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Construction of Comprehensive Metabolic Network for Glycolysis with Regulation Mechanisms and Effectors

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Abstract Glycolysis has a main function to provide ATP and precursor metabolites for biomass production. Although glycolysis is one of the most important pathways in cellular metabolism, the details of its regulation mechanism and regulating chemicals are not well known yet. The regulation of the glycolytic pathway is very robust to allow for large fluxes at almost constant metabolite levels in spite of changing environmental conditions and many reaction effectors like inhibitors, activating compounds, cofactors, and related metal ions. These changing environmental conditions and metabolic reaction effectors were focused on to understand their roles in the metabolic networks. In this study, we have investigated for construction of the regulatory map of the glycolytic metabolic network and tried to collect all the effectors as much as possible which might affect the glycolysis metabolic pathway. Using the results of this study, it is expected that a complex metabolic situation can be more precisely analyzed and simulated by using available programs and appropriate kinetic data.

Key words: Glycolysis, metabolic pathway, inhibitors, activating compounds, regulation mechanism

Glycolysis is a central pathway in almost all organisms. It has very important functions to connect with many metabolic pathways such as TCA cycle, pentose phosphate pathway, amino acid biosynthesis pathways, lipid biosynthesis pathways, and ATP generating pathways, and it also provides many precursors to off-central metabolic pathways.

Simply, glycolytic metabolism is represented whereby one 6-carbon molecular glucose is converted to two 3-carbon molecular pyruvates while ATP and NADH are produced [1]. But in the glycolytic metabolic system, numerous metabolic materials, enzymes, and effectors (directly and/or indirectly influencing factors to metabolism) are composed of the whole metabolic reaction. Then it is organized and acts as one unit of an important metabolic system [22]. Nowadays, the study of the glycolysis metabolic pathway is very actively being investigated more than other metabolic pathways, and we can easily collect the glycolysis metabolic information such as metabolic reaction type and metabolic reaction mechanisms using the data from many databases and literatures [5–7].

Although enormous studies have been devoted to glycolysis so far, the details of the control and regulation mechanisms of enzymes, metabolites, and related effectors, have not been well elucidated yet. Even the kinds of effectors and the links of control loops have not been classified and summarized enough to be used in the study of metabolic analysis.

Recently, the study of regulation for the metabolic network through the systematic approach is considered to effectively produce useful products [23]. But the research adopting the systematic approaches did not always give satisfactory results. One reason is that most research did not consider enough operating factors except major reaction factors such as substrate, enzyme, and metabolite. Another is that influence of operating factors to the major glycolytic reactions was not sufficiently considered in the metabolic network model. These operating factors are able to affect indirectly to enzyme catalyzed reactions and have very important roles such as leading the regulation of the desired reactions. Therefore, we need to consider and study these factors for more precise understanding of the glycolysis metabolic network. In this study, we have made an effort to

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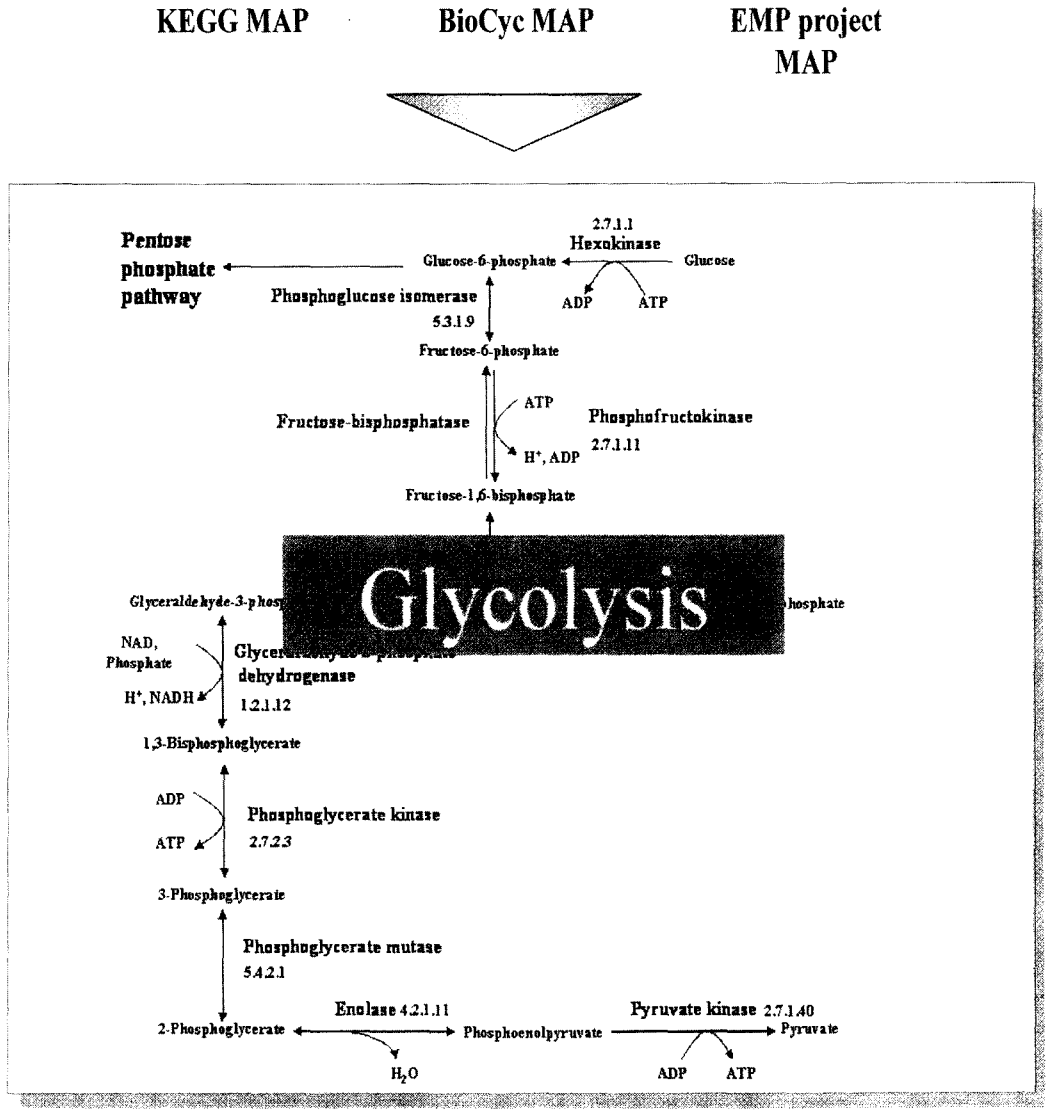
make a strict glycolysis metabolic network model by considering regulators, and effectors as much as possible. Then, we have analyzed effectors and constructed the new regulation maps for the glycolysis metabolic network.

METHODS

Analysis of Glycolytic Enzymes

Most metabolic reactions of living cells are composed of substrates, enzymes, and effectors, which affect individual reactions in a living cell. Sometimes metabolic reactions of microorganisms are composed of some complex reactions and many influencing factors [29]. In most cases, enzymes have very important functions that lead and regulate internal metabolic reactions [2].

Therefore, the study for the enzyme reaction is the core to establishing a metabolic regulation network and elucidating its role on a cell's behavior. In this study, we have performed an analysis of individual enzyme reaction characteristics and mechanisms for nine glycolytic enzymes. These nine enzymes are as follows; hexokinase (2.7.1.1), phosphoglucose isomerase (5.3.1.9), phosphofructokinase (2.7.1.11), aldolase (4.1.2.13), glyceraldehyde 3-phosphate dehydrogenase (1.2.1.12), phosphoglycerate kinase (2.7.2.3), phosphoglycerate, mutase (5.4.2.1), enolase (4.2.1.11), and pyruvate kinase



Construction of synthetic methionine metabolic pathway

Fig. 1. Procedure of constructing the glycolytic metabolic network was taken from the KEGG, BioCyc, EMP projects, and some published literatures were used for establishing an adequate glycolysis metabolic network.

