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METAL-ION CATALYSIS OF ALDOSE–KETOSE ISOMERIZATIONS IN ACIDIC SOLUTIONS*

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ABSTRACT

A comparative study has been made of the ability of metal ions to catalyze the Lobry de Bruyn–Alberda van Ekenstein transformation An analytical enzymic assay system, which is highly sensitive and selective, was developed to monitor the rates of isomerization of hexose and triose phosphates The metal ions catalyze the isomerization of hexose phosphates by virtue of their Lewis acid character with the first transition series being most effective The metal ions also appear to have no ability to direct the course of the reactions Amino acids also catalyze the isomerization apparently by virtue of the free carboxylate anion

INTRODUCTION

One of the earliest recognized reactions of carbohydrates was the interconversion of aldoses and ketoses, known as the Lobry de Bruyn–Alberda van Ekenstein transformation[1] These transformations have been demonstrated for a variety of aldehydes and ketones, but perhaps the best known is the interconversion of D-glucose, D-fructose, and D-mannose

Early studies of these transformations were handicapped by the lack of convenient, quantitative techniques for monitoring the course of the reactions Usually, the products of these reactions were determined by isolation of derivatives such as the phenylhydrazones With the availability of the hydrogen isotopes came the first advances in the elucidation of the mechanisms of these transformations Since that time, much effort has gone into establishing the validity of the enediol mechanism first proposed by Wohl and Neuberg[2] (see review by Speck[3])

Although Lobry de Bruyn–Alberda van Ekenstein transformations have usually been carried out in basic media, enolization is subject to general acid base catalysis,

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and, indeed, catalysis by acid was reported[4] as early as 1905 Interest in the effect of metal ions on the transformation was also established early, dating back to the original work by Lobry de Bruyn and Alberda van Ekenstein[1]. Later it became clear that certain alkaline earth metals were capable of catalyzing the transformation and, when a basic species such as acetate was present, the reaction was of the first order with respect to the metal ion

Biological systems catalyze the interconversion of the hexose 6-phosphates by virtue of two very specific enzymes, glucosephosphate (EC 5 3 1 9, D-glucose-6-phosphate aldo–ketoisomerase) and mannosephosphate (EC 5 3 1 8, D-mannose-6-phosphate aldo–ketoisomerase) isomerases Whereas glucosephosphate isomerase shows no requirement for a metal ion, we have previously shown mannosephosphate isomerase to be a zinc metalloprotein[5] The metal may be removed from the enzyme and replaced by certain other metals and still yield an active enzyme Similarly, Mn^{2+}, Mg^{2+}, and Co^{2+} have been reported to be essential or to activate several other aldo–ketoisomerases[6] Thus, it would appear that the aldose–ketose isomerases may be divided into two classes those which utilize a metal in the catalytic mechanism and those which do not The present study was conducted to provide information relating to the mechanisms of aldose–ketose isomerizations, with hopes of better understanding the role of the metal ions

EXPERIMENTAL

Reaction conditions — All metal ion solutions were prepared by dissolving the chloride salts in acetate buffers The reactions were conducted in specially constructed, sealed-glass ampules having only a small area above the surface of the solution and designed to allow adjustment of the pH with a small combination electrode In all experiments the reaction solutions (1 0 ml) had a 50mM concentration of metal ion, 30mM of sugar phosphate, and 10mM of acetate with a constant ionic strength being maintained by addition of sodium chloride The pH was adjusted and the ampules were sealed with rubber septums and flushed with nitrogen for 60 sec through 2-syringe needles inserted into the septum. The ampules were placed in a thermostatically regulated water-bath at 50° and covered with a heavy black cloth Aliquots (100 μl) were removed at various time intervals with a syringe and added to a measured amount of cation-exchange resin (Dowex 50W-X8, 200–400 mesh, H^+) to remove the metal ions This step was required since the metal ions interfere with the assay either by inactivating the enzymes used in the assay (*v i*) or by their absorbance at the wavelength being monitored* Aliquots of this solution were then assayed as described in the following paragraphs

