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Activation by calcium of AMP deaminase from the human red cell

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We have investigated the effects of Ca^{2+} on AMP deaminase from human red cells. At variance with the other known modulators, Ca^{2+} increased the apparent affinity for AMP without modifying the characteristic positive cooperativity of the enzyme towards the substrate. Ca^{2+} sensitivity was not modified by dialysis, but dilution of the haemolysate produced an activation of the enzyme similar to that induced by Ca^{2+}. Simultaneously, the Ca^{2+} dependence was lost. The sensitivity to other modulators, such as ATP, diphosphoglycerate or phosphate, was not modified by dilution. Partial purification of the enzyme produced the same effects as haemolysate dilution. These results may be interpreted to mean that Ca^{2+} acts by antagonizing an endogenous inhibitor present in red cell lysates.

AMP deaminase; Ca^{2+} dependence; Nucleotide metabolism; (Red cell)

1. INTRODUCTION

The presence of high levels of AMP deaminase (AMP aminohydrolase, EC 3.5.4.6) activity in human erythrocytes has been known for a long time [1]. Mature erythrocytes do not have the ability to reconvert IMP to AMP or to synthesize nucleotides from purine ring precursors [2]. For these reasons the control of AMP deaminase activity may be crucial to the preservation of the adenine nucleotide content of these cells. The enzyme is subjected to allosteric regulation by several metabolites. ATP is a powerful stimulator [3] and P_i [4] and 2,3-diphosphoglycerate [5] are strong inhibitors. AMP deaminase is nearly inactive in the intact erythrocyte, but can be stimulated by the addition of several metabolic inhibitors [6,7] or increase in cytoplasmic Ca^{2+} levels [8,9]. This leads to an irreversible depletion of the adenine nucleotides [10]. Here, we have examined the mechanism of the effect of Ca^{2+} on AMP deaminase activity of human red cell lysates.

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2. EXPERIMENTAL

Freshly drawn packed human red cells were lysed with 5 vols ice-cold distilled water containing 0.1 mM EGTA. Membranes were sedimented by centrifugation at 35000 × g and 4°C for 30 min and the supernatant was dialyzed vs 100 vols distilled water containing 0.1 mM EGTA at 4°C for 24 h. The partially purified enzyme was prepared by treatment of the dialyzed enzyme with DEAE-cellulose [11]. Purification was about 10-fold.

AMP deaminase activity was measured at 37°C in a medium containing, in mM: KCl, 150; imidazole-HCl buffer, 20 (pH 7). Unless otherwise stated dialyzed lysates were diluted to 1:2 in the final incubation medium, the concentration of AMP was 1 mM, and Ca^{2+}, when present, was at 1 mM. This concentration of Ca^{2+} has been shown previously to produce maximal stimulation of AMP deaminase [9]. Other additions are specified in each experiment. The incubation period was terminated by the addition of perchloric acid (final concentration, 0.5 N). Measurements of activity were based on the decrease in absorbance at 265 nm or formation of ammonia [11] and performed under conditions of initial velocity.

3. RESULTS

Addition of Ca^{2+} increased AMP deaminase activity of red cell lysates, typically to 3–10-times the control value without Ca^{2+}. About one out of every five preparations showed little or no Ca^{2+} sensitivity. No correlation with blood donors or

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other obvious factors was found. These preparations were discarded. On storage at 4°C for several days, loss of Ca^{2+} sensitivity together with an increase in enzyme activity was observed. The Ca^{2+} sensitivity of lysates was unmodified by dialysis, so that dialyzed lysates were systematically used for all the experiments reported here. Fig.1 shows the reversibility of the effect of Ca^{2+}. Addition of Ca^{2+} increased AMP deaminase activity approx. 10-fold and the effect was quickly and fully reversed by chelation of Ca^{2+} with EGTA. Other divalent cations were also tested at 1 mM. Mg^{2+} did not have a significant effect, lanthanum was as powerful as Ca^{2+}, Mn^{2+} had a weaker effect and Co^{2+} seemed to slow IMP formation.

