THE INFLUENCE OF Mg^{2+}/ADENINE NUCLEOTIDE RATIOS AND ABSOLUTE CONCENTRATION OF Mg^{2+}/ADENINE NUCLEOTIDE ON THE OBSERVED VELOCITY OF SOME KINASE REACTIONS*

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Received 14 June 1973

1. Introduction

A great deal has been written concerning the importance of ATP:ADP ratios and the adenylate energy charge in kinase reactions as possible modifiers in the control of metabolism [1, 12, 16]. The role of various metals, especially divalent cations, and particularly magnesium has also been widely discussed [2, 5, 7, 9, 16–18].

During a recent investigation [14, 15] in which we were concerned with developing assay procedures for fluorometric assay of enzyme pathways, we became aware of the extreme sensitivity of some of these reactions to the ratio of Mg^{2+}/ATP or Mg^{2+}/ADP and to the concentrations of these substances in a fixed ratio. In determining optimal concentration of assay ingredients for Embden–Meyerhof pathway enzymes, we found it expedient to vary each assay ingredient in turn while holding all others constant. In many instances ingredients exhibited distinct concentration optima for observed enzyme velocity, instead of increasing to a point where a change in concentration had no further effect.

Where kinase enzymes were concerned, or where assays were coupled through kinase reactions with ATP or ADP held constant, variations in Mg^{2+} concentration exhibited broad velocity maxima. With Mg^{2+} constant, and changes in ATP or ADP, sharper maxima could be observed with rapid inhibitory changes at concentrations above the maximum. With Mg^{2+} and ATP or ADP varied in a fixed ratio, sharp maxima were also obtained with strong inhibition at higher concentrations.

* Supported by funds from Temple University and from the Temple University, School of Dentistry.

2. Methods and materials

Enzyme velocities between 0.3–0.4 nmoles per minute were measured using an Aminco fluorometer microphotometer fitted with a 4-sample automatic changer, temperature control, and recorder readout. Total sensitivity of the instrument was adjusted to 10^{-5} M NADH. Biochemical ingredients, including coupling enzymes, were obtained from the Sigma Company (St. Louis, Mo.). The plan was to initially vary ingredients using a reconstituted enzyme system with crystalline enzymes and then to repeat this procedure using a low-speed supernatant fraction of bovine gingiva homogenate [13]. In the assay procedures, hexokinase was coupled to the reduction of $NADP^+$ by glucose 6-phosphate dehydrogenase; phosphofructokinase was coupled to the oxidation of NADH by glycerolphosphate dehydrogenase; phosphoglycerate mutase and phosphoglycerate kinase were coupled to the oxidation of NADH by glyceraldehyde phosphate dehydrogenase; and phosphopyruvate hydratase and pyruvate kinase coupled to the oxidation of NADH by lactate dehydrogenase.

3. Results

Figs. 1–6 show variations in observed velocity of enzyme reactions as a result of varying $MgCl_2$, adenine nucleotide, or Mg^{2+}/adenine nucleotide in a fixed ratio

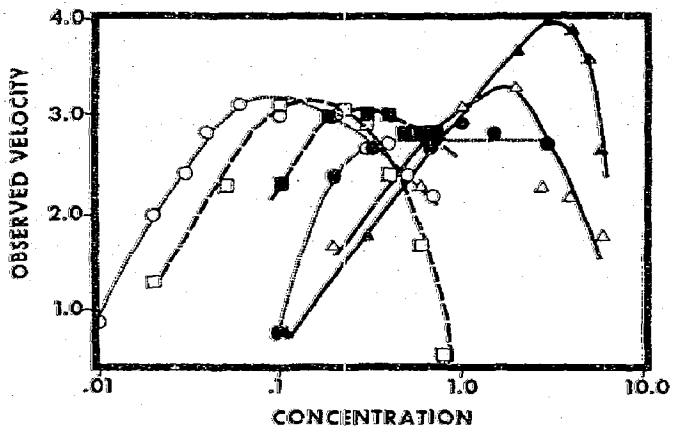

Fig. 1. Hexokinase. ○ = $MgCl_2$, μmol × 10^{-2} (ATP = 1.5 μmol); △ = ATP, μmol ($MgCl_2$ = 5.0 μmol); □ = Mg^{2+}/ATP 6:1 ATP, μmol × 10^{-1}; ● = $MgCl_2$, μmol × 10^{-1} (ATP = 1.0 μmol); ▲ = ATP, μmol ($MgCl_2$ = 10 μmol); ■ = Mg^{2+}/ATP 3:1 ATP, μmol × 10^{-1}.