(2.7.1.40). The study of these enzymes was performed by collecting useful information from available databases and literatures [12- 16].

Collection of Effector Information

General metabolic reactions are composed of metabolic materials, enzymes, and effectors such as inhibitors, activating compounds, cofactors, and metal ions. Each effector can be defined as the following: inhibitor has a function of inhibiting enzyme reaction, activating compound has a function of activating enzyme reaction, cofactor has a function of assisting enzyme reaction, and metal ion has a function of leading enzyme reaction [15]. Most effectors operate to regulate enzyme reactions in order to lead and

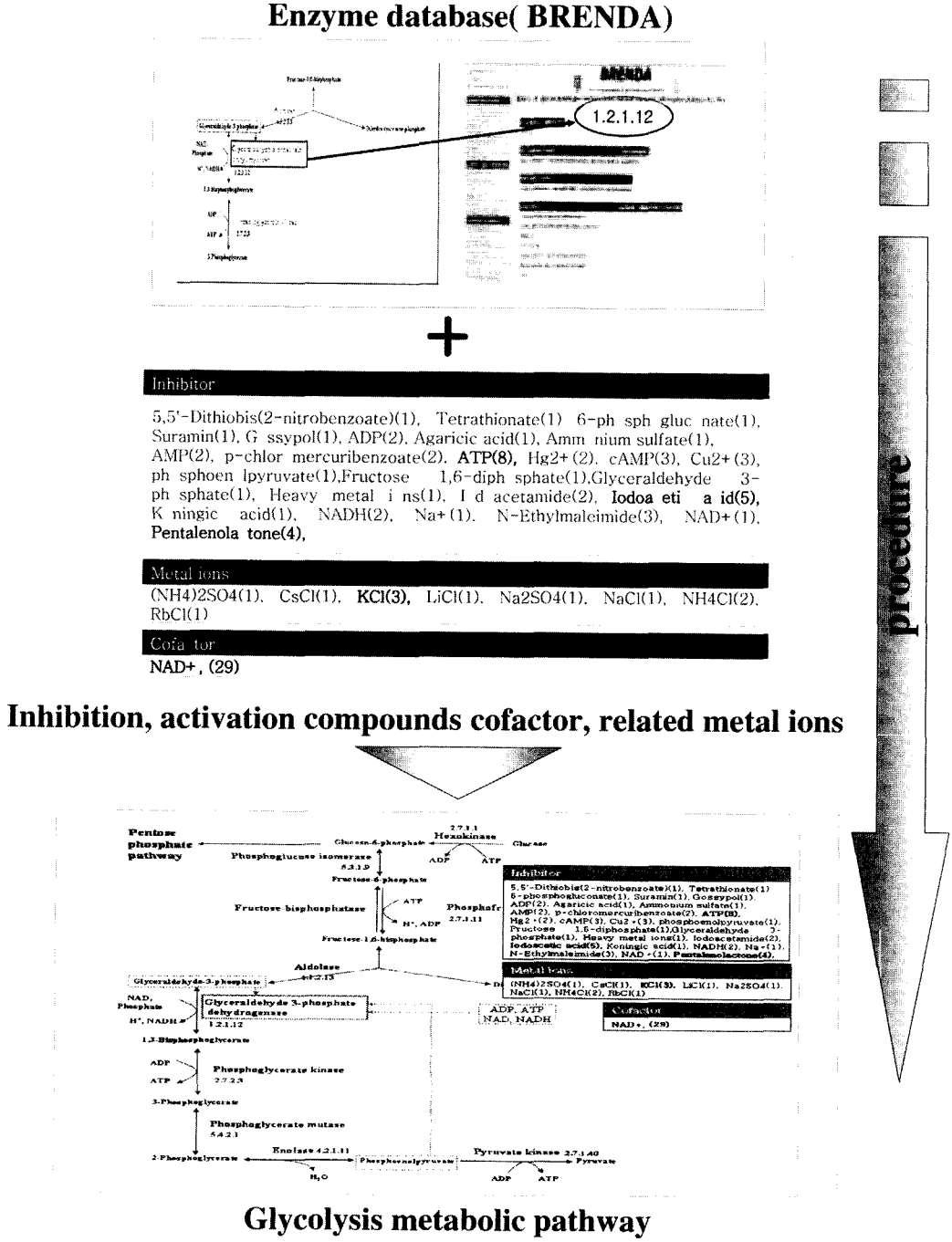


Fig. 2. Collection of enzyme (pyruvate kinase) data from the BRENDA database and literatures: Effectors were mostly inhibitors, activating compounds, cofactors, and related metal ions, which affect directly and/or indirectly on the function of individual metabolism.

assist metabolic reaction to desirable metabolites production and maintaining the homeostasis condition in a cell.

Our research has been mainly focused on the analysis of effectors which are able to influence enzyme catalyzed reactions in glycolytic metabolism. Most effector information was collected by examining databases in Web and published literatures [12- 16].

Glycolysis Metabolic Network

The study of glycolysis metabolic pathway is very actively being investigated more than others and the information about it could be easily obtained from many databases and published literatures. But most information [11- 17] has some difference in reaction types, experimental condition, and analytical methods. Moreover, changing environmental conditions and active effectors are not properly considered in many cases. Therefore, we needed to strictly examine the overall glycolytic metabolism with the emphasis on influencing effectors and regulatory mechanisms.

In this study, we have tried to construct a stricter glycolysis metabolic network based on regulator information and the analysis of various effectors. Information from the KEGG [14], BioCyc [10], and EMP [13] projects, and some other published literatures [1, 18] were used for establishing an adequate glycolysis metabolic network. Figure 1 shows the whole procedure of constructing the glycolysis metabolic