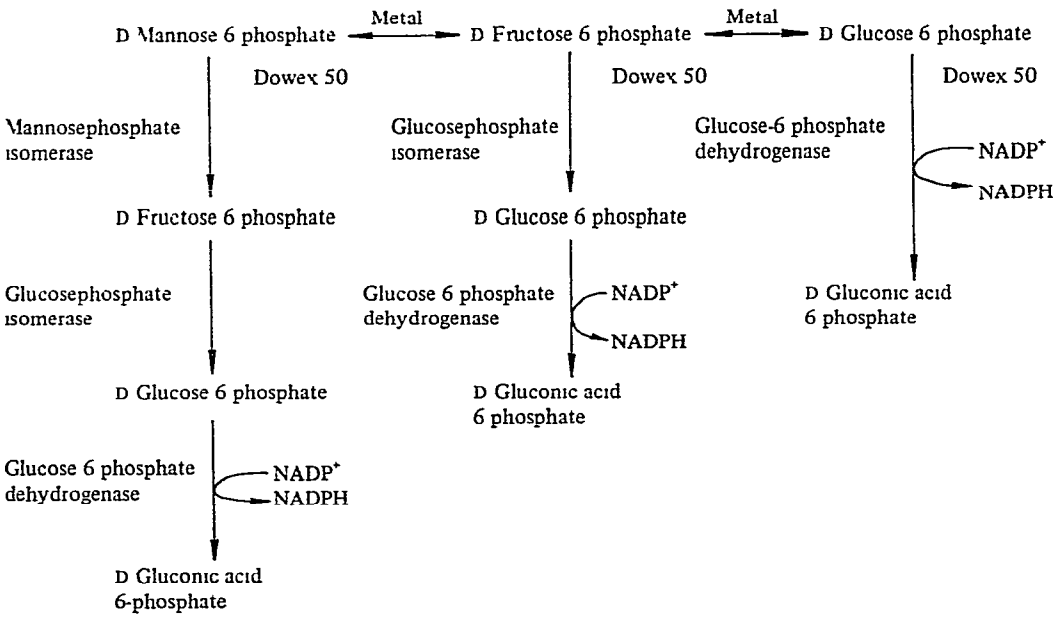
Purification of D-*mannose 6-phosphate* — Most commercial sugar phosphates contain a significant amount (*i e*, 1–6%) of the contaminating epimeric hexose

*It should be noted that a small proportion of Al^{3+} can interfere with the enzyme assay by directly oxidizing nicotinamide–adenine dinucleotide, reduced (NADH) and nicotinamide–adenine dinucleotide phosphate, reduced (NADPH)

phosphates, and thus purification was necessary. The following method was developed for purifying D-mannose 6-phosphate from contaminating D-glucose and D-fructose 6-phosphates The barium salt of D-mannose 6-phosphate (500 mg) was treated with Dowex 50 (8X, 2 g, H^+) cation-exchange resin to remove the barium ions. The pH of the resulting solution was adjusted to 8 0 with M sodium hydroxide. Glucose-6-phosphate dehydrogenase (EC 1 1 1.49, D-glucose-6-phosphate NADP oxidoreductase) and glucosephosphate isomerase (50 units of each) were added to the solution along with a slight excess of nicotinamide–adenine dinucleotide phosphate, oxidized ($NADP^+$) necessary for removal of the contaminants This solution was stirred for 1 h at room temperature with the pH being maintained between 7 5 and 8 0 The pH was then adjusted to 1–2 with M hydrochloric acid, and the solution stirred for 2 min to denature and precipitate the enzymes After the pH had been readjusted to 6–7, activated charcoal (0 5 g) was added, and the mixture stirred for 2 min and filtered The resulting solution was free of $NADP^+$, NADPH, enzyme activity, and contained less than 0 2% of D-glucose and D-fructose 6-phosphates as contaminants

RESULTS AND DISCUSSION

The hexose phosphate assay — The nonenzymic isomerization of D-mannose 6-phosphate was studied as follows (Scheme 1) After removal of the metal ions from



