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High selective isomerization of glucose into fructose catalyzed by a mimic glucose isomerase

Ni Zhang,[a] Xiang-Guang Meng,*[a] Yan-Yan Wu,[a] Hong-Jin Song,[a] Hong Huang,[a] Fei Wang,[a] and Jing Lv[a]

Abstract: An amphoteric phenolic compound with carboxyl and amino functional groups was synthesized and used as mimic glucose isomerase to catalyze the isomerization of glucose into fructose. The mimic enzyme displayed excellent catalytic activity for isomerization of glucose to fructose under mild conditions. The yield of fructose and selectivity of fructose could reach 33.0% and 93.5% after reacting 4 h, respectively, at pH 8.5 and 80 °C. The catalysis reaction rate constant k_{cat} and Michaelis constants K_{mG}, K_{mF} were calculated. The activation energy of glucose to fructose was evaluated as 65.9 kJ mol^{-1}. The possible catalysis reaction mechanism was proposed. The acid-base groups on the catalyst play an important role in proton extraction and transfer, as well as in the rotation of local carbon chain, which leads to the highly selective isomerization of glucose into fructose under mild conditions.

Introduction

The utilization of renewable lignocellulosic biomass in the world is extremely important to solve the problems of resource shortage, for example fossil fuels depletion and the pollution of environment,[1-3] and glucose is the main product of degradation of biomass. Compared with glucose, fructose is easier to convert into important platform compounds such as hydroxylmethylfurfural (HMF), levulinic acid, and other versatile platform chemicals and liquid fuels.[4,5] Therefore, conversion of glucose into fructose was regarded as a key intermediate step in biomass valorization.[6] In past years, considerable efforts have been devoted to improving yields and selectivity of fructose by investigating various homogeneous and heterogeneous catalytic systems. Early investigations for the isomerization of glucose involved the utility of soluble alkalis, such as sodium hydroxide and calcium hydroxide at high pH values and room temperature.[7-9] Under these conditions, the reaction suffered from a low rate of isomerization and numerous byproducts. To improve the selectivity of fructose, an alternative method was to replace inorganic bases with organic bases, for example triethylamine. Recently Tessonnier et al. reported several organic amines catalyzed isomerization of glucose to fructose. As a result, yield of 17-31% and selectivity of 43-62% were obtained with 10 mol% catalyst at 100 °C.[10] For separation and easy recovery of catalyst, many natural and man-made solid materials such as hydrotalcites,[11,12] immobilized amines,[13] zeolites in alkaline-exchange form,[14] mesoporous ordered molecular sieves of the M41S family,[15] zirconium carbonate,[16] and anion-exchanged resins[17] have been reported as efficient solid base catalysts. Michailof et al. used MgO as heterogeneous catalyst for isomerization of glucose into fructose, leading to 44% conversion of glucose and 75.8% selectivity of fructose were obtained in H_2O solvent. The addition of organic solvents DMSO, DMA and sulfolane significantly reduced the selectivity of fructose.[18,19] Many Lewis acids catalysts were also employed for the isomerization reaction, such as $CrCl_3$, $AlCl_3$ and $SnCl_4$.[20] The successful applications of materials containing Ti, Sn β-zeolites and mesoporous silica were reported.[21-23] It is desirable to use solid heterogeneous catalysts in industrial large-scale processes due to the advantage of the easy recycling and reuse.[18] Recently, Saravanamurugan and Tsapatsis proposed an alternative route to achieve high yield fructose through combining glucose isomerization, fructose ketalization and fructosidehydrolysis.[24,25] Although considerable efforts have been devoted to the isomerization of glucose to fructose in the past years, the selectivity in fructose was usually less than 75%, which is particularly unsatisfactory.[20]

Glucose isomerase which catalyzes aldoses to ketoses, was found in various biological microorganisms, such as *Streptomycessp., S. rubiginosus, A. missouriensis, Thermus neapolitana*, etc. The enzymatic isomerization of glucose into fructose has attracted much attention beacuse of the highly selectivity and yield of fructose.[26] An sweetener high-fructose corn syrup is produced via bioconversion approach using immobilized glucose isomerase at 58 to 60°C, the process gives 43% yield of fructose.[27] Phosphoglucose isomerase (PGI; EC 5.3.1.9), one glucose isomerase, catalyses the interconversion of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P). The crystal structure of PGI from the mouse phosphoglucose isomerase at 1.6 Å has been determined, which core fold of protomer and the interprotomer spatial arrangement of the dimer are similar to those of already reported crystal structures of other PGIs.[28,29] The active site involved in isomerization comprises highly conserved residues, including glutamic acid, histidine, lysine and arginine residues. In the enzyme catalyzed isomerization of glucose into fructose, several steps are involved as follows: (i) ring opening of glucose; (ii) formation of a cis-enediol intermediate by abstracting proton on C2 of glucose; (iii) transfer of proton; (iv) ring closure of chain fructose. The residues histidine and lysine were thought to be related to the ring opening and closing of monosaccharides, and residues glutamic

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acid and arginine to proton abstraction and transfer, respectively.[28,30] Despite the high activity and selectivity of natural enzymes, the use of natural enzymes is too expensive due to the difficulties in isolation and purification and the sensitivity to environmental conditions.[31]

Here we constructed a novel compound to mimic the PGI. The carboxyl, phenolic and positive groups in the active site of PGI were introduced as illustrated in Figure 1. The synthesized catalyst 2,2',2'',2'''-(((2-hydroxy-5-methyl-1,3-phenylene)bis(methylene))-bis(azanetriyl))tetraacetic acid (BBTA) was employed to catalyze the isomerization of glucose to fructose. The catalyst displayed great catalytic activity for the isomerization reaction, as well as high selectivity in fructose in aqueous solution at relative low temperature (70-90 ºC).