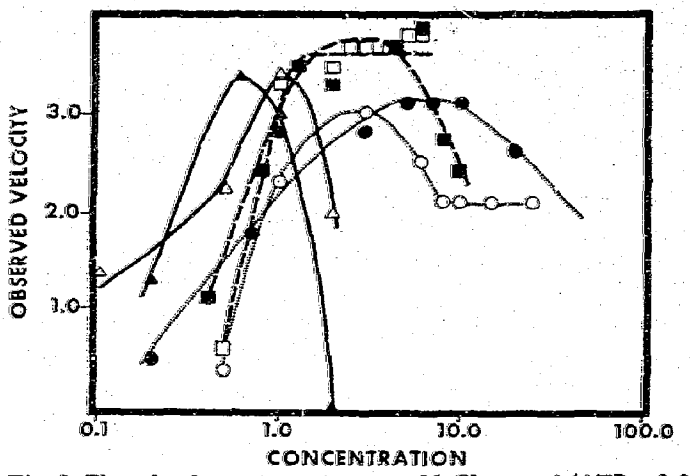

Fig. 3. Phosphoglycerate mutase. ○ = $MgCl_2$, μmol (ATP = 3.0 μmol); △ = ATP, μmol ($MgCl_2$ = 30 μmol); □ = Mg^{2+}/ATP 5:1 $MgCl_2$, μmol; ● = $MgCl_2$, μmol (ATP = 5.0 μmol); ▲ = ATP, μmol ($MgCl_2$ = 10 μmol); ■ = Mg^{2+}/ATP 2:1 $MgCl_2$, μmol × 10^{-1}.

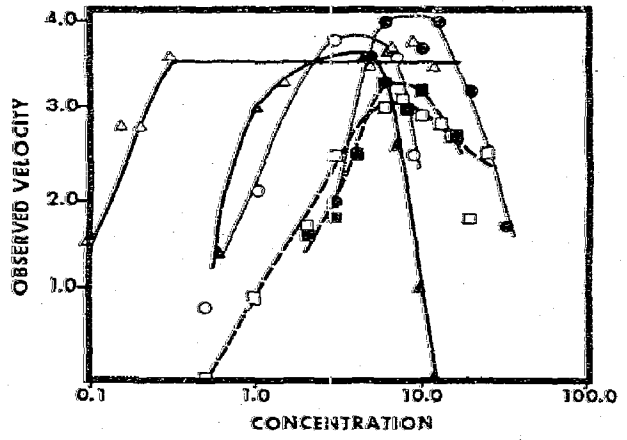

Fig. 2. Phosphofructokinase. ○ = $MgCl_2$, μmol (ATP = 0.6 μmol); △ = ATP, μmol ($MgCl_2$ = 5.0 μmol); □ = Mg^{2+}/ATP 5:1 $MgCl_2$, μmol; ● = $MgCl_2$, μmol (ATP = 0.6 μmol); ▲ = ATP, μmol ($MgCl_2$ = 20 μmol); ■ = Mg^{2+}/ATP 2:1 $MgCl_2$, μmol.