The effect of Ca^{2+} on the substrate saturation curve for AMP is shown in fig.2. Ca^{2+} increased the apparent affinity of the enzyme for AMP without modifying the maximal velocity. The characteristic cooperative kinetics of the enzyme were not modified by Ca^{2+}. Hill plots of the data of fig.2 showed n values of 2.4 for the control and 2.3 for the Ca^{2+}-activated enzyme. In another 2 similar experiments the estimated values of n were 2.7 and 2.6 for the controls vs 2.2 and 2.8 in the presence of 1 mM Ca^{2+}. The activating effect of Ca^{2+} differs from that previously reported for ATP, which transforms the sigmoidal kinetics of AMP deminase into hyperbolic [3]. This difference is also documented in fig.2. The estimated value of n in this experiment was 1.1 for the ATP-activated enzyme. Addition of Ca^{2+} to the ATP-activated enzyme did not produce an additional increase in activity (not shown). Fig.2 also shows the inhibitory effect of 2,3-diphosphoglycerate, shifting the sigmoidal kinetics towards the right, as described [5]. Addition of Ca^{2+} to the 2,3-diphosphoglycerate-inhibited enzyme increased the activity, but again without modification of the cooperativity. In the experiment of fig.2 the value of n in the presence of 2,3-diphosphoglycerate was 3.5 and addition of Ca^{2+} did not significantly modify it (n = 3.9).

Table 1 lists the values of AMP deaminase activity obtained at different lysate concentrations. Dilution of the lysate increased the activity of the enzyme. At the same time the effect of Ca^{2+} became weaker. Fig.3 shows the effects of dilution on the kinetics of saturation by AMP. Dilution increased the activity in a fashion reminiscent of the

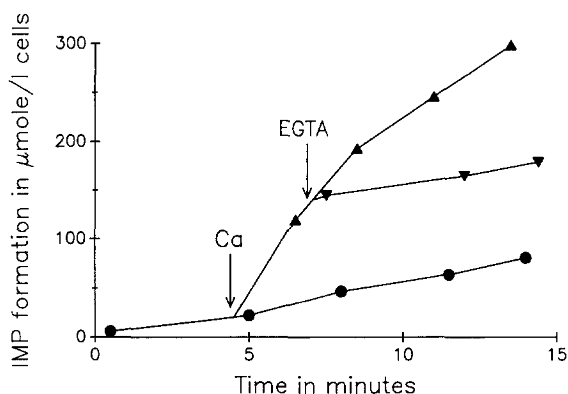

Fig.1. Reversibility of the effect of Ca^{2+} on AMP deaminase activity of dialyzed human red blood cell lysates. Ca^{2+} (1 mM) and EGTA (2 mM) were added at the times shown by the arrows. The concentration of AMP was 1 mM.

effect of Ca^{2+}, since sigmoidal kinetics were preserved. The values for the Hill coefficient in this experiment were 2.2 for the diluted enzyme vs 2.6 in the undiluted control and 2.2 for the same condition in the presence of Ca^{2+} (not shown). Fig.3 also shows that the sensitivity to Ca^{2+} was lost on dilution, even though sensitivity to ATP was preserved. The effect of ATP was, as usual, to transform the sigmoidal kinetics into hyperbolic.

Concentration of red cell lysates, previously diluted 1:20 with distilled water, by centrifugation over Centricon concentrators (exclusion size, 10000 Da), yielded a preparation in which Ca^{2+} sensitivity was preserved. In contrast, Ca^{2+} sen-

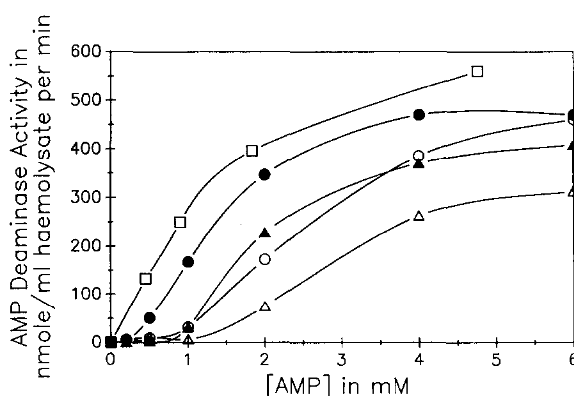

Fig.2. Effects of Ca^{2+}, 2,3-diphosphoglycerate and ATP on AMP deaminase activity of dialyzed human red blood cell lysates. (○) Control; additions: (●) 1 mM Ca^{2+}; (□) 2 mM ATP; (△) 1 mM 2,3-diphosphoglycerate; (▲) 2,3-diphosphoglycerate and Ca^{2+} (both at 1 mM).