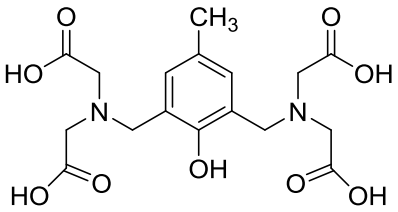


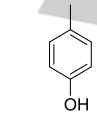
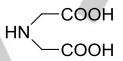
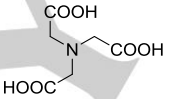
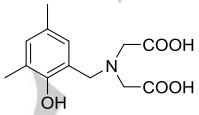
Figure 1. The chemical structure of BBTA.

Results and Discussion

Comparison of various catalytic systems

Glucose is difficult to be conversed to fructose in a neutral aqueous solution. In previous reports, the non-enzyme catalyzed isomerizations of glucose to fructose were generally carried out under acidic or alkaline conditions. In this work, we focused on the nearly neutral and low temperature conditions for the isomerization of glucose. In glucose isomerase, carboxyl, amino, imidazole and phenolic groups were generally found in the catalytic active domain. To design and construct a mimic isomerase, some compounds with the groups above were selected and their catalytic ability for the isomerization of glucose into fructose was studied and the results were listed in Table 1. From Table 1 it can been found that only 3.7% of glucose could be conversed after reacting 4 h at pH 8.5 and 80 ºC in the absence of any catalyst. It was also observed that both single functional compounds (acetic acid and *p*-methylphenol) and bifunctional groups compounds (iminodiacetic acid and aminotriacetic acid) showed weak catalytic activity of isomerization of glucose, and the conversion of glucose was less than 10%. Interestingly, the macromolecule composed of carboxyl, amino and phenol groups exhibited excellent catalytic performance for the isomerization reaction. From Table 1 it can be seen that 2,2'-((2-hydroxy-3,5-dimethylbenzyl)azanediyl)-diacetic acid exhibited much better catalytic performance compared with single functional and bifunctional groups compounds, which indicated good synergistic effect among the functional groups. The yield of fructose reached 33.0% in BBTA catalyzed system for a reaction time of 4 h at pH 8.5 and 80 °C, which is even higher than 2,2'-((2-hydroxy-3,5-dimethylbenzyl)-azanediyl)diacetic acid, but less than twice. This indicated that phenol group played an important role in the catalytic process. It is noted that for the BBTA catalyzed system, the selectivity of fructose reached 93.5%.

Table 1. Conversion of glucose and selectivity of fructose in various systems[a]

Catalyst	Conversion of glucose / %	Yield of fructose / %	Selectivity of fructose / %
Bulk solution	3.7	3.3	89.2
CH_3COOH	7.6	7.2	94.7
	8.2	7.8	95.1
	4.5	4.3	95.6
	9.0	8.2	91.1
	20.5	22.7	90.3
BBTA	35.3	33.0	93.5

[a]$[glucose]_0$ = 0.2775mol L^{-1}, [catalyst] = 0.014mol L^{-1}, pH 8.5, 80 °C, 4h.

Influence of solvent

To investigate the influence of solvent, the isomerization reaction catalyzed by BBTA was carried out in several mixed solvents. The experimental results were listed in Table 2.

Table 2. Conversion of glucose and yield of fructose in various solvents[a]

Solvent	Conversion of glucose / %	Yield of fructose / %	Selectivity of fructose / %
H_2O	35.3	33.0	93.5
THF-H_2O	3.4	3.1	91.2
DMF-H_2O	6.0	5.0	83.3
DMSO-H_2O	6.5	5.2	80.0

[a]The ratio of mixed solvents is 1:1(volume ratio). $[glucose]_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}, pH 8.5, 80 °C, 4h.

From Table 2 it can be seen that solvents cause a significant effect on the isomerization reaction. When organic solvent THF, DMF or DMSO was added to the aqueous solution, the conversion of glucose and yield of fructose decreased significantly. It seemed that the larger polarity of the solvent, the greater conversion of glucose and the yield of fructose. The order is H_2O>>DMSO>DMF>THF, which is related to the proton transfer ability of solvents. This implied that the isomerization reaction involved a proton transfer process. In the cases of other ratios of organic solvent to water, such as 7:3 and 3:7, the conversion of glucose and yield of fructose were also in line with the order. It is noted that the addition of organic solvent not only reduced the reaction rate, but also reduced the selectivity of fructose. Therefore, aqueous solution is the best medium for this work's catalytic system.

