinase-1 inactivation in the serum.

**Materials and methods**

Pure human creatine kinase-1 and immunoglobulin-G were prepared and their purity was verified as described by Prabhakaran et al. (1979). The creatine kinase was stored at 4°C and used within 8 h of final purification.

Creatine kinase activity was measured, at 37°C, with the BMC-NAC kits (BMC Diagnostics/Biochemicals Ltd, St Laurent, Quebec, Canada), whose composition is based on the optimized method of Szasz, Gruber & Bernt (1976). This assay contains 10 mmol of N-acetylcysteine/l and 2 mmol of ethylenediaminetetraacetate (disodium salt)/l (Gruber, 1978). The reaction was monitored on an LKB 8600 Reaction Rate Enzyme Analyzer at 37°C.

The creatine kinase-immunoglobulin G complex was prepared as described by Prabhakaran et al. (1979) except that a Sephadex G-100 column (2.5 cm x 88 cm) was used instead of a Sephacryl S-200 Superfine column (Pharmacia-Canada Ltd, Dorval, Quebec, Canada). The fractionation was carried out at 4°C with a flow rate of 48 ml/h. The eluting buffer was Tris/HCl (50 mmol/l; pH 7.0 at 25°C) containing, per litre, 10 mmol of thioglycerol and 2 mmol of ethylenediaminetetraacetate (disodium salt). Under these conditions the complex was eluted within 5–6 h.

The stability of the complex was studied by adding it either to buffer (described above) or to human serum which had been heat-inactivated (Nealon & Henderson, 1977) to contain no endogenous creatine kinase activity (60°C for 1 h). The complex, in either of the two diluents, was then incubated at 37°C, 4°C and -20°C with frequent sampling to determine residual enzyme activity. To avoid repeated freezing and thawing, the samples stored at -20°C were kept in 0.2 ml aliquots (Nealon & Henderson, 1977).

The stability of the activity of the enzyme associated with the complex was also studied, in detail, in heat-inactivated serum and in buffer at 37°C. The estimations of the decay constant, $K_d$, and the half-life in vitro, $t_{1/2}$, were based on accepted formulae (Stevens, 1961) for first-order decay reactions. The first-order decay constant is thus defined as

$$K_d = 2.303 \times \text{slope}$$

when $\log(\text{activity}_{t=0}/(\text{activity}_{t=0} - \text{activity}_{t\rightarrow0}))$ is plotted as a function of time, $t$. The reaction half-life is defined as,

$$t_{1/2} = \log_e 2/K_d \quad (1)$$

**Results**

The conditions under which the creatine kinase-immunoglobulin G complex could be isolated by column chromatography were established by examining the stability of the enzyme activity of the complex both in buffer and in heat-inactivated serum. Enzyme activity was found to decrease on incubation in the sequence 4°C, 37°C and -20°C for both buffer and serum (Fig. 1). The complex in buffer alone was about twice as stable as in serum at any temperature. Thereafter all isolations of the complex were carried out by column chromatography in buffer at 4°C. After column chromatography, the activity of creatine kinase-1 eluted in the void volume of the Sephadex G-100 column, i.e. creatine kinase in complex, corresponded to approximately 10–20% of the total activity. This percentage value of association was estimated by comparing the initial total activity with the activity of 'free' creatine kinase estimated by summation of the activities in the appropriate fractions from the column. At 4°C, 'free' creatine kinase-1 loses no activity in thiol-containing buffer over a 24 h period (Nealon & Henderson, 1977).

The loss of the activity at 37°C of the enzyme associated with the complex in both heat-inactivated serum and in buffer (Fig. 2) followed biphasic first-order decays (Stevens, 1961).

**Discussion**

The calculations from the data (Fig. 2) indicate that under the conditions in vitro most closely representative of the situation in vivo, i.e. in heat-inactivated serum at 37°C, the $t_{1/2}$ of the enzyme activity associated with the creatine kinase-immunoglobulin G complex is 15-3 min. Since, as described above, 10–20% of the creatine kinase-1 is involved at any particular time in its decay reaction with human immunoglobulin G, then, assuming this binding to be the only route of inactivation, the overall biological half-life value for the decay of all (i.e. 100%) the creatine kinase-1 activity can be calculated.
Creatine kinase-1–immunoglobulin-G complex

Fig. 1. Stability of the creatine kinase-1–immunoglobulin G complex. The complex, eluted from the Sephadex G-100 column as described in the text, was stored in either the eluting buffer (○, △ □) or in heat-inactivated serum (■, □) at 4°C (△), 37°C (○, ●) or −20°C (□, ■). The complex had a creatine kinase activity of about 400 units/l.