Scheme 1

the aliquot to be assayed (Dowex 50), the solution (50 μl) was added, in a 1 0-ml quartz cell, to an assay solution (0 8 ml) containing triethanolamine [tri(2-hydroxyethyl)amine, 100mM, pH 9 0] and 0 5mM $NADP^+$ After the baseline absorbance at 340 nm had been established, a 10-μl aliquot (1 0 unit) of D-glucose-6-phosphate dehydrogenase was added This enzyme converts D-glucose 6-phosphate present into D-gluconic acid 6-phosphate with the stoichiometric reduction of one mole of $NADP^+$ Thus, the concentration of D-glucose 6-phosphate was determined from the increase in absorbance at 340 nm A molar absorbance coefficient of $6\ 22 \times 10^3 M^{-1}\ cm^{-1}$ for NADPH was used[7] The concentration of D-fructose 6-phosphate was then determined by the addition of glucose phosphate isomerase (one unit) Finally, the concentration of D-mannose 6-phosphate was obtained by the addition of mannose-phosphate isomerase*

In the study of the isomerization of D-glucose 6-phosphate a slight modification of the assay procedure was necessary to measure the concentration of D-fructose and D-mannose 6-phosphates After removal of the metal, a 50-μl aliquot was added to triethanolamine (200 μl, 0 1M, pH 11 0) and $NADP^+$ (100 μl, 1 7 μmoles) Glucose-6-phosphate dehydrogenase (2 units in 25 μl) was added, the reaction allowed to proceed for 20 min, and then passed through a pad of activated charcoal (50 mg) on a Swinny filter The filtrate was assayed for the concentrations of D-fructose and D-mannose phosphates as just described

The triose phosphates were assayed by a method similar to that for the hexose phosphates the 1,3-dihydroxyacetone 1-phosphate concentration was determined by the NADH-dependent glycerol-phosphate dehydrogenase (EC 1 1 1 8, L-glycerol-3-phosphate NAD oxidoreductase), and the resulting D-glyceraldehyde 3-phosphate by the subsequent addition of triose phosphate isomerase (EC 5 3 1 1, D-glyceraldehyde-3-phosphate aldo-ketoisomerase)

The enzymic assays just described provide a rapid, simple, and quantitative technique for the study of these reactions Although g l c was initially utilized to analyze these solutions, several problems and difficulties made this technique less desirable It is inherently slower and less quantitative than the enzymic method since a volatile derivative must be prepared prior to analysis When the trimethylsilyl ether glycosides were prepared with *N,O*-bis(trimethylsilyl)acetamide[8] and the derivatives chromatographed (Chromosorb W H P , 100–120 mesh, 3% OV-17), the anomers of the individual sugars were resolved However, g l c of a mixture of the per(trimethylsilylated) hexose phosphates showed overlapping peaks which could not be resolved Although preparation of the alditol acetate derivatives[9] would overcome the problem of anomerization, D-mannitol is produced from both D-mannose and D-fructose upon reduction with sodium borohydride

Catalysis by metal ions — Although the monovalent cations exhibited essentially no catalytic effect in the nonenzymic isomerization of D-mannose 6-phosphate at

*A smaller aliquot of the reaction mixture was usually necessary in order to obtain a suitable change of absorbance for D-mannose 6-phosphate

pH 6.0, most of the divalent cations were capable of catalyzing the isomerizations (Fig 1) This capability, however, varied over an order of magnitude The linearity of these data indicates the quantitative nature and reproducibility of the assay Only