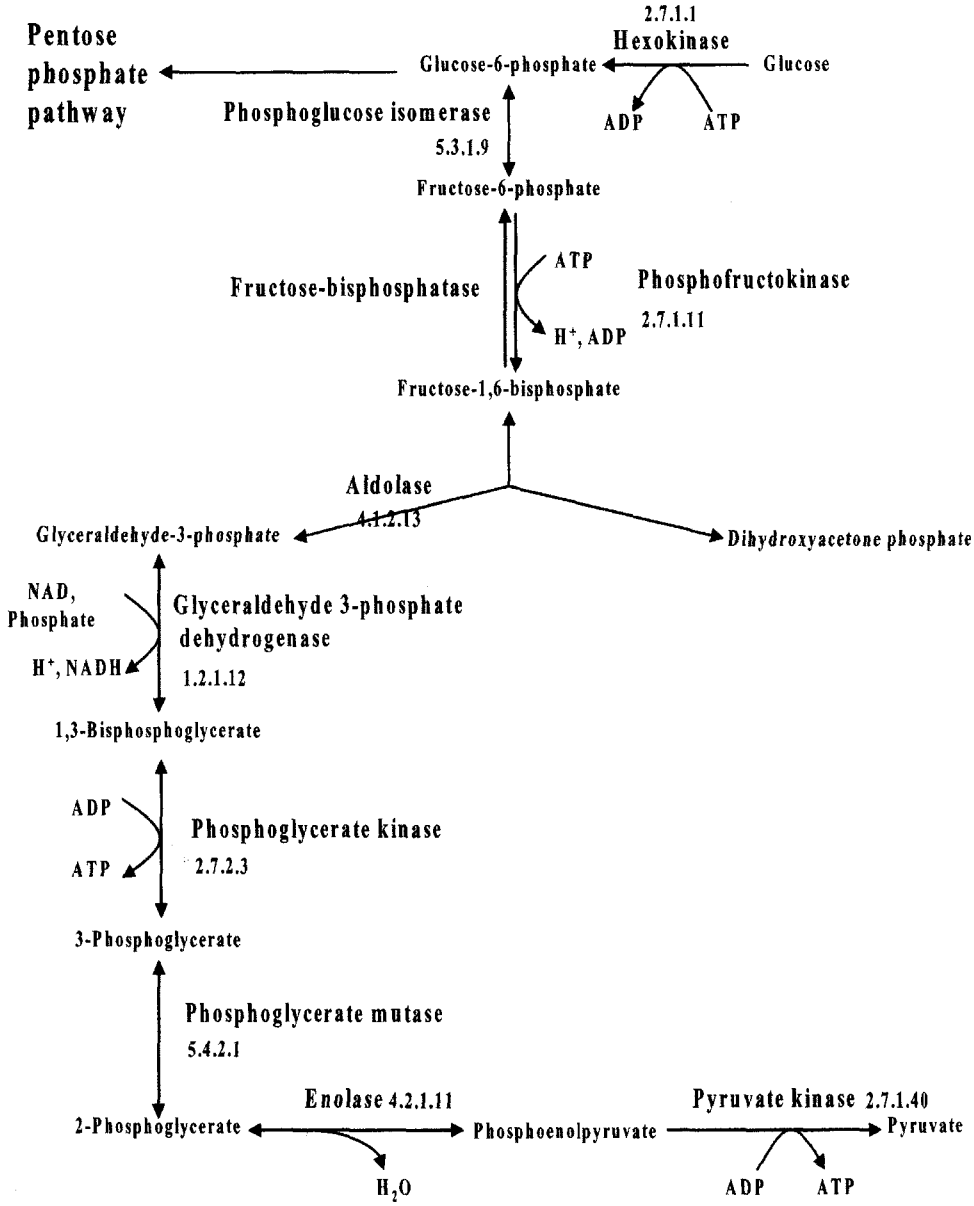


Fig. 3. Constructed glycolysis metabolic network: it is consisted of one branch point, ten metabolites, and nine enzyme reactions.

network with regulatory loops and effector information. We have tried to find the most common metabolic pathways and regulation mechanisms from the database information. Also, we have tried to add effectors information for the enzyme reactions in the glycolysis metabolic network as much as possible. Figure 2 shows the procedure of the detailed method for the construction of the glycolysis metabolic network. First, we collected enzyme information from the BRENDA database and some literatures. It is being shown that pyruvate kinase information was collected from the BRENDA database [12]. The BRENDA database has various information which include nomenclature, reactions and specificity, stability, enzyme structure, and functional parameters. In this study, reactions and specificity information were mainly used in construction of the glycolysis network. Collected effector information from BRENDA and other databases was added to the glycolysis metabolic network. The roots affected by inhibitors and activating compounds to enzyme reactions were presented using the dotted line in the map.

RESULTS AND DISCUSSION

Collection of Effectors in Glycolysis Metabolic Network

There are many kinds of effectors which affect metabolic reactions in the glycolysis metabolic network. So in this

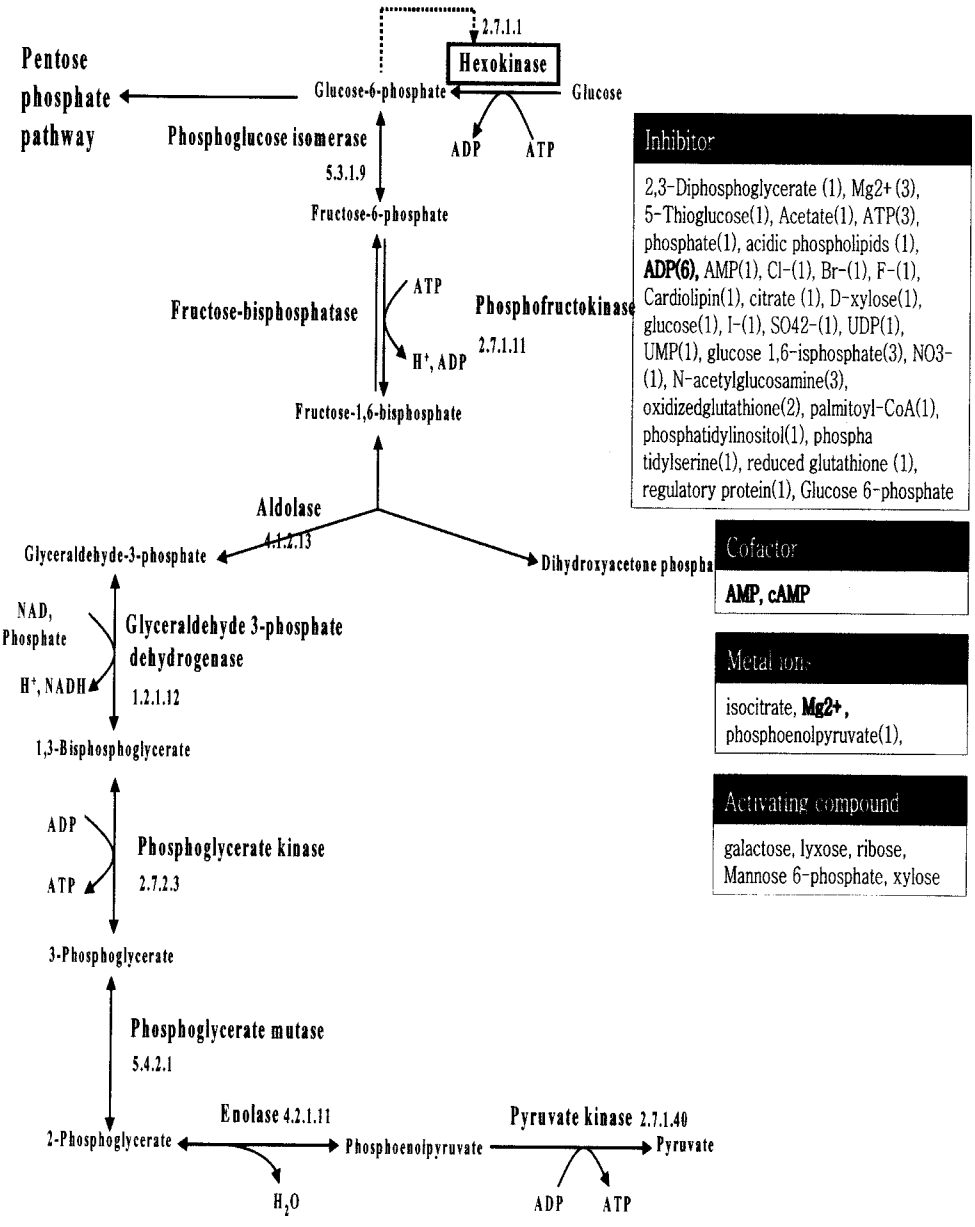


Fig. 4. Regulation of the hexokinase (2.7.1.1) reaction step in the glycolysis metabolic network.

research, we have focused on the effectors which mainly affect enzyme reactions in the glycolysis metabolic network. Because *E. coli* has currently plentiful information, most data were taken from *E. coli* data, and additional information was fulfilled from other species like *Aerobacter*, *Bacillus*, *Salmonella*, *Vibrio*, *etc.* Various kinds of effectors were collected for nine glycolysis enzyme reaction steps in this study. We have selected an inhibitor which is able to have inhibition effect on individual enzyme reactions. Some information for inhibitors was expressed by quantitative values, and the other was expressed as only the level of influence. Specifically, inhibitors were divided into internal inhibitors which exist in the glycolysis metabolic network and inhibit the glycolysis enzyme reaction, and external inhibitors which do not exist in the glycolysis metabolic network but inhibit the individual glycolysis enzyme reaction. Activating compounds were divided by the same method.