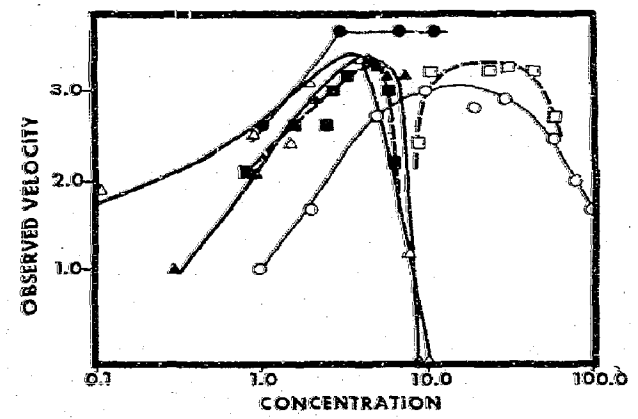

Fig. 4. Phosphoglycerate kinase. ○ = $MgCl_2$, μmol (ATP = 3.0 μmol); △ = ATP, μmol ($MgCl_2$ = 10 μmol); □ = Mg^{2+}/ATP 8:1 $MgCl_2$, μmol; ● = $MgCl_2$, μmol × 10^{-1} (ATP = 3.0 μmol); ▲ = ATP, μmol ($MgCl_2$ = 4.0 μmol); ■ = Mg^{2+}/ATP 8:1 $MgCl_2$, μmol × 10^{-1}.

with all other ingredient concentrations held constant. The open symbols represent the reconstituted system, and the closed symbols represent the gingiva supernatant. Velocities are given in nmoles per minute × 10, and concentrations are indicated on the figure legends. When the concentration is μmol × 10^{-2}, and 0.1 is indicated on the chart, the actual concentration is 10 μmol. All assay volumes were 2.0 ml.

Fig. 1 illustrates the hexokinase assay system. Although variations in most ingredients exhibit velocity maxima, the most striking results can be seen with variations of ATP. The optimal Mg^{2+}/ATP ratio was 6:1 with the reconstituted system and 3:1 with gingiva.

Fig. 2. shows the phosphofructokinase assay system. Variations in ATP again produce dramatic results, with an increase of 1.0 μmol producing complete inhibition in gingiva. The Mg^{2+}/ATP ratio is 5:1 for crys-

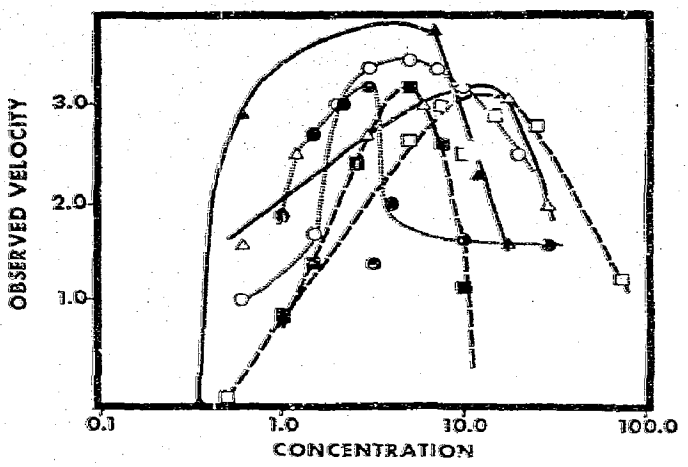

Fig. 5. Phosphopyruvate hydratase. ○ = $MgCl_2$, μmol (ADP = 6.0 μmol); △ = ADP, μmol × 10 ($MgCl_2$ = 5.0 μmol); □ = Mg^{2+}/ADP 5:1 $MgCl_2$, μmol; ● = $MgCl_2$, μmol (ADP = 0.6 μmol); ▲ = ADP, μmol × 10 ($MgCl_2$ = 5.0 μmol); ■ = Mg^{2+}/ADP 1:1 $MgCl_2$, μmol.