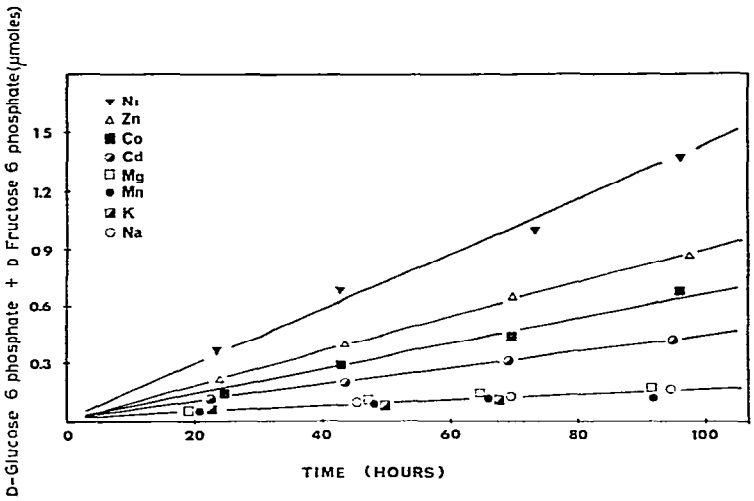


Fig 1 Effect of metal ions on the nonenzymic isomerization of D-mannose 6-phosphate at pH 6 0 In each solution, the concentration of metal ion was 50mM, of D-mannose 6-phosphate 30mM, of acetate 10mM The reactions were carried out at 50°, under nitrogen, and in the dark

extremely small proportions of D-glucose 6-phosphate were produced (Table I) It is also clear that essentially no side reactions occurred under these conditions, since the amount of D-glucose and D-fructose 6-phosphates formed equals the amount of D-mannose 6-phosphate that disappeared It is not clear whether the divalent metals prevent the side reactions normally observed in the nonmetal-catalyzed transformations[3] or the shorter reaction times and milder conditions preclude their formation

TABLE I

PRODUCTS FORMED IN THE NONENZYMIC ISOMERIZATION OF D-MANNOSE 6-PHOSPHATE[a]

Metal ion	*Products formed* (mM)		
	D-*Fructose 6-phosphate*	D-*Glucose 6-phosphate*	*Other*[b]
Na^+	0 10	0 03	0 17
Co^{2+}	0 64	0 04	0 06
Zn^{2+}	0 81	0 05	0 05
Ni^{2+}	1 40	0 05	0 02

[a]Reaction conditions 95 h, 50°, pH 6 0, with initial concentration of D-mannose 6-phosphate 30 0mM The concentration of metal ions was 50mM [b]Other, side reactions were estimated from the difference in the initial concentration of D-mannose 6-phosphate and the sum of the concentrations of D-mannose, D-glucose and D-fructose 6-phosphates after 95 h

When the isomerization of D-glucose 6-phosphate was studied under conditions identical to those used for D-mannose 6-phosphate (Fig 1), only trace amounts of D-mannose 6-phosphate were formed during the first 100 h On the other hand, D-fructose 6-phosphate appeared at a rate comparable to that of its production from D-mannose 6-phosphate (*e g* $11\,0\times10^{-9}$ moles of D-fructose 6-phosphate h^{-1} in the Zn^{2+} catalyzed reaction from D-glucose 6-phosphate *vs* $13\,7\times10^{-9}$ moles of D-fructose 6-phosphate h^{-1} in the Zn^{2+} catalyzed isomerization from D-mannose 6-phosphate) Thus, under these conditions, the metals do not show a specificity for either of the two epimeric aldohexoses, since only the more stable product (D-fructose 6-phosphate) is formed Therefore, it appears that no "directive uniqueness" is afforded to mannosephosphate isomerase by its metal ion alone, but rather that the enzyme has taken advantage of the natural catalytic ability of the metal ion as a super acid catalyst in these isomerizations A second group of metal ions, which includes some of the metals from the group studied at pH 6 0 for comparison, were studied at pH 3 5 because of their limited solubility at higher pH ranges (Table II)

TABLE II

EFFECT OF METAL IONS ON THE ISOMERIZATION OF D-MANNOSE 6-PHOSPHATE[a]

Metal ion	*Rate (mμmoles h^{-1})*
Fe^{3+}	9 0
Cr^{3+}	9 0
Al^{3+}	7 4
Cu^{2+}	6 1
Ni^{2+}	3 1
Fe^{2+}	1 5
Na^{+}	1 3

[a]The reaction conditions were identical to those described in Table I, except that all reactions were performed at pH 3 5 The rate reflects the sum of the D-glucose and D-fructose phosphates produced