Effect of pH

Natural glucose isomerases generally exhibit the best catalytic activity in weak alkaline solution, with the optimal pH of 7 to 9.[32,33] In order to achieve high conversion of glucose, the non-enzymatic isomerizations of glucose to fructose were usually carried out under acidic or alkaline conditions. However the selectivity of fructose was low.[7-9,22]

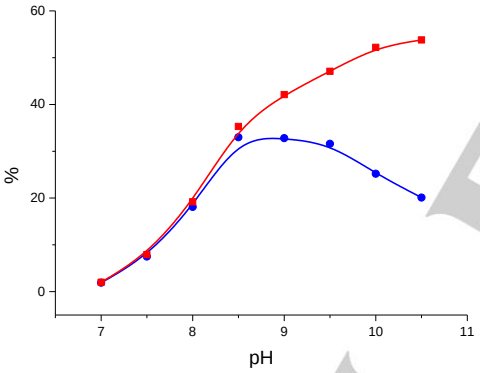


Figure 2. Plots of conversion of glucose (■) and yield of fructose (●) versus pH at 80 °C for 4h. $[glucose]_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}.

From Figure 2 it can be seen that in the presence of BBTA, pH effect was obvious. The conversion of glucose increased with increasing pH and arrived 53.8% for reaction of 4 h at 80 °C. It can be further observed that the variation of the fructose yield was different from glucose conversion. Firstly the yield of fructose rapidly increased with increasing pH and up to a yield of 33.0% and selectivity of 93.5% at pH 8.5. Subsequently the yield decreased slowly in the range of pH 8.5 to 9.5. Although this phenomenon is unusual, it was also observed in isomerization reaction catalyzed by natural enzyme *Thermus oshimai* (ToGI).[32] Then the yield and selectivity of fructose decreased rapidly with increasing pH.

It was noted that the BBTA catalyzed system gave a satisfactory selectivity of fructose (S>93%) in the range of pH 7.0 to 8.5, which compared with the value 8.0 of optimal pH of nature *Streptomyces* enzyme and *Bacillus* enzyme.[34]

Effect of temperature

The isomerization of glucose into fructose is slow due to the relatively large reaction active energy under neutral aqueous solution, so the isomerization reactions were usually carried out at high temperatures (T>100 ºC) to speed up the reaction process.[20] However, glucose and fructose are unstable and easy to decompose and result in the low selectivity (S<70%) at high temperature. In this work, to avoid a low selectivity of fructose, the reaction of glucose isomerization was carried out in the range of temperature 70-90 ºC. Figure 3 illustrates the influence of temperature on both conversion of glucose and yield of fructose. It can be seen that high temperature promotes the reaction rate of conversion of glucose. However we further observed that the yield of fructose reached a maximum in 3-5 h, and the higher the temperature, the earlier the maximum appeared (Figure 3(B)). This implied that two processes involving generation and decomposition of fructose occurred simultaneously during the catalytic reaction. Expectedly, the effect of temperature on reaction rate of the generation and decomposition of fructose is different. The maximum yield of fructose was observed at 80 ºC and reaction 4h.

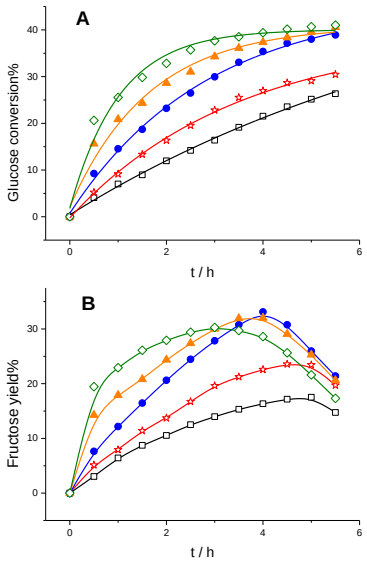


Figure 3. Conversion of glucose (A) and yield of fructose (B) with time at pH 8.5 at different temperature. (□)70°C, (☆)75°C, (●)80°C, (▲) 85°C, (◇) 90°C. $[glucose]_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}.

Products analysis and reaction kinetics

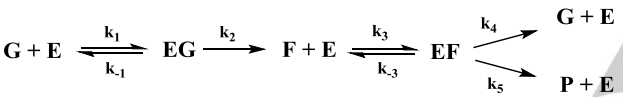
To explore the products of isomerization reaction, HPLC, chromatographic analysis and GC-MS were employed. The

results showed that besides fructose, there were three other products including mannose, 1,3-dihydroxyacetone and HMF in the reaction solution. The products 1,3-dihydroxyacetone and HMF may be derived from the further reaction of fructose，and the mannose may come from the isomerization of glucose.[35] The content of mannose, 1,3-dihydroxyacetone and HMF in reaction solution were listed in Table 3. Based on these observations, the catalytic reaction kinetic model of glucose isomerization was suggested in Scheme 1.