Analysis of Enzyme Reaction in Glycolysis Metabolic Network

Generally, most microorganisms lead and regulate their metabolic reaction to continue their life [25], and they have many kinds of reaction mechanisms and specific reaction types. Glycolytic reactions are composed of five different enzyme reactions like phosphorylation-kinases, phosporyl-shift-mutase, isomerization-isomerase, dehydration-dehydratase, and aldol cleavage-aldolase. These five different enzyme catalyzed reactions can be classified into specific enzyme reaction types in the glycolysis metabolic network [19]. In this study, we have newly

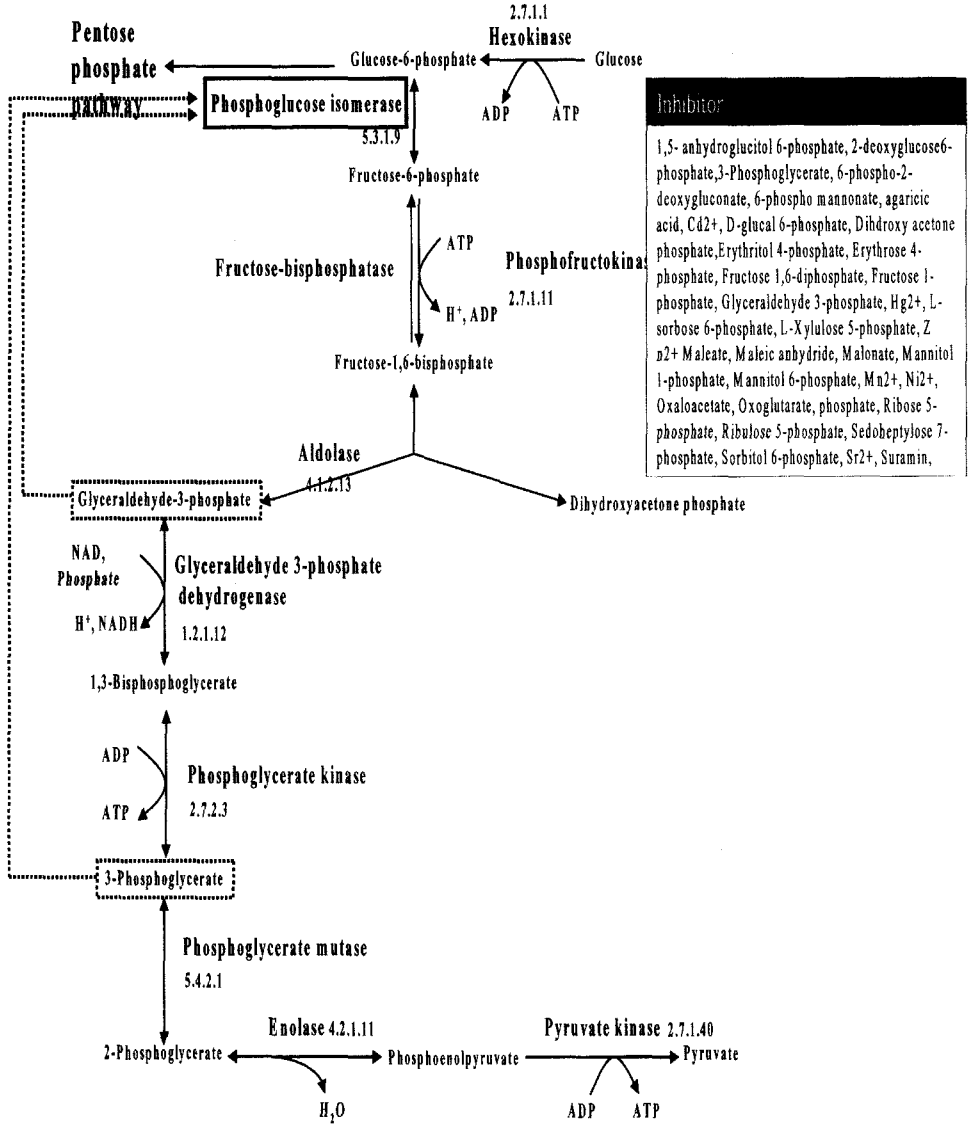


Fig. 5. Regulation of the phosphoglucose isomerase (5.3.1.9) reaction step in the glycolysis metabolic network.

established the glycolysis metabolic network and it is shown in Fig. 3. The developed glycolytic enzyme reaction map is composed of nine enzyme reactions and each enzyme reaction step has the characteristics as follows:

First Step. This step is a type of phospho group transfer reaction, and it was catalyzed by hexokinase. Hexokinase reaction mechanism follows where D-hexose is converted into D-hexose consuming 1 mol of ATP and producing 1 mol of ADP. This reaction is inhibited by its product glucose-6-phosphate which has feedback type inhibition. Glucose-6-phosphate inhibits by competition at the active site, as well as by allosteric interactions at a separate site on the enzyme. Glucose and ATP are not observed as an inhibitor in a normal condition, but when glucose and ATP concentration get to be above 0.2 mM and 1 mM, these metabolites have inhibition feature [30]. Figure 4 shows the hexokinase metabolic reaction step developed in this study. Mg^{2+} seemed to be metal ion and there is no activating compound in this reaction step [8, 12].

Second Step. This reaction type is intramolecular oxidoreduction, and it was catalyzed by phosphoglucose isomerase. Phosphoglucose isomerase reaction mechanism is where D-glucose 6-phosphate is converted into D-fructose 6-phosphate and this mechanism involves acid/base catalysis, with ring opening, isomerization via an enediolate intermediate, and then ring closure. This reaction was inhibited by internal inhibitors, such as glyceraldehyde 3-phosphate, 3-phosphoglycerate. Fructose 1,6-diphosphate and fructose 1-diphosphate act as external inhibitors when their concentrations get to be above 1 mM [31]. Figure 5 shows

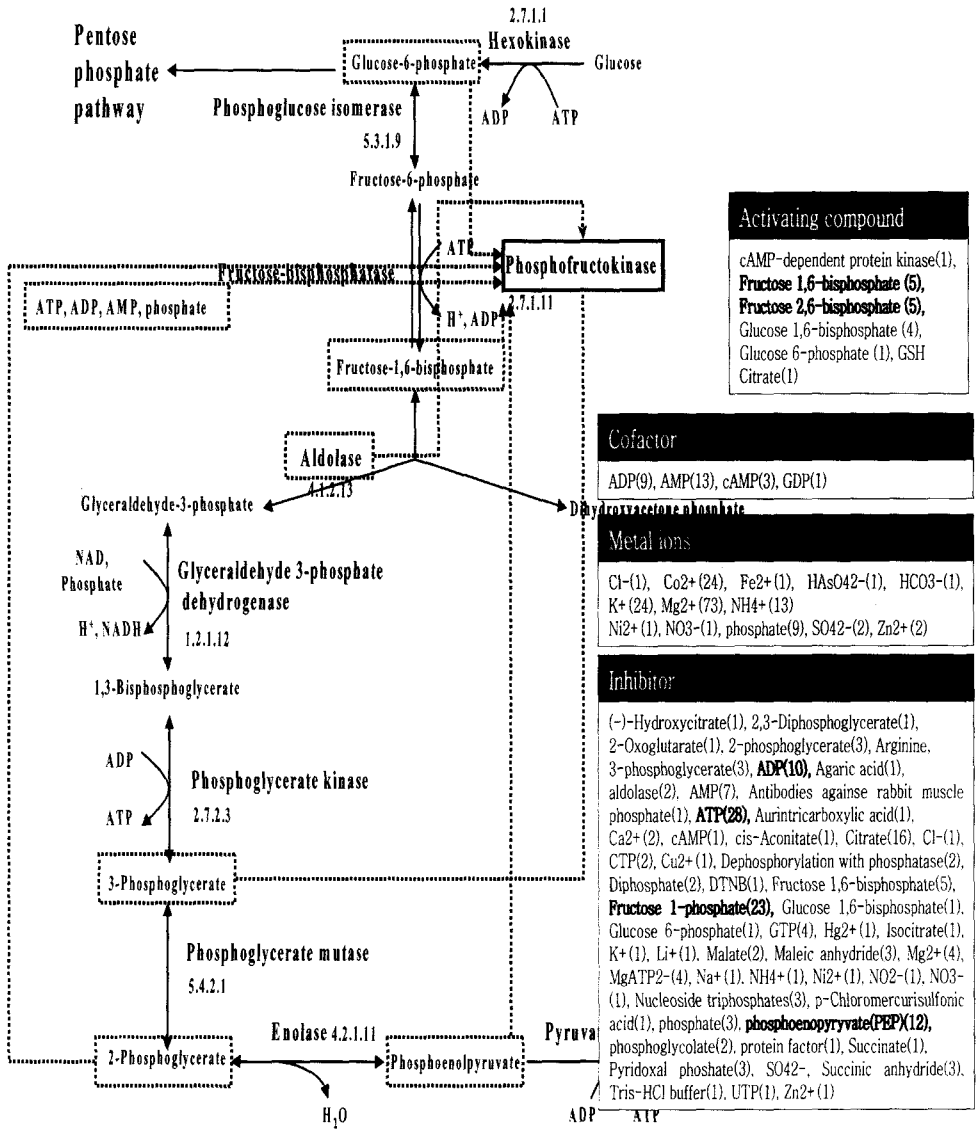


Fig. 6. Regulation of the phosphofructokinase (2.7.1.11) reaction step in the glycolysis metabolic network.

the phosphoglucose isomerase metabolic reaction step through this study. There was no other internal effector in the second reaction step [7, 12].

