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Note

An Organogermanium Compound Enhances the Initial Reaction Rate of Alkaline Isomerization of an Aldose into a Ketose through Enediol Complex Formation

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Abstract: We previously demonstrated that the organogermanium compound 3-(trihydroxygermyl)propanoic acid (THGP) enhances the enzymatic and alkaline isomerization of an aldose to a ketose through *cis*-diol complex formation by multiple mechanisms. Its higher affinity for the ketose than the aldose protects the ketose complex from alkaline decomposition. Furthermore, it has been reported that the aldose-ketose alkaline isomerization pathway includes 1,2-enediol. Therefore, we speculated that the complex-forming ability of THGP could also be applied to enediol, a transient intermediate of alkaline isomerization. To test this prediction, we analyzed the initial rates of glucose or lactose isomerization in a region where there was no substantial difference in pH with and without THGP addition. The results showed that THGP enhanced the rate of fructose or lactulose formation per unit time by approximately 2-fold compared to the control. This finding indicated that THGP could form a complex with the transition state of aldose-ketose alkaline isomerization.

Key words: organogermanium compound, 3-(trihydroxygermyl)propanoic acid, aldose-ketose, alkaline isomerization, initial kinetics, enediol intermediate

Previously, we reported that 3-(trihydroxygermyl)propanoic acid (THGP) enhances the yields of glucose-to-fructose enzymatic and alkaline isomerization reactions, as well as that of lactose-to-lactulose alkaline isomerization.[1)2)3)] THGP is a hydrolysate of the organogermanium compound poly-*trans*-[(2-carboxyethyl)germasesquioxane] (Ge-132), the biological safety of which has been confirmed. We also revealed that the driving force for the isomerization-promoting effect of THGP is its high complex formation ability for the *cis*-diol moieties of saccharides.[1)2)4)5)] The complex formation ability of THGP with fructose, which is *cis*-diol rich, is 40-fold higher than that with glucose, and the complex formation ability with lactulose is 24-fold higher than that with lactose.[1)2)] Due to the difference in affinity, the reverse isomerization reaction is suppressed in glucose-fructose enzymatic isomerization so that the fructose yield increases 1.6-fold.[1)]

In the alkaline isomerization of glucose into fructose and of lactose into lactulose, the product ketose-THGP complex has an anionic charge because the ketose and the negatively charged THGP form a stable complex. The negative charges protect the ketose from alkaline anion ($^{-}$OH) attack. As a result, alkaline decomposition of ketose was suppressed, enabling a 2.4-fold increase in fructose yield and a 3.2-fold increase in lactulose yield relative to that of the control.[1)2)] In the alkaline decomposition of ketose, the pH of the reaction is lowered by the organic acid origin of the Nef–Isbell mechanism,[6)] suppressing the alkaline isomerization reaction. However, the protection of ketose by THGP suppresses the generation of organic acids and prevents a decrease in the pH. As mentioned above, the ability of THGP to form a complex with the *cis*-diol group enhances enzymatic and alkaline isomerization reactions from glucose to fructose and from lactose to lactulose by multiple mechanisms.

On the other hand, Nagorski *et al.* reported that the paths of the aldose-ketose alkaline isomerization reaction in aqueous solution proceed through both 1,2-enediol and hydride transfer from the C-2 position to the C-1 position.[7)] Moreover, it has been reported that the ability of THGP to form a complex with catechol's 1,2-enediol is several tens of times higher than of its complex-forming ability with other *cis*-diol compounds.[8)] Stereochemically, *cis*-enediols arranged on a plane have the same structure as catechol diols. From these facts, we speculated that the complex-forming ability of THGP can be applied to *cis*-enediol, which is an intermediate in the alkaline isomerization reaction. The purpose of this study is to test this prediction. We investigated whether THGP could form a complex with a *cis*-enediol intermediate, whether the transition state was stabilized and whether the activation energy decreased, as in the case of an enzyme or

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Abbreviations: THGP, 3-(trihydroxygermyl)propanoic acid; HPLC, high-performance liquid chromatography.

other catalyst. This prediction could be confirmed by the observation of an enhancement in the reaction kinetics. Therefore, to distinguish this complexation from the alkaline degradation protection of the saccharide by the THGP complex, this study compared the initial ketose-formation rates in a region where the differences from the initial pH in the aldose-ketose alkaline isomerization were substantially negligible with and without THGP.