Table 3. Conversion of glucose and distribution of products[a]

Reaction time / h	Conversion of glucose / %	Yield / %			
		Fructose	1,3-dihydroxyacetone	HMF	Mannose
1	14.5	14.1	0.2	0.08	0.1
2	23.2	22.1	0.5	0.1	0.3
3	30.0	28.2	0.7	0.11	0.9
4	35.4	33.0	0.9	0.12	1.2

[a][glucose]$_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}, pH 8.5, 80°C.



Scheme 1. The proposed kinetic model of catalysis reaction.

According to our experimental results, a kinetic reaction pathway for isomerization of glucose was proposed: in terms of kinetics, the binding process of glucose and catalyst should not be ignored because of the larger molecules of glucose and catalyst. Hence substrate glucose G first reversibly combines with catalyst E to generate an intermediate compound EG, whose reversible rate constants are k_1 and k_{-1}, respectively. Then EG reacts into fructose F and releases catalyst E, which is the rate-limiting step and the rate constant is k_2. Consequently, fructose F combines with catalyst E to form another intermediate EF, whose reversible rate constants are k_3 and k_{-3}, respectively. Lastly, the intermediate EF generates G and release E, and also yields by-products P. Based on the theory of reaction kinetics, the kinetic equations (1) and (2) were deduced, the detailed derivation process is described in the Supplementary material. Equation (1) represent the relationship between time t and glucose concentration [G]. Equation (2) represent the relationship between time t and fructose concentration [F].

$$t = \frac{[a_1a_2 - (a_1 - k_4E_TK_{mG}S_aC_0)a_1k_4E_T - a_2a_3a_4k_4E_T]}{a_1a_4k_4E_T}\ln\left(\frac{a_1[G] + a_2}{a_1C_0 + a_2}\right) + a_3([G] - C_0) \quad (1)$$

$$t = -\frac{(a_5K_{mG} + a_5C_0 - a_6a_7K_{mG})}{{a_5}^2}\ln\left(\frac{a_7 - a_6[F]}{a_7}\right) + \frac{a_6}{a_5}[F] \quad (2)$$

where $a_1=(k_{-1} - k_4 - k_1K_{mG})E_TK_{mF} + k_4E_TK_{mG}S_aC_0$, $a_2=(K_{mF} - K_{mG}S_a)k_4E_T$, $a_3=\frac{(K_{mG}K_{mF}+K_{mG}S_aC_0)}{k_4E_TK_{mG}S_aC_0}$, $a_4=k_4E_TK_{mG}S_aC_0$, $a_5=\frac{k_2E_T}{K_{mG}S_a} + \frac{(k_4+k_5)E_T}{K_{mF}}$, $a_6=\frac{1}{K_{mF}} - \frac{1}{K_{mG}S_a}$, $a_7=\frac{k_2E_TC_0}{K_{mG}}$. $K_{mG} = \frac{k_{-1}+k_2}{k_1}$, $K_{mF} = \frac{k_{-3}+k_4+k_5}{k_3}$ are the Michaelis constants for the first and second reaction, respectively. $k_2 = k_{cat}$， and E_T is the total concentration of catalyst. S= [F]/(C_0-[G]), where C_0 is the initial concentration of glucose. S represents the selectivity of fructose. Under this work's conditions, the value of S changed little within 4 h reaction and approximating a constant S_a. The products increase gradually with reaction proceeding and the products may influence the reaction rate. To avoid this interference, we used the data of initial reaction time of 3 h to evaluate the values of k_{cat}, K_{mG} and K_{mF} in this work. K_{mG}, K_{mF} and k_{cat} were obtained by nonlinear fitting according to kinetic equations (1) and (2). The calculated results are listed in Table 4. From Figure 4 it can be found that the theoretical fitting curves are in good agreement with the experimental data, and both the multiple correlation coefficients of glucose conversion and that of fructose yield were all above 0.97. This indicated that the reaction kinetic model proposed as Scheme 1 was reasonable.

Table 4. K_{mG}, K_{mF} and k_{cat} of glucose isomerization catalyzed by BBTA[a]

T / °C	70	75	80	85	90
K_{mG}/mol L^{-1}	0.07	0.16	0.21	0.28	0.46
K_{mF}/mol L^{-1}	0.05	0.13	0.19	0.23	0.30
10^4k_{cat}/s^{-1}	1.05	1.38	1.98	2.73	3.71

[a][glucose]$_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}, pH 8.5.

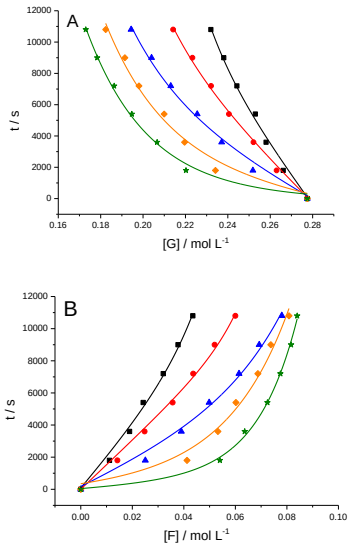


Figure 4. Plots of reaction time vs. concentration of glucose (A) and fructose (B). [glucose]$_0$ = 0.2775mol L^{-1}, [BBTA] = 0.014mol L^{-1}, pH 8.5. (■)70°C, (●)75°C , (▲)80°C, (◆)85°C, (★)90°C. Solid lines in A and B are the theoretical fitting curves based on equations (1) and (2), respectively.