Third Step. Phospho group transfer reaction type is the third reaction feature, and this reaction was catalyzed by phosphofructokinase. Phosphofructokinase reaction mechanism is where D-fructose 6-phosphate converted into D-fructose 1,6-bisphosphate with 1 mol ATP consumption and 1 mol ADP production. This reaction has a mechanism similar to that of hexokinase which has a highly spontaneous reaction feature. This enzyme reaction step is usually the rate-limiting step of glycolysis and this enzyme is allosterically inhibited by ATP. ATP has a strong inhibition feature when ATP concentration gets to be above 1 mM [20]. Glucose 6-phosphate, fructose 1,6-bisphosphate, 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate also acted as internal inhibitors which have feedback type inhibition. Glucose 6-phosphate has a weak inhibition feature. Figure 6 shows the phosphofructokinase metabolic reaction step through this study. Glucose 6-phosphate, glucose 1,6-bisphosphate, and fructose 1,6-phosphate were operated activating compounds. Fe^{2+}, K^{+}, Mg^{2+}, Zn^{2+} were operated metal ions in the third reaction step [8, 12].

Fourth Step. This reaction was catalyzed by aldolase, and the reaction type is elimination reaction of some metabolic materials. Aldolase reaction mechanism is where D-fructose 1,6-bisphosphate is converted into glycerone phosphate, D-glyceraldehyde 3-phosphate. This reaction

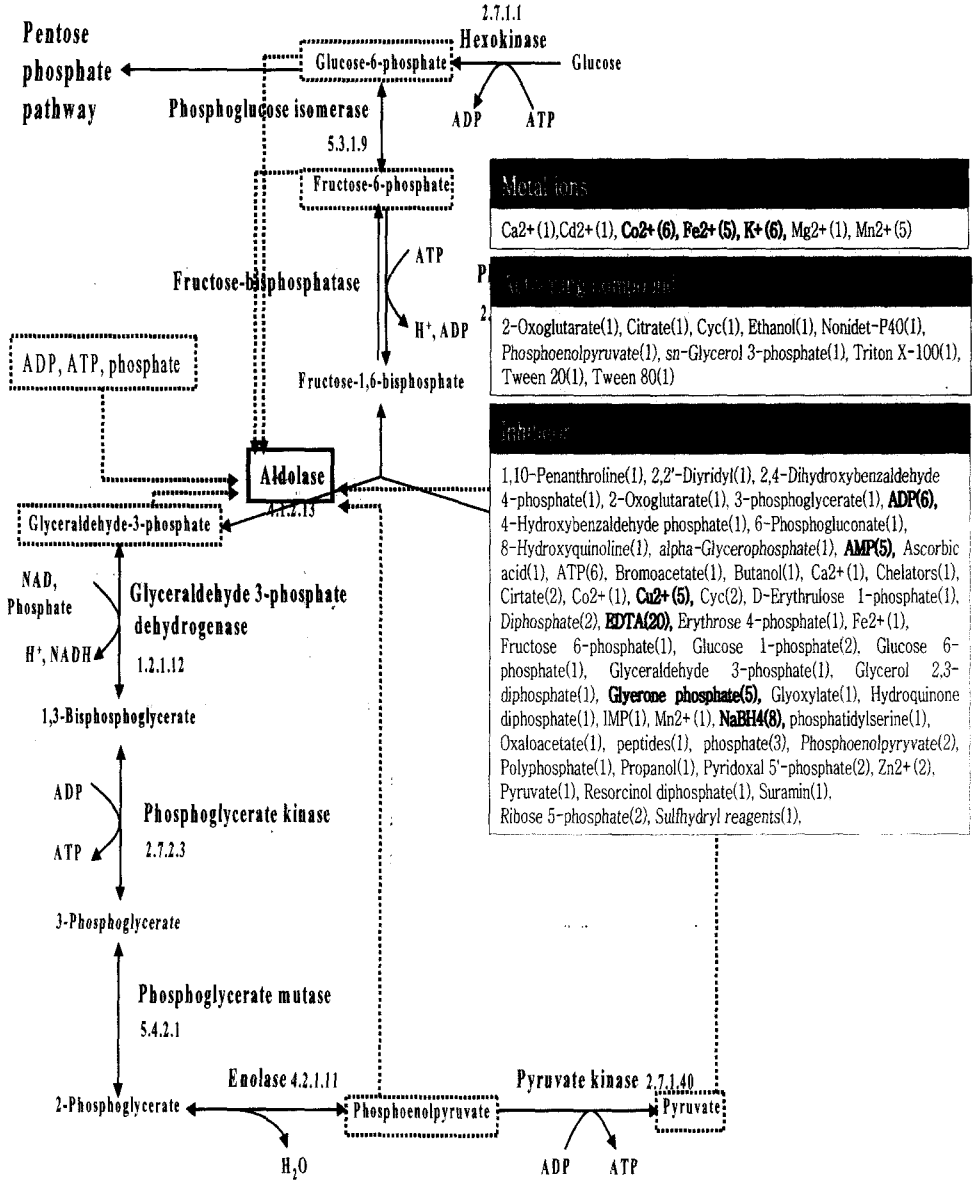


Fig. 7. Regulation of the aldolase (4.1.2.13) reaction step in the glycolysis metabolic network.

was inhibited by internal inhibitors such as glucose 6-phosphate, fructose 6-phosphate, glyceraldehyde 3-phosphate, phosphoenolpyruvate, and pyruvate. Zn^{2+} and Cu^{2+} could be operated as external inhibitors when their concentration gets to be above 0.5 mM [26]. Figure 7 shows the aldolase metabolic reaction step through this study. PEP (Phosphoenolpyruvate) is operated internal activating compounds. Ethanol could be external activating compounds when ethanol w/v came to be 10%. Citrate also could be external activating compounds when citrate concentration gets to a high level [24]. Fe^{2+}, K^{+}, Mg^{2+} were operated metal ions in the fourth reaction step [8, 12].