Fig. 2. Decay of the enzyme activity of the creatine kinase-1–immunoglobulin G complex. The complex was suspended in heat-inactivated serum (●) or in buffer (○) and incubated at 37°C with sampling as shown. The initial enzyme activity was 320 units/l.

The basis for this statement is the fact that free creatine kinase-1 can be isolated from the G-100 column separation, concentrated, and caused to react with a fresh solution of immunoglobulin-G. In other words, the reaction between the enzyme and the immunoglobulin is a continuing process limited only, in vitro, by the availability of immunoglobulin G.

It is evident that, even at 4°C, the complex is rapidly decaying on the column before the K_d determination is made (Fig. 1). It is therefore necessary to use very high enzyme activities to prepare a complex with sufficient residual activity to conduct the experiments shown in Fig. 2.

If, as an example of the calculations involved, 10% of the enzyme activity decays in serum by formation of a complex with immunoglobulin G in 30.6 min (twice the t_0.5 value for serum shown in Fig. 2) then the fraction of free creatine kinase remaining is 90% or 0.9. These values can be substituted into the integrated first-order rate equation (Franklin & Newman, 1973):

\[ 2.030 \log \left[ A_t / A_0 \right] = -K_t \tag{2} \]

where \( A_0 \) is the activity of the enzyme originally present (100%) and \( A \) is the activity remaining after time \( t \) (90% after 30.6 min). Thus the overall decay constant of this process is 0.00345 min⁻¹ and the half-life of this overall process will, from eqn. (1) above, be 201 min.

If 20% of the creatine kinase activity complexes with immunoglobulin G then by the same reasoning the overall process will have a half-life of 95.0 min. This range of values (95–201 min) is of the same order as Morin’s (1977) value of 112 min (calculated from the \( K_d \) value of 0.0062).

Morin (1977) demonstrated that the decay in vitro of human creatine kinase isoenzymes in heat-
inactivated human serum was similar to data for these isoenzymes in vivo. It is on the basis of his work that we believe our studies in vitro have relevance to the fate of creatine kinase-1 activity in blood. Thus we suggest that the initial inactivating reaction is formation of a complex of a small (10–20%) proportion of the enzyme with immunoglobulin G.

It should be noted that our initial studies (Prabhakaran et al., 1979) were conducted with immunoglobulin G at a concentration of 10 g/l (or 62.5 µmol/l), which is within the physiological range of immunoglobulin G concentrations in human serum. Serum concentrations of creatine kinase-1 will nearly always be low (never exceeding one-third, i.e. about 40 units/l at 37°C) of the upper limit of total serum creatine kinase activity. Assuming a maximal specific activity of 200 reaction is formation of a complex of a small (10–20%) proportion of the enzyme with immunoglobulin G.

As immunoglobulin G is synthesized at about 2 g/day (13 µmol/day) in the adult (Waldman, Blaese & Strober, 1970), it would appear that the capacity of the immunoglobulin G reservoir is not likely to be exceeded by the amount of available creatine kinase-1.

The different $K_d$ values for serum and buffer are presumably due to potent inhibitors of creatine kinase known to be present in serum, such as cystine (Jacobs, Philipp, Sundmark & Weselake, 1978), urate (Warren, 1975) and other, unidentified, substances (Jacobs et al., 1978). The magnitude of this effect is considerable as it has been shown that cystine, for example, is present in a 10 000-fold molar excess over creatine kinase in serum (Jacobs et al., 1978). However, the effect of these serum inhibitors appears to be reversible by thiol agents, whereas we have shown previously, and in the present paper, that both thiol agents and ethylenediaminetetra-acetate appear to have little stabilizing influence on the loss of enzyme activity from the enzyme–immunoglobulin complex (Prabhakaran et al., 1979). Presumably the conformational rearrangement of the enzyme when bound to the immunoglobulin does not allow reactivation of the enzyme. Be that as it may, the demonstration of an interaction between an enzyme and immunoglobulin G suggests a new role for immunoglobulin G as an enzyme scavenger.

With the appreciation that creatine kinase-1 is present in small concentrations in the serum of healthy adults and children (Jung, Neumann, Cobet, Nugel & Egger, 1979), it seems likely that this complexing reaction could also be involved in the removal of physiological quantities of creatine kinase-1.

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References