Effect of temperature

It can be seen from Table 4 that values of K_{mG} and K_{mF} were all relatively small, which showed that the glucose and fructose have obvious association with catalyst in the kinetic reaction process. Compared with those values of 0.14-1.02 in natural enzyme catalyzed isomerization systems,[36-38] the range of values of K_{mG} and K_{mF} calculated in this work is consistent with that of natural enzyme. Although the reaction rate constants kcat were 10^2-10^6 folds smaller than those catalyzed by natural glucose isomerase,[36,37] the catalyst BBTA displayed considerable catalytic activity and excellent selectivity of fructose under mild reaction conditions.

Since the values of k_{cat} at various temperature were obtained, the activation energy E_a and pre-exponential factor could be evaluated by the Arrhenius plots of ln k_{cat} vs. 1/T, as illustrated in Figure 5. The activation energy and pre-exponential factor of glucose isomerization were calculated as 65.9 kJ mol^{-1} and 1.09×10^6, respectively. The enzyme catalyzed reaction activation energy of glucose isomerization were reported in the range of 35-75 kJ mol^{-1},[38-40] while those E_a of nonenzyme catalyzed isomerization were in the range of 30-121 kJ mol^{-1}.[41] The low reaction activation energy under mild condition indicates that this is an efficient catalytic system for the isomerization of glucose into fructose.

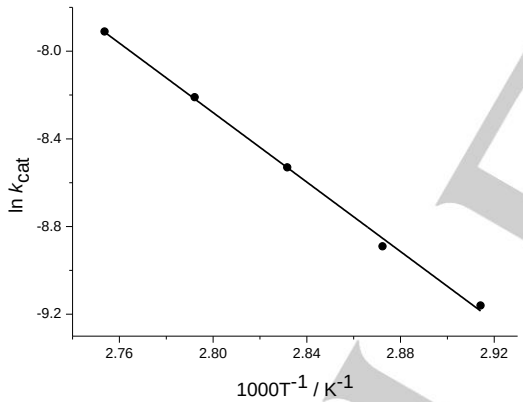
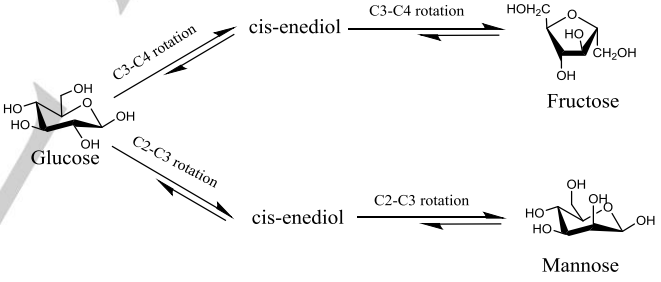


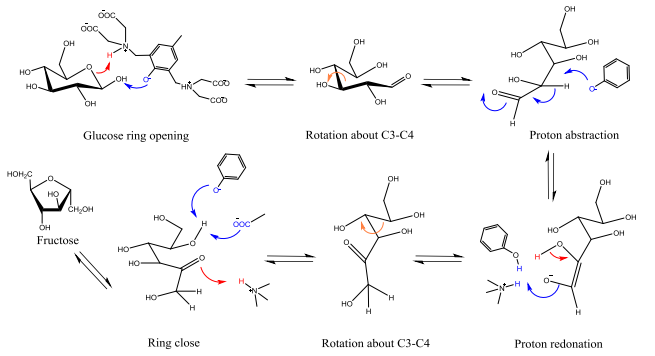
Figure 5. Plot of ln k_{cat} vs. 1/T for glucose isomerization. [BBTA]=0.014mol L^{-1}, pH 8.5.

Possible catalytic mechanism

In enzyme or non-enzyme catalyzed reaction, cis-enediol was believed as the intermediate in isomerization of glucose into fructose, and which formation is the rate-limiting step.[26,42] In this work, the catalyst contains several functional groups which may play different role in catalysis reaction. The values of pKa of these functional groups were determined by titration analysis. The values of pKa of the mono compound 2,2'-((2-hydroxy-3,5-dimethylbenzyl)azanediyl)diacetic acid were evaluated as 2.4 and 6.4 of the carboxylic acids, 8.7 of the phenol and 10.4 of the tertiary amine. The pKa of the BBTA on one side of benzene ring were evaluated, the values of the carboxylic acids were 2.3 and 6.5, respectively, and pKa of the phenol and tertiary amine were evaluated as 8.9 and 10.7, respectively, in aqueous solution at 25 ºC. The experimental results show that the groups on both sides of the benzene ring of catalyst are independent and do not affect each other. Hence, in aqueous solution of about pH 8.5, the phenolic group exists in both neutral and anionic forms, carboxyl in anionic form and tertiary amine in cationic form. Negative carboxyl and phenoxy anions could play important roles in abstracting proton. Although carboxyl groups are considered to be the key to hydrogen extraction in isomerization catalyzed by natural enzyme, in this work it is likely that phenoxy group plays a major role in hydrogen extraction, which can be evidenced by the obvious change of yield of fructose near pH 8 (Figure 2). Tertiary amine carrying a proton may play role in proton transfer, as neutral phenoxy group acts. Furthermore, the electric field can be formed between the positive and negative groups, which can induce obviously the substrate atoms and lead to the rotation of carbon chain, such as C2-C3 and C3-C4, and this promoted the generation of products fructose (rotation on C3-C4) and mannose (rotation on C2-C3). The electrical effect can be supported by a reduction of the glucose conversion by adding inorganic salts (Table S1). Based on the analyses above and experimental data, a possible catalysis mechanism was proposed as illustrated in Scheme 2 and Scheme 3.