We conducted three types of experiments to compare the initial ketose formation rates in glucose or lactose alkaline isomerization in the presence and absence of THGP. Each alkaline isomerization and composition analysis was performed as described in a previous paper.[1)2)] In experiments 1−1 and 1−2, glucose-fructose alkaline isomerizations were performed twice independently under the conditions of glucose (0.5 M) -THGP (0.5 M), 80 ˚C, pH 11. To prepare the reaction mixture of glucose-THGP solution, 6.25 mL of a 2 M THGP (pH 10) solution was added to 12.5 mL of a 1 M glucose solution. Then, 0.48 mL of a 10 M NaOH solution was added to this solution to adjust the pH to 11. Subsequently, the volume was adjusted to 25 mL (actual pH of 11.08). The final concentrations of D-glucose and THGP in the reaction mixture were 0.5 M. The control solution was prepared using distilled water in place of the THGP solution. All preparation steps were performed under ice-cold conditions. Then, 16 (and 8) 1.0 mL reaction mixture samples were dispensed into 1.5 mL microcentrifuge tubes and incubated at 80 ˚C with shaking (120 rpm) for 1, 2, 3, 4, 5, 6, 7 or 8 min (and 2, 4, 6, or 8 min). Each sample was quickly placed in ice water. Each 30 μL reaction solution was subjected to high-performance liquid chromatography (HPLC) analysis (LC-10A instrument equipped with an RID-6A refractive index detector, Shimadzu)[1)], and the residual liquid was used for pH measurements. In experiments 2 and 3, lactose-lactulose alkaline isomerization was performed independently in a lactose-THGP solution (0.3 M each) at 80 ˚C, pH 10 and 11, respectively. To prepare the lactose-THGP solution for the reaction mixture, 1.35 mL of a 4 M THGP (pH 10.5) solution was added to 12 mL of a 0.45 M lactose solution, and 4 mL of distilled water was added. Then, 0.01 mL (and 0.11 mL) of a 10 M NaOH solution was added to this solution to adjust the pH to 10 (and pH 11). Subsequently, the volume was adjusted to 18 mL, and the actual pH was 10.09 (and actual pH of 10.98). The final concentrations of lactose and THGP in the reaction mixture were 0.3 M. The control solution was prepared using distilled water in place of the THGP solution. All preparation steps were performed under ice-cold conditions. Next, 32 (and 32) 1.0 mL reaction mixture samples were dispensed into 1.5 mL microcentrifuge tubes and incubated at 80 ˚C with shaking (120 rpm) for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 45, or 60 min and then quickly placed in ice water. After sampling 30 μL from each reaction solution for HPLC,[2)] the pH of the residual liquid was measured.

The results are shown in Figs. 1, 2, 3, and Table 1. Each figure shows the overall picture of the alkaline isomerization in the presence and absence of THGP: the change in the aldose concentration, the ketose concentration, and the pH with the reaction time. The deviation from the mean of each saccharide concentration between the two results of experiments 1−1 and 1−2 was reproducible with a maximum of less than 5 %. Table 1 shows the change in pH over the reaction time (ΔpH), the total saccharide ratio (remaining aldose and formed ketose), and the ketose-formation rate (M/min) with its coefficient of determination (R^2) in the initial reaction region of each experiment.

The total saccharide ratio in this region was more than 95 %, and the amount of alkaline decomposition was less than 5 % in the initial reaction region. Even in the control sample, the amount of alkaline decomposition of the saccharides was negligible. These results indicate that alkaline degradation of the saccharides proceeded only slightly in the initial reaction. The average coefficients of determination in the presence and absence of THGP in the experiments were both high, 0.99 and 0.98, respectively. The ratios of the ketose-formation rates in the presence/absence of THGP