The ability of metal ions to catalyze the isomerization appears dependent on their Lewis acid character, the trivalent ions being especially effective catalysts (Table II) It also appears that larger ions are not as effective in catalyzing the isomerization as smaller ions within the same group It is undoubtedly the influence of several additional properties (*e g* electronegativity, coordination number, and symmetry of the coordination sphere) which endows each metal with some degree of uniqueness Although the relative catalytic abilities of Ni^{2+}, Co^{2+}, and Mn^{2+} correlate well with their size to charge ratio, no single parameter exhibits correlation with the catalytic ability of all the metals This is to be expected from such a variety of transition and nontransition metals

In the study of the effect of metals on the nonenzymic isomerization of the triose phosphates (Table III), little or no catalysis was observed for the conversion of D-glyceraldehyde 3-phosphate into 1,3-dihydroxyacetone 1-phosphate Even under

the mild conditions employed, side reactions occured as indicated by the decrease in concentration of the aldehyde

TABLE III

EFFECT OF METAL IONS ON TRIOSE PHOSPHATES[a]

Metal ion	*Initial conc (mM)*		*Final conc (mM)*	
	G3P[b]	*DHAP*[c]	*G3P*[b]	*DHAP*[c]
Na^+	9 28	0 331	6 01	0 300
Co^{2+}	9 12	0 364	6 13	0 364
Ni^{2+}	9 57	0 464	4 73	0 381
Mn^{2+}	9 05	0 464	7 33	0 331

[a]D-Glyceraldehyde 3-phosphate was incubated for 50 h at 25°, under nitrogen, in the dark, and at pH 6 0 [b]Abbreviation G3P, D-glyceraldehyde 3-phosphate [c]Abbreviation DHAP, 1,3-dihydroxyacetone 1-phosphate

Catalysis by amino acids — Since in enzymes one amino acid fulfills the function of the base catalyst in the isomerizations, we have studied the effect of free amino acids on the nonenzymic isomerization of D-mannose 6-phosphate (Table IV) A small catalytic effect was observed when a free carboxylate ion was present At neutral pH, the zwitterion form predominates and charge–charge interaction between the α carboxyl and α amino groups render the amino acid catalytically inactive Upon increase of the pH to a point where the zwitterion is disrupted, the catalysis by hydroxide ions swamps out the effect of the amino acid However, blocking of the α amino group afforded catalysis by virtue of the basicity of the carboxylate ion Esterification or amidation destroyed the catalytic effect of the amino acids In the hexosephosphate isomerases the imidazole nitrogen atom of histidine[10] and the γ carboxyl group of glutamic acid[11] have been proposed to function as the base catalyst in promoting proton abstraction In triosephosphate isomerase a γ carboxylate group is believed to fulfill this role[12] It is interesting to note that although the catalytic effect of amino acids on the nonenzymic reaction is small, no additional catalysis could be attributed to the imidazole nitrogen of histidine, and yet the carboxylate group of the glutamate anion does stimulate the rate of reaction

TABLE IV

EFFECT OF AMINO ACIDS ON MANNOSE 6-PHOSPHATE ISOMERIZATION[a]

Amino acid	*Rate (mμmoles h^{-1})*
Control	1 34
Glycine	1 34
Glycinamide	1 33
N-Acetylglycine	2 35
Histidine	1 34
Glutamic acid	2 15

[a]The conditions were identical to those described in Fig 1

Mechanism — Mechanisms considering the involvement of metal ions in aldose–ketose isomerizations[3 13 14] have depicted the carbonyl oxygen atom and another hydroxyl group of the monosaccharide molecule as forming a chelate with the metal ion, which results in labilization of the α proton and subsequent stabilization of the enediolate ion While the present studies provide no direct mechanistic evidence, they are in accord with this proposed role It may also be significant that those divalent ions which best catalyze this isomerization (Ni, Zn, Co) favor tetrahedral coordination Furthermore, two of these metal ions (Co^{2+} and Zn^{2+}) have been shown to function in the enzymic interconversion of D-mannose 6-phosphate and D-fructose 6-phosphate[5] Thus, it would appear that a number of analogies of the nonenzymic and enzymic systems exist, and that studies of this model system may provide additional insight into the enzymic isomerizations

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