Scheme 2. Possible reaction pathways.



Scheme 3. Propose catalytic mechanism for the generation of fructose.

The catalytic mechanism of glucose into fructose may involve the following processes: ring-opening of glucose, rotation about C3-C4, loss and gaining of proton (formation of cis-enediol),

rotation about C3-C4 again and ring-closure of fructose. The ring- opening of glucose and the ring-closure of fructose may due to the transfer of proton by the combining action of positive tertiary amines and negative carboxyl and phenoxy. The formation of intermediate cis-enediol is attributed to the extraction of hydrogen from C2 by phenoxy anion and subsequent proton delivery from tertiary amine or phenol. The C3-C4 may occur two times before and after the formation of intermediate cis-enediol, respectively. The rotation makes the formation of both cis-enediol and chain fructose in a more favorable state. It is noted that the rotation of C2-C3 would result in generation of mannose, this has been supported by products of reaction in this work. It is obvious that catalysts with positive and negative groups cause the easily rotation of substrate chains, and this results in the good selectivity of fructose. As far as we know, this is the first example to achieve fructose with a selectivity of more than 90% for the isomerization of glucose in non-enzymatic catalytic system under mild conditions.

Conclusions

In this work, a novel catalyst with multiple groups was designed and synthesized and used as a mimic glucose isomerase. The mimic isomerase system exhibited a great catalytic activity for the isomerization of glucose into fructose in weakly alkaline aqueous solutions at relatively low temperature (70-90 °C). The high selectivity (S>93%) of fructose was achieved before 4 h reaction time. The functional groups phenoxy, carboxyl and tertiary amino showed an obvious synergistic effect on the isomerization reactions. The kinetic model of catalysis reaction was suggested, and the catalysis rate constant k_{cat} and Michaelis constants K_{mG} and K_{mF} were calculated. The activation energy E_a= 65.9 kJ mol^{-1} for the isomerization of glucose into fructose was evaluated. This work will provide a new technology and method for the isomerization of glucose into fructose under mild conditions.

Experimental Section

Materials

Iminodiacetic acid, paraformaldehyde, p-Cresol, fructose, glucose, mannose, 1,3-dihydroxyacetone, HMF , 2,4-dimethylphenol were purchased from Adamas Company. Sodium chloride, sodium hydroxide, sodium sulfate potassium iodide were purchased from Kelong reagent Company. Solvents Tetrahydrofuran (THF), Dimethylac-etamide (DMA), Dimethylsulfoxide (DMSO), methanol, hydrochloric acid and formaldehyde were purchased from Kelong reagent Company. Acetonitrile was purchased from Adamas Company, chromatographic pure grade. All above reagents were used without further purifcation.

Instruments

NMR (AM-400, Bruker, Switzerland), Elemental Analyzer (Euro-EA-3000, America), UV-5300 spectrophotometer (Yuanxi Comp-any, China), High performance liquid chromatography (HPLC) (LC-10T, Shodex, Japan), with a RI detector (RI-201R, Shodex, Japan) and a sugar-D chromatographic column. HPLC also with a UV-vis detector and a C_{18} column.

Synthesis

The catalyst 2,2'-((2-hydroxy-3,5-dimethylbenzyl)azanediyl)-diacetic acid was synthesized according to previous report.[43] The white solid product was obtained. ^{1}H NMR (400 MHz, DMSO-d6): δ 6.81 (s, 0H), 6.59 (s, 0H), 3.75 (s, 1H), 3.38 (s, 1H), 2.10 (s, 1H), 2.07 (s, 1H). ^{13}C NMR (101 MHz, DMSO): δ 172.4, 152.5, 130.7, 127.6, 126.7, 123.7, 120.8, 55.0, 53.3, 19.9, 15.6.