Fifth Step. This reaction type is a redox reaction, and it was catalyzed by glyceraldehyde-3-phosphate dehydrogenase. Glyceraldehyde-3-phosphate dehydrogenase reaction mechanism is where D-glyceraldehyde-3-phosphate and phosphate converted into 3-phospho-D-glyceroyl phosphate; here, 1 mol NAD consumption and 1 mol NADH production reactions occur. This reaction is the only step in glycolysis in which NAD^{+} is reduced to NADH. This reaction was inhibited by internal inhibitor, such as phosphoenolpyruvate which has feedback type inhibition. ATP and AMP could be operated as external inhibitors when ATP and AMP concentrations get to be above 1 mM

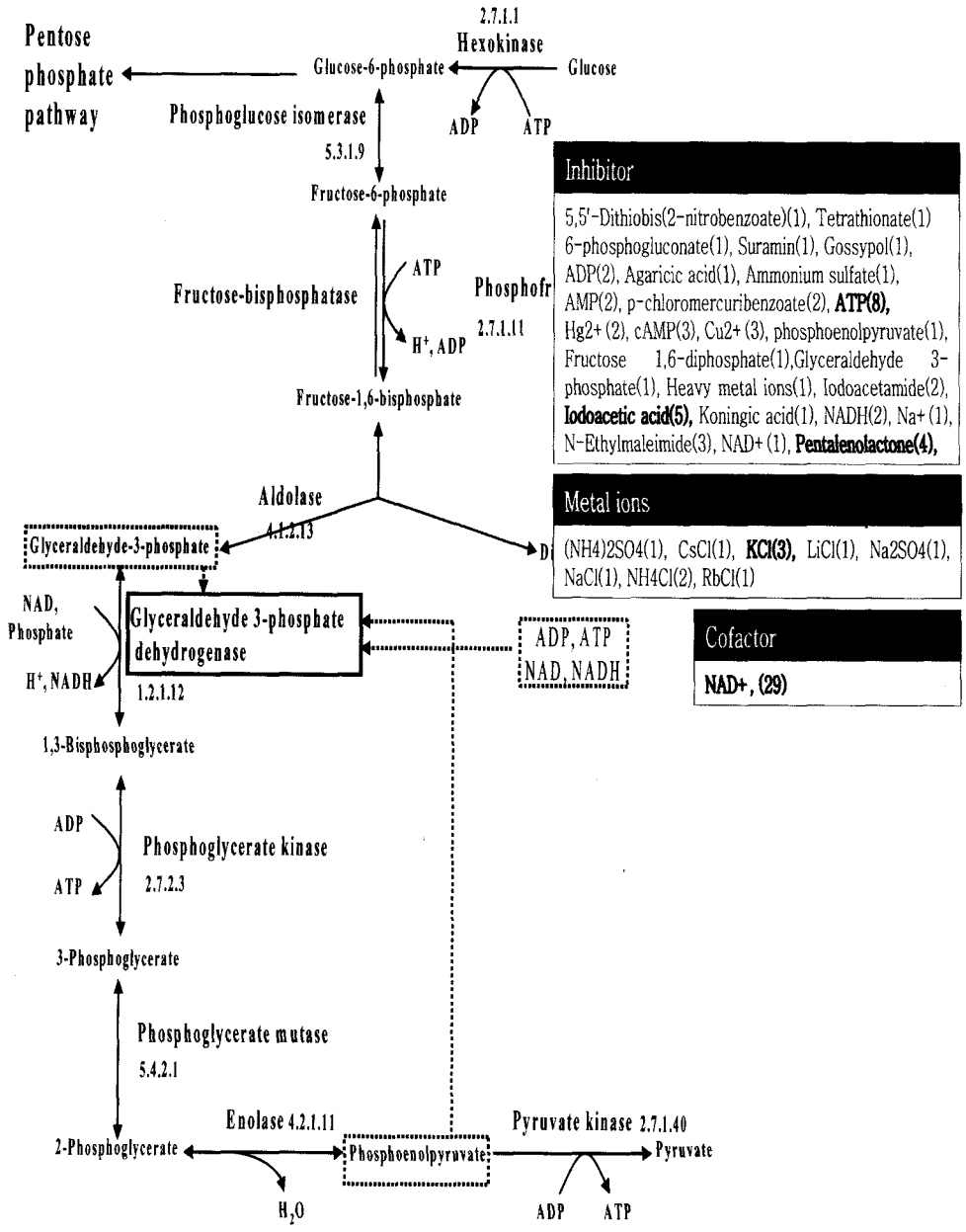


Fig. 8. Regulation of the glyceraldehyde 3-phosphate dehydrogenase (1.2.1.12) reaction step in the glycolysis metabolic network.

and 18 mM [9]. Figure 8 shows the glyceraldehyde-3-phosphate dehydrogenase metabolic reaction step through this study. Ethanol could be external activating compounds when ethanol concentrations increase 30% [4]. There was nothing to report to be influencing effector except metal ions such as NaCl in the fifth reaction step [12, 19].

Sixth Step. This reaction has the characteristic that it is a phospho group transfer, and it was catalyzed by phosphoglycerate kinase. Phosphoglycerate reaction mechanism is where 3-phospho-D-glycerate is converted into 3-phospho-D-glyceroyl phosphate with 1 mol ATP consumption and 1 mol ADP production. This reaction was inhibited by internal inhibitor such as 3-phosphoglycerate, and it has feedback type inhibition. Ca^{2+} could be an external inhibitor when Ca^{2+} concentration gets to be above 1 mM [21]. Figure 9 shows the phosphoglycerate kinase metabolic reaction step through this study. There was not influencing factors except metal ions such as Mg^{2+}, NaCl, and Ca^{2+} in the sixth reaction step [8, 12].

Seventh Step. Isomerization reaction type is the seventh reaction feature, and it was catalyzed by phosphoglycerate mutase. Phosphoglycerate mutase reaction mechanism is where 2-phospho-D-glycerate is converted into 3-phospho-D-glycerate. There were no inhibitors, or activating compounds except metal ions such as Mn^{2+} in the seventh reaction step [7, 12]. Figure 10 shows the phosphoglycerate mutase metabolic reaction step through this study.

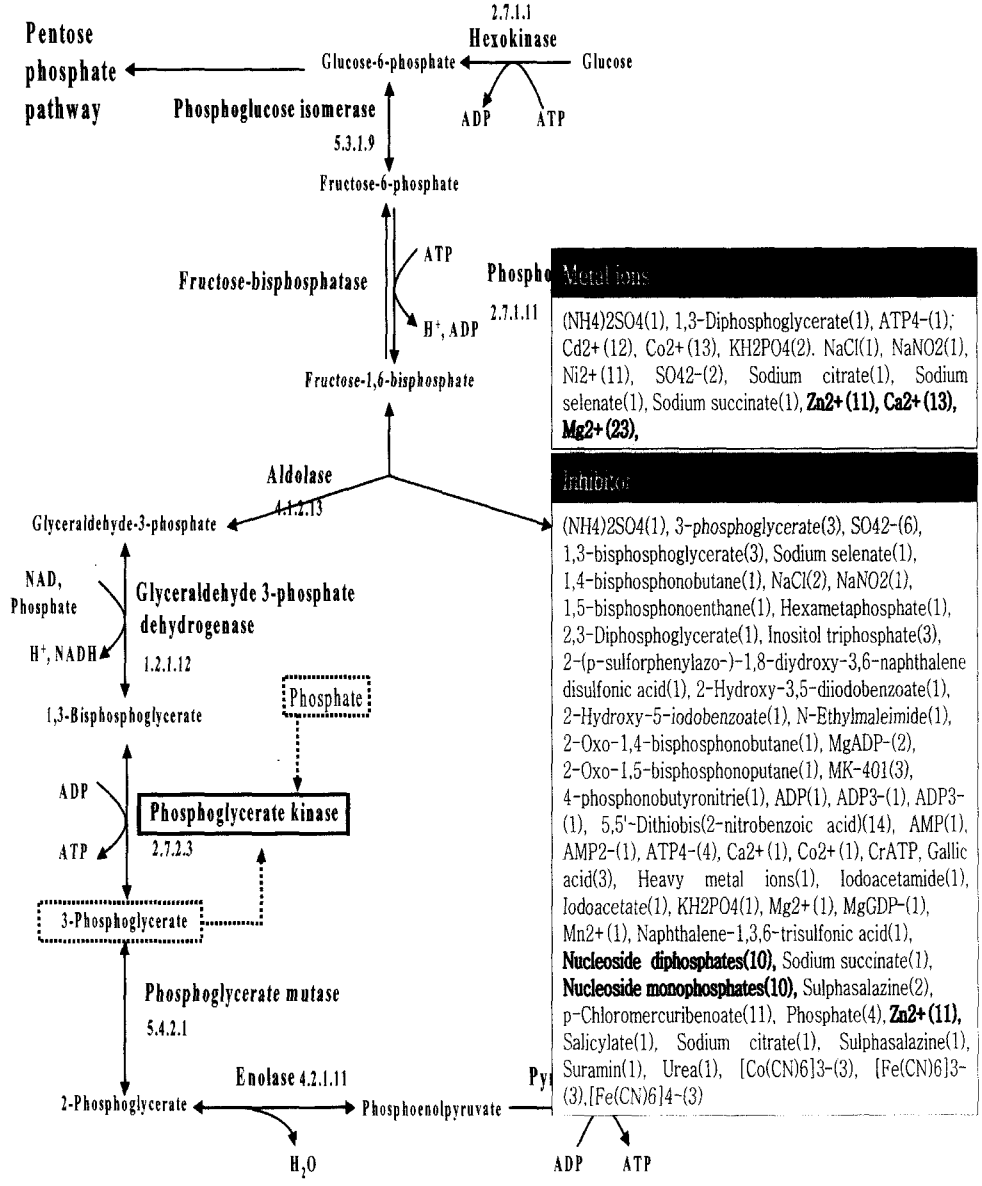


Fig. 9. Regulation of the phosphoglycerate kinase (2.7.2.3) reaction step in the glycolysis metabolic network.