Table 1

Effects of dilution on AMP deaminase activity of dialyzed human red cell lysates

Dilution of the lysate	AMP deaminase activity (nmol/ml haemolysate per min)	
	Control	+ 1 mM Ca^{2+}
1:2	14	150
1:4	50	197
1:8	105	264
1:20	178	222

AMP was at 1 mM. Except for Ca^{2+}, the ionic composition of the assay medium was the same for all the conditions

sitivity was largely lost and enzyme activity increased when 150 mM KCl and 1 mM $CaCl_2$ were added to the lysate prior to centrifugation over the concentrator.

The kinetic characteristics of the partially purified enzyme were similar to those of the diluted enzyme. Saturation kinetics by AMP were sigmoidal (n = 2.1) and were transformed into hyperbolic by ATP (n = 1.3). The sensitivity to Ca^{2+} was lost, but the enzyme was still sensitive to inhibition by P_i and 2,3-diphosphoglycerate. With the purified enzyme, the inhibition by 2,3-diphosphoglycerate was not antagonized by Ca^{2+} (not shown).

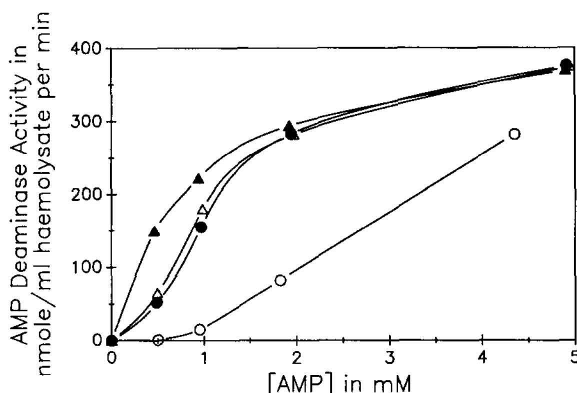

Fig.3. Effect of dilution on AMP deaminase activity of dialyzed human red blood cell lysates. (○) Undiluted enzyme without Ca^{2+}; (△) enzyme diluted 1:20 without Ca^{2+}; (▲) same plus 1 mM Ca^{2+}; (●) diluted enzyme plus 2 mM ATP. Note that the same ionic strength and medium composition, except for the concentration of the lysate, applies to diluted and undiluted enzymes. IMP is referred to per l packed cells (about 80% haematocrit).

4. DISCUSSION

Our results confirm the activation of AMP deaminase activity by the increase in cytoplasmic Ca^{2+} concentration reported previously in intact red cells [8,9]. The mechanism differs from those reported previously for other known modulators. Thus, whereas those either decreased (ATP [3]) or increased (P_i and 2,3-diphosphoglycerate [4,5]) the Hill coefficient, Ca^{2+} increased the apparent affinity for AMP without affecting the Hill coefficient. The effect of Ca^{2+} should then involve a different regulatory site. This conclusion was reinforced by the finding that dilution or purification of the enzyme led to insensitivity to Ca^{2+}, whereas sensitivity to ATP, diphosphoglycerate and phosphate was preserved. The observation that dilution of the haemolysate increased activity in the same fashion as Ca^{2+} did with a simultaneous loss of the sensitivity to Ca^{2+} suggests that its effect may be indirect. The purified enzyme preparation behaved much in the same way as the diluted preparation. The simplest interpretation of these results would be to postulate the existence of an endogenous inhibitor in the haemolysate which is lost on purification or weakened in its action by dilution. Ca^{2+} (and other divalent cations) would antagonize the effect of this inhibitor thus releasing the enzyme activity.

The nature of this hypothetical inhibitor is obscure at present. Askari and Rao [4] reported that dilution of red cell lysates produced an increase in AMP deaminase activity, which was attributed to dilution of 2,3-diphosphoglycerate. The endogenous inhibitor reported here could not be diphosphoglycerate since it is not lost on dialysis and addition of diphosphoglycerate to the purified enzyme did not restore the sensitivity to Ca^{2+}. We have no explanation for the discrepancy between the present results and the findings of Askari and Rao [4], who were unable to observe an increase in AMP deaminase activity on dilution of dialyzed red cell lysates. We observed spontaneous stimulation of AMP deaminase, with loss of Ca^{2+} sensitivity, in some cell batches. The enzyme also becomes activated and loses Ca^{2+} sensitivity with time in stored lysates. Perhaps subtle changes in metabolism, pH or other factors could be responsible for this activation. The role of the calcium stimulation in the degradation of adenine

nucleotides in stored blood [2] and the possible similarities with stimulation of AMP deaminase induced by metabolic inhibitors [6,7] remains to be determined.

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