The catalyst BBTA was synthesized by modification of previous published procedures[44,45] as follows: NaOH (10 g) was added to 140 mL aqueous solution containing iminodiacetic acid (0.125 mol) and p-cresol (0.063 mol) under ice water bath. Then paraformaldehyde (0.063 mol) was added slowly into the mixture and the solution was stirred for 30 min at room temperature. Then the solution was heated to 70 ºC and stirred for 4 h. The solvent was acidized and removed by vacuum distillation. Then the pure white solid of BBTA was obtained by recrystallization of crude products in methanol. The structure of BBTA was given in Figure 1. Elemental analysis: calcd (%) for BBTA (398.1): C 51.26, H 5.57, N 7.03; found: C 51.24, H 5.54, N 7.02. ^{1}H NMR (400 MHz, D_2O): δ 6.97 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 1.2 Hz, 1H), 6.66 (d, J = 8.2 Hz, 1H), 4.71 (s, 8H), 3.70 (s, 2H), 3.16 (s, 4H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, D_2O): δ 178.4, 153.9, 130.9, 129.6, 129.2, 122.7, 115.7, 57.0, 55.2, 19.4. The ^{1}H and ^{13}C NMR spectra of BBTA were illustrated in Figure S1 and Figure S2.

Experimental methods

Typically, 0.25 g glucose and 0.028 g catalyst were added into 5 mL aqueous solution, the solution was sealed, heated and kept at required temperature. Initially, the pH was adjusted by NaOH and the N_2 was introduced into solution for 30 min, then the solution was sealed. The reactor was cooled by an ice bath after a certain reaction time. The glucose, fructose and other products in the reaction solution were analyzed by GC-MS, chromatographic analysis. And the concentrations were detected by HPLC.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: Mimic enzyme • Glucose • Fructose • Kinetics • Mechanism