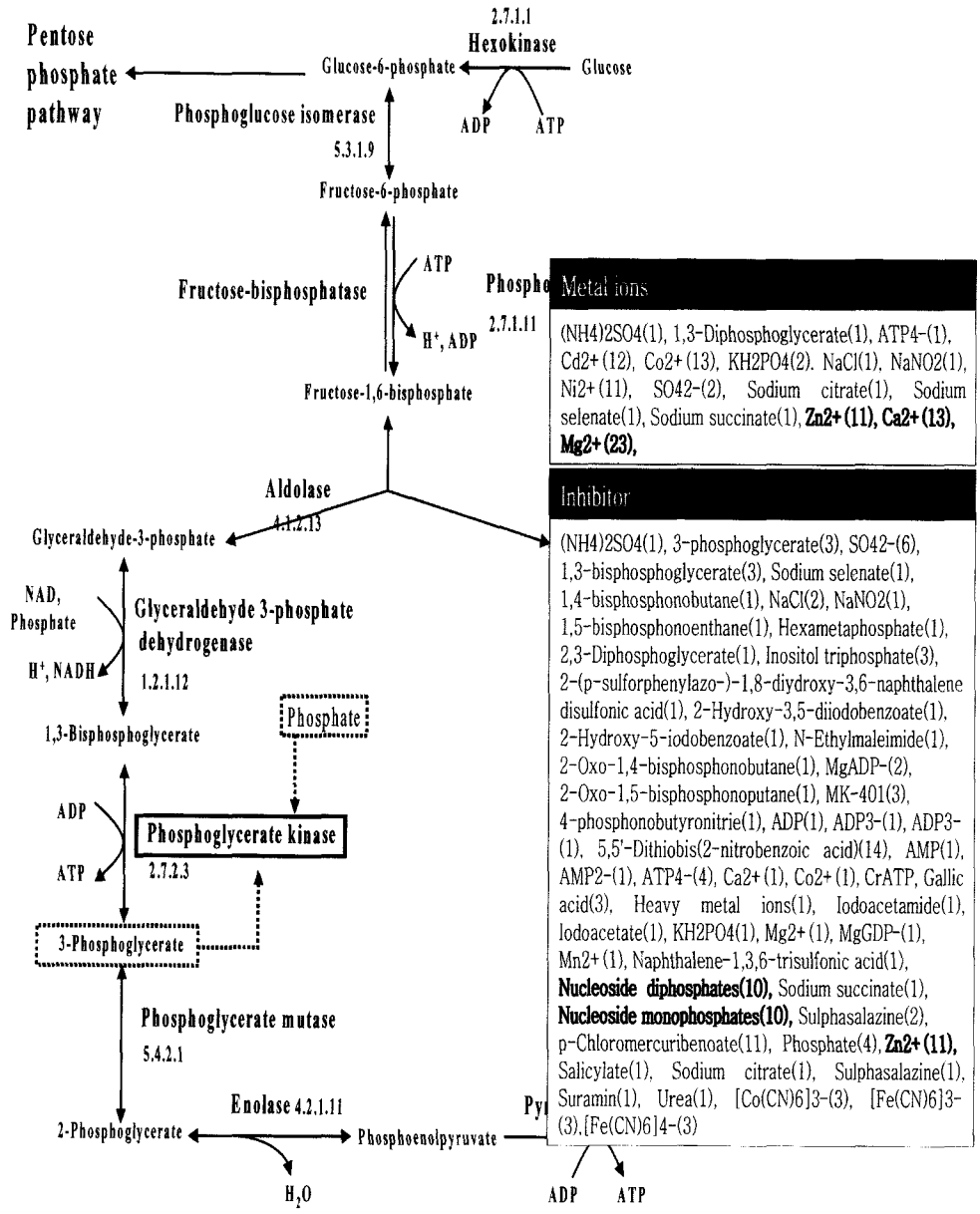


Fig. 10. Regulation of the phosphoglycerate mutase (5.4.2.1) reaction step in the glycolysis metabolic network.

Eighth Reaction Step. This enzyme reaction was catalyzed by phosphoenolpyruvate hydratase, and it has elimination and addition reaction types. Phosphoenolpyruvate hydratase reaction mechanism is where 2-phospho-D-glycerate is converted into phosphoenolpyruvate and H_2O. F^- could be the external inhibitor, when F^- concentration gets to be below 10 mM or above 10 mM, being of noncompetitive and competitive inhibition type, respectively [28]. Figure 11 show the phosphoenolpyruvate hydratase metabolic reaction step through this study. Phosphate is the activating compound which has a slight stimulation feature. Mg^{2+}, Mn^{2+}, Zn^{2+} were the operative metal ions in this reaction [6, 12].

Final Reaction Step. The final reaction has a phospho group transfer reaction type, and this reaction was catalyzed by pyruvate kinase. Pyruvate kinase reaction mechanism is where pyruvate is converted into phoshoenolpyruvate with 1 mol ATP consumption and 1 mol ADP production. This reaction was inhibited by internal inhibitors such as glucose-6-phosphate, 3-phosphoglycerate, 2-phosphoglycerate, and pyruvate. Specifically, glucose-6-phosphate is the internal inhibitor at high concentration. Glucose 6-phosphate, 3-phosphoglycerate, and 2-phosphoglycerate have feedback type inhibition and pyruvate has feedforward type inhibition [3]. Figure 12 show the pyruvate kinase metabolic reaction step through this study. Glucose 6-phosphate, glyceraldehyde-