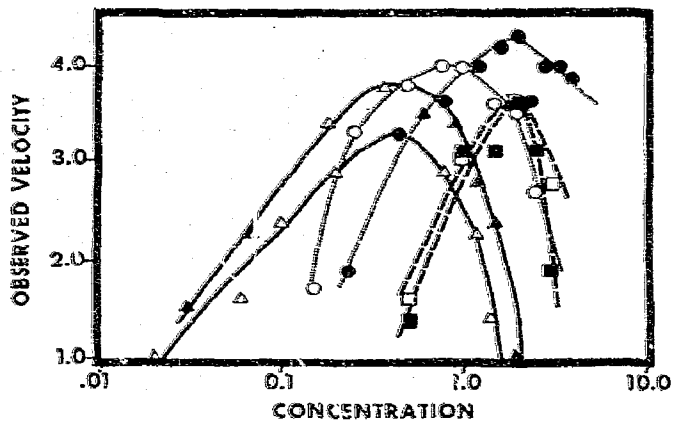

Fig. 6. Pyruvate kinase. ○ = $MgCl_2$, μmol × 10^{-3} (ADP = 1.0 μmol); △ = ADP, μmol × 10^{-1} ($MgCl_2$ = 8.0 μmol); □ = Mg^{2+}/ADP 2:1 $MgCl_2$, μmol × 10^{-1}; ● = $MgCl_2$, μmol × 10^{-1} (ADP = 6.0 μmol); ▲ = ADP, μmol × 10^{-1} ($MgCl_2$ = 8.0 μmol); ■ = Mg^{2+}/ADP 2:1 $MgCl_2$, μmol × 10^{-1}.

talline enzyme and 2:1 for gingiva. Increase in concentration of Mg^{2+}/ATP 2:1, inhibits gingiva extract.

Fig. 3 depicts the phosphoglucomutase assay system. Here, variations of the Mg^{2+}/ATP ratio and variations in absolute concentration in a fixed ratio inhibit sharply above the maxima. The Mg^{2+}/ATP ratio is 5:1 for crystalline phosphoglucomutase and 2:1 for gingiva.

Fig. 4 represents the phosphoglycerate kinase assay system. The Mg^{2+}/ATP ratio is 8:1 for both enzymes, with strong inhibition in gingiva in higher concentrations of ATP or Mg^{2+}/ATP 8:1.

Fig. 5 displays the phosphopyruvate hydratase system. Mg^{2+}/ADP ratios are 5:1 for crystalline enzyme and 1:1 for gingiva. Higher concentrations of ADP or Mg^{2+}/ADP 1:1 inhibit gingiva.

Fig. 6 summarizes the pyruvate kinase assay system. The Mg^{2+}/ADP ratio is 2:1 for both enzymes, and higher concentrations of all ingredients are inhibitory.

4. Discussion

Our observations indicate that, in assay systems for kinase reactions or in systems coupled through certain kinase reactions, the relative concentrations of Mg^{2+}/ATP, Mg^{2+}/ADP, and the absolute concentrations of these materials in a fixed ratio, have a profound influence on the observed velocity of the reaction.

These effects were all observed at high concentrations of substrate, other cofactors, and coupling enzymes—sufficiently high to observe maximal velocity of the enzyme being assayed. Control experiments using only the coupling enzymes ensured that the effects shown could only be observed when the kinases were present.

If this is true *in vitro*, then it also should be true *in vivo*. This, then, raises the interesting possibility that metabolic controls may be mediated by the influence of such changes outlined above on the many kinase reactions known in living systems as well as such reactions as pyruvate carboxylase, phosphopyruvate carboxylase, and oxidative phosphorylation in the mitochondrion.

The ratio of Mg^{2+}/ATP is known to influence the velocity of adenyl cyclase [3, 4, 6], and MgADP complexes of various ratios are optimal for kinase reactions [9]. Moreover, cobalt, which interferes with Mg^{2+} transport [10], can inhibit early methylcholanthrene tumors in mice [8, 11]. Bachelard [2] presents evidence that changes in Mg^{2+}/ATP ratios in hexokinase produce allosteric effects which may function as a metabolic regulator. We have found that variation in Mg^{2+}, ATP, or ADP, or in the concentrations of Mg^{2+}/adenine nucleotide in a fixed ratio, all exert a profound effect on the observed velocity of several kinase reactions

and offer these relationships as possible control mechanisms in intermediary metabolism

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