[1] S. K. Maity, *Renew. Sust. Energ. Rev.* **2015**, *43*, 1446-1466.
[2] X. H. Liao, Y. Liu, X. Peng, C. Mi, X. G. Meng, *Catal. Lett.* **2016**, *146*, 1249-1255.
[3] I. Bodachivskyi, U. Kuzhiumparambil, D. B. G. Williams, *ChemSusChem* **2018**, *11*, 642-660.
[4] I. Thapa, B. Mullen, A. Saleem, C. Leibig, R. T. Baker, J. B. Giorgi, *Appl. Catal. A-Gen.* **2017**, *539*, 70-79.
[5] S. Caratzoulas, M. E. Davis, R. J. Gorte, R. Gounder, R. F. Lobo, V. Nikolakis, S. I. Sandler, M. A. Snyder, M. Tsapatsis, D. G. Vlachos, *J. Phys. Chem. C* **2014**, *118*, 22815-22813.
[6] S. Lima, A. S. Dias, Z. Lin, P. Brandão, P. Ferreira, M. Pillinger, J. Rocha, V. Calvino-Casilda, A. A. Valente, *Appl. Catal. A-Gen.* **2008**, *339*, 21-27.
[7] G. de Wit, A. P. G. Kieboom, H. van Bekkum, *Carbohydr. Res.* **1979**, *74*, 157-175.
[8] C. Kooyman, K. Vellenga, H. G. J. de Wilt, *Carbohydr. Res.* **1977**, *54*, 33-44.
[9] H. S. El Khadem, S. Ennifar, H. S. Isbell, *Carbohydr. Res.* **1987**, *169*, 13-21.
[10] C. Liu, J. M. Carraher, J. L. Swedberg, C. R. Herndon, C. N. Fleitman, J. P. Tessonnier, *ACS Catal.* **2014**, *4*, 4295-4298.
[11] G. Lee, Y. Jeong, A. Takagaki, J. C. Jung, *J. Mol. Catal. A-Chem.* **2014**, *393*, 289-295.
[12] I. Delidovich, R. Palkovits, *J. Catal.* **2015**, *327*, 1-9.
[13] Q. Yang, S. F. Zhou, T. Runge, *J. Catal.* **2015**, *330*, 474-484.
[14] C. Moreau, R. Durand, A. Roux, D. Tichit, *Appl. Catal. A-Gen.* **2000**, *193*, 257-264.
[15] R. O. L. Souza, D. P. Fabiano, C. Feche, F. Rataboul, D. Cardoso, N. Essayem, *Catal. Taday* **2012**, *195*, 114-119.
[16] P. A. Son, S. Nishimura, K. Ebitani, *Reac. Kinet. Mech. Cat.* **2014**, *111*, 183-197.
[17] D. Mun, N. T. T. Huynh, S. Shin, Y. J. Kim, S. Kim, Y. G. Shul, J. K. Cho, *Res. Chem. Intermed.* **2017**, *43*, 5495-5506.
[18] A. A. Marianou, C. M. Michailof, A. Pineda, E. F. Iliopoulou, K. S. Triantafyllidis, A. A. Lappas, *ChemCatChem* **2016**, *8*, 1100-1110.
[19] A. A. Marianou, C. M. Michailof, D. K. Ipsakis, S. A. Karakoulia, K. G. Kalogiannis, H. Yiannoulakis, K. S. Triantafyllidis, A. A. Lappas, *ACS Sustain. Chem. Eng.* **2018**, *6*, 16459-16470.
[20] I. Delidovich, R. Palkovits, *ChemSusChem* **2016**, *9*, 547-561.
[21] M. Moliner, Y. R. Leshkov, M. E. Davis, *Pans* **2010**, *107*, 6164-6168.
[22] C. M. Lew, N. Rajabbeigi, M. Tsapatsis, *Micropor. Mesopor. Mater.* **2012**, *153*, 55-8.
[23] A. Bayu, S. Karnjanakom, K. Kusakabe, A. Abudula, G. Q. Guan, *Chinese. J. Catal.* **2017**, *38*, 426-433.
[24] S. Saravanamurugan, M. Paniagua, J. A. Melero, A. Riisager, *J. Am. Chem. Soc.* **2013**, *135*, 5246-5249.
[25] Q. Guo, L. Ren, P. Kumar, V. J. Cybulskis, K. A. Mkhoyan, M. E. Davis, M. Tsapatsis, *Angew. Chem. Int. Ed.* **2018**, *57*, 4926-4930.
[26] D. H. Yu, H. Wu, A. J. Zhang, L. Tian, L. D. Liu, C. M. Wang, X. X. Fang, *Process Biochem.* **2011**, *46*, 599-603.
[27] B. Fatima, Z. Hussain, *J. Anim. Plant Sci*. **2015**, *25*, 10-18.
[28] J. T. G. Solomons, E. M. Zimmerly, S. Burns, N. Krishnamurthy, M. K. Swan, S. Krings, H. Muirhead, J. Chirgwin, C. Davies, *J. Mol. Biol.* **2004**, *342*, 847-860.
[29] H. Yamamoto, H. Miwa, N. Kunishima, *J. Mol. Biol.* **2008**, *382*, 747-762.
[30] J. E. D. Dyson, E. A. Noltmann, *J. Biol. Chem.* **1968**, *243*, 1401-1414.
[31] K. Buchholz, J. Seibel, *Carbohydr. Res.* **2008**, *343*, 1966-1979.
[32] D. X. Jia, L. Zhou, Y. G. Zheng, *Enzyme. Microb. Technol.* **2017**, *99*, 1-8.
[33] S. H. Bhosale, M. B. Rao, V. V. Deshpande, *Microbiol. Rev.* **1996**, *60*, 280-300.
[34] M. Suekane, M. Tamura, C. Tomimura, *Agric. Biol. Chem.* **1978**, *42*, 909-917.
[35] D. M. Gao, T. Kobayashi, S. Adachi, *Biosci. Biotechnol. Biochem.* **2015**, *79*, 1005-1010.
[36] A. Converti, M. D. Borghi, Enzyme. Microb. *Technol*. **1997**, *21*, 511-517.
[37] H. Karaoglu, D. Yanmis, F. A. Sal, A. Celik, S. Canakci, A. O. Belduz, *J. Mol. Catal. B: Enzym.* **2013**, *97*, 215-224.
[38] R. L. C. Giordano, R. C. Giordano, C. L. *Bioproc. Biosyst. Eng.* **2000**, *23*, 159-166.
[39] H. S. Lee, *J. Hong, J. Biotechnol.* **2000**, *84*, 145-153.
[40] C. A. Smith, M. Rangarajan, B. S. Hartley, *Biochem. J.* **1991**, *277*, 255-261.
[41] Q. Yang, M. Sherbahn, T. Runge, *ACS Sustain. Chem. Eng.* **2016**, *4*, 3526-3534.
[42] M. H. Meng, H. Y. Lin, C. J. Hsieh, Y. T. Chen, *FEBS Letters* **2001**, *499*, 11-14.
[43] E. Licsandru, E. Petit, S. Moldovan, O. Ersen, M. Barboiu, *Eur. J. Inorg. Chem.* **2015**, *2015*, 3637-3641.
[44] L. Ma, L. P. Lu, M. L. Zhu, Q. M. Wang, F. Gao, C. X. Yuan, Y. B. Wu, S. Xing, X. Q. Fu, Y. H. Mei, X. L. Gao, *J. Inorg. Biochem.* **2011**, *105*, 1138-1147.
[45] S. S. Feng, L. Ma, G. Q. Feng, Y. Jiao, M. L. Zhu, *J. Mol. Struct.* **2014**, *1059*, 27-32.

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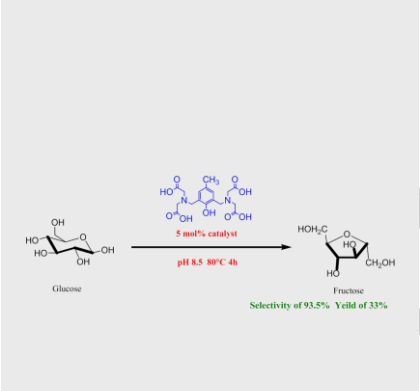

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High selective isomerization of glucose into fructose catalyzed by a mimic glucose isomerase

An amphoteric phenolic compound with carboxyl and amino functional groups was synthesized and used as mimic glucose isomerase to catalyze the isomerization of glucose into fructose. The mimic enzyme displayed excellent catalytic activity for isomerization of glucose to fructose under mild conditions. The yield of fructose and selectivity of fructose could reach 33.0% and 93.5% after reacting 4 h, respectively, at pH 8.5 and 80 °C.

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