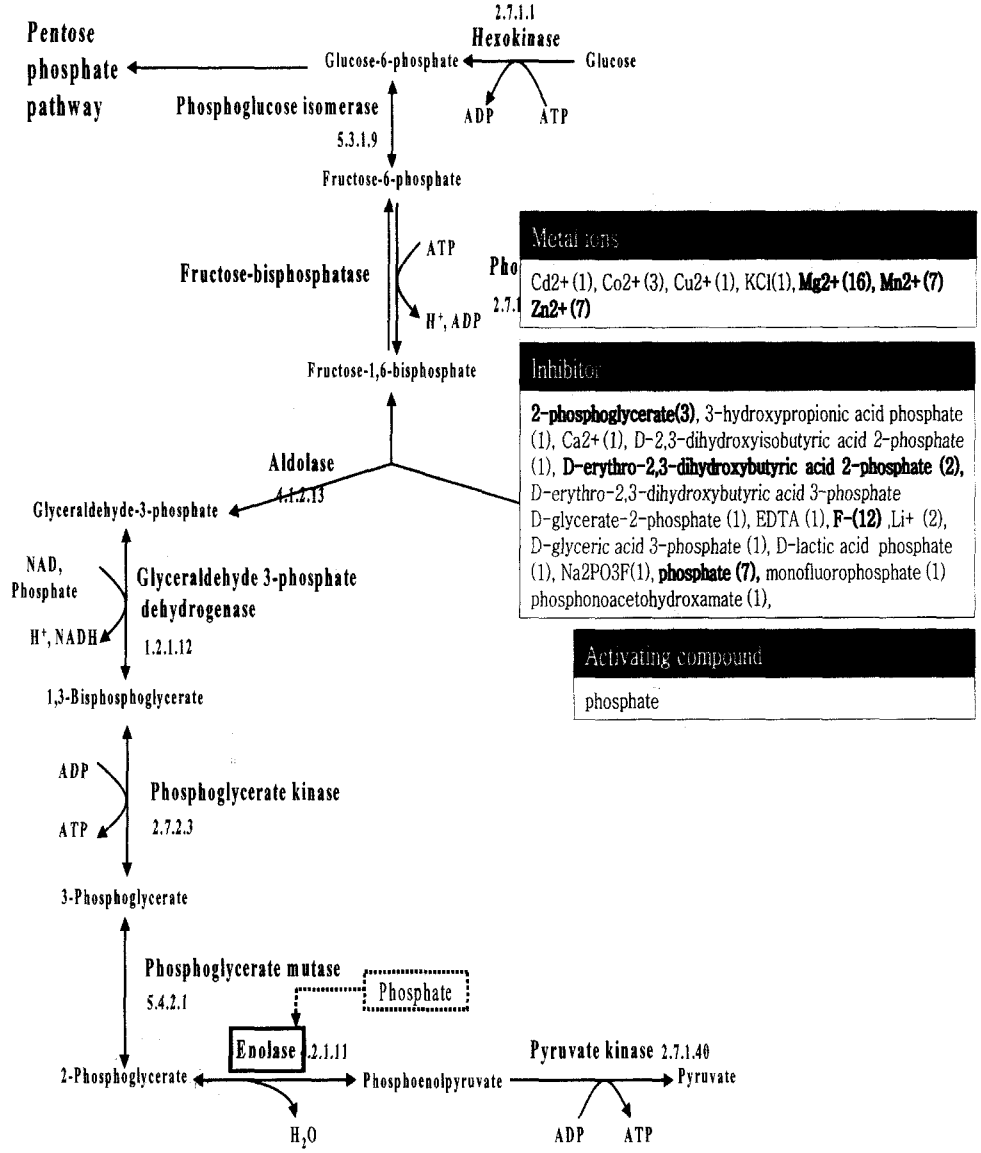


Fig. 11. Regulation of the enolase (4.2.1.11) reaction step in the glycolysis metabolic network.

3-phosphate, fructose-1,6-diphosphate were operated activating compounds. Glucose 6-phosphate and glyceraldehyde-3-phosphate were observed to have slight activation, fructose-1,6-diphosphate was observed to have strong activation [27]. Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+} operated as metal ions in the final reaction step [8, 12].

Here, we believe we have established more precise regulatory metabolic mechanisms for the glycolysis metabolic network through the study of these enzyme reactions [22].

CONCLUSION

Finally, we could achieve modeling of the metabolic network and analyzing of metabolic mechanisms in the glycolysis metabolic network. The constructed glycolysis metabolic network was composed of one branch point, substrate (glucose), ten metabolites, and nine enzyme reactions which could separate to seven reversible reactions and two irreversible reactions. Our research has two distinct points; one is a try to more strictly construct the glycolysis metabolic network from an overall analysis of reactions and mechanisms. The other point is that we have considered the influence of effectors in the glycolysis metabolic network. From Figs. 3 to 12, we show the progress of enzyme reaction steps in the glycolysis metabolic network. Moreover, our newly constructed metabolic network contains the major reaction mechanisms showing inhibitory controlling loops and activating loops. Therefore, we can effectively consider the influence of inhibitors and activating compounds in individual enzyme

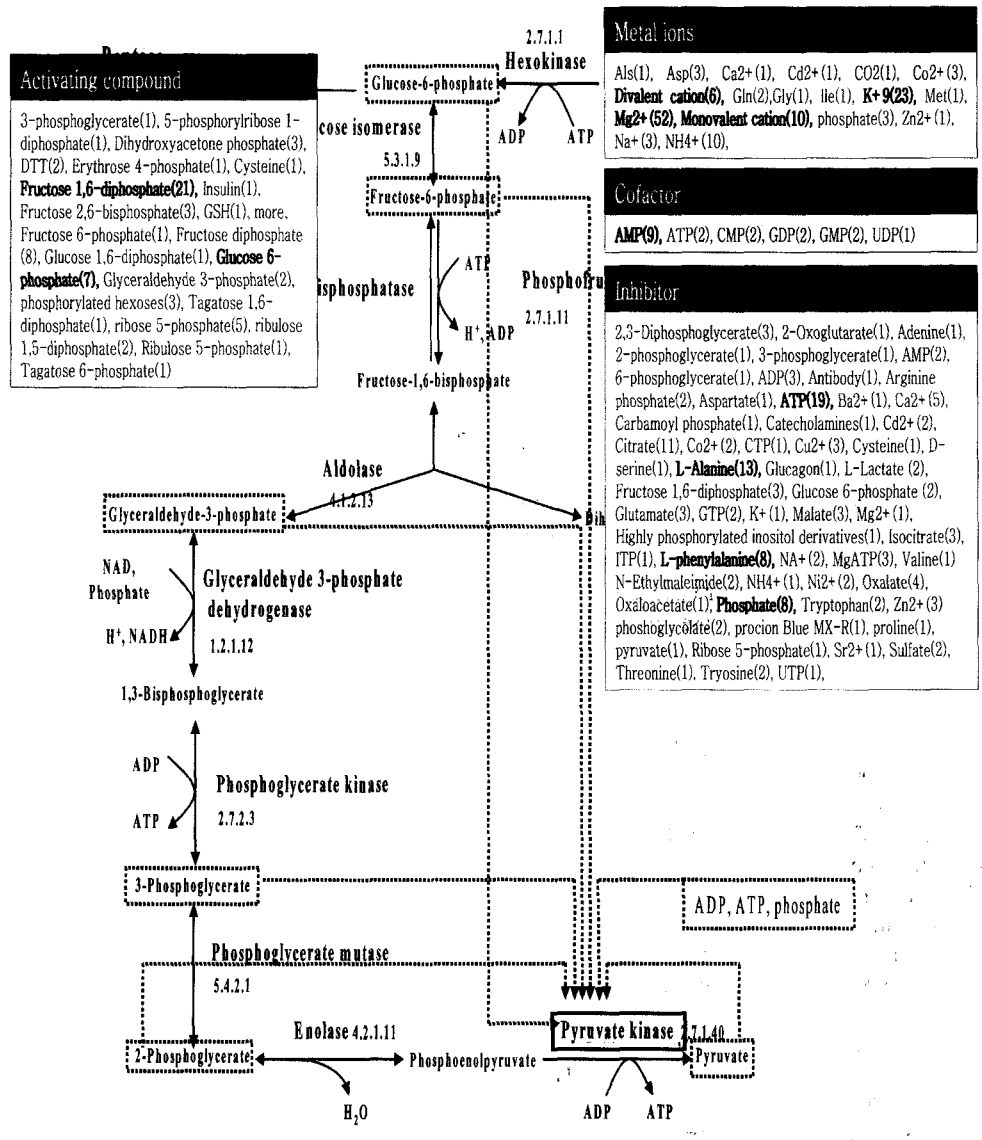


Fig. 12. Regulation of the pyruvate kinase (2.7.1.40) reaction step in the glycolysis metabolic network.

reactions. Furthermore, it can give us useful information to analysis of the glycolysis system. One is analysis of flux distribution and the other is the study of enzyme kinetics.

There are many databases and literatures which serve information for the glycolysis metabolic network and mechanism. But most data obtained from these databases and literatures have many differences in types, species, experimental conditions, and analytical methods. Moreover, these data sources do not consider the influence of effectors and variable environmental condition. Therefore, it was needed to examine more carefully, to collect biosynthetic information to construct the glycolysis metabolic network. We have tried to construct a strict glycolysis metabolic network in this study. Finally, we have achieved a new construction of the glycolysis metabolic network and the collection of important effectors through this study.

Acknowledgments

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