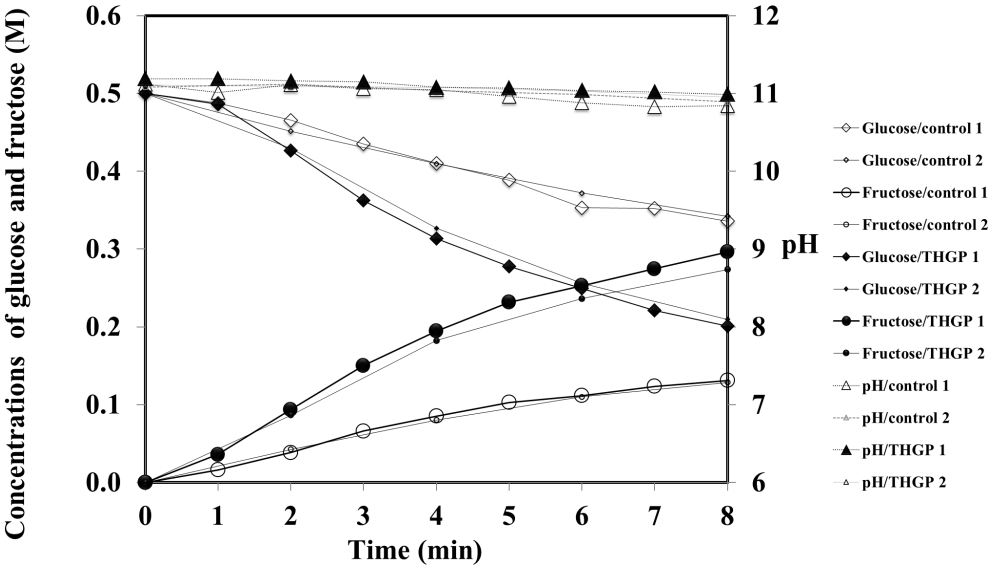

Fig. 1. Effects of the organogermanium compound THGP on alkaline isomerization from glucose to fructose at 0.5 M, 80 ˚C, and pH 11.

Time course of the remaining glucose content, the amount of fructose formed, and the pH during alkaline isomerization with and without THGP. The alkaline isomerizations were performed twice (experiment 1−1 and 1−2).

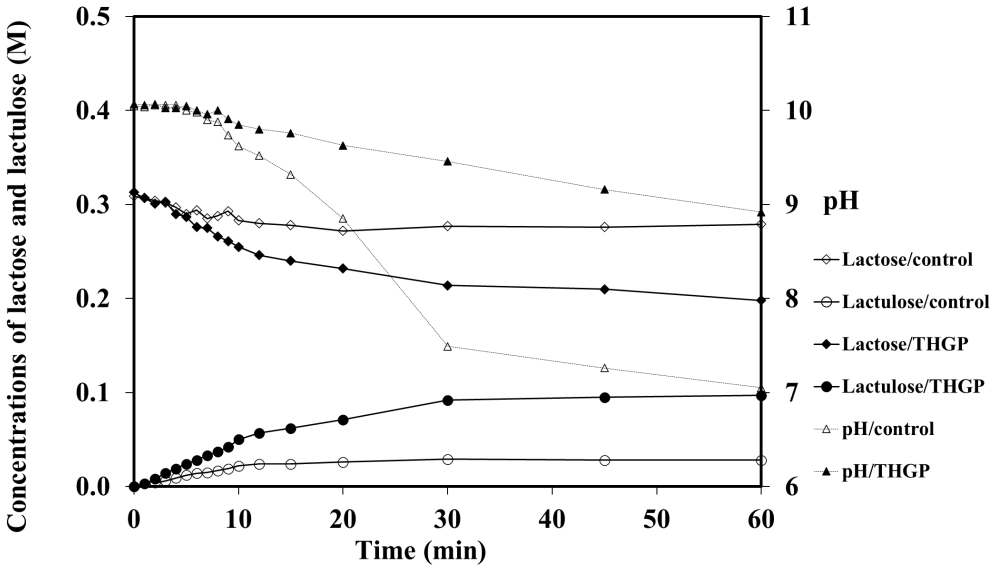

Fig. 2. Effects of the organogermanium compound THGP on alkaline isomerization from lactose to lactulose at 0.3 M, 80 °C, and pH 10.

Time course of the remaining lactose content, the amount of lactulose formed, and the pH during alkaline isomerization with and without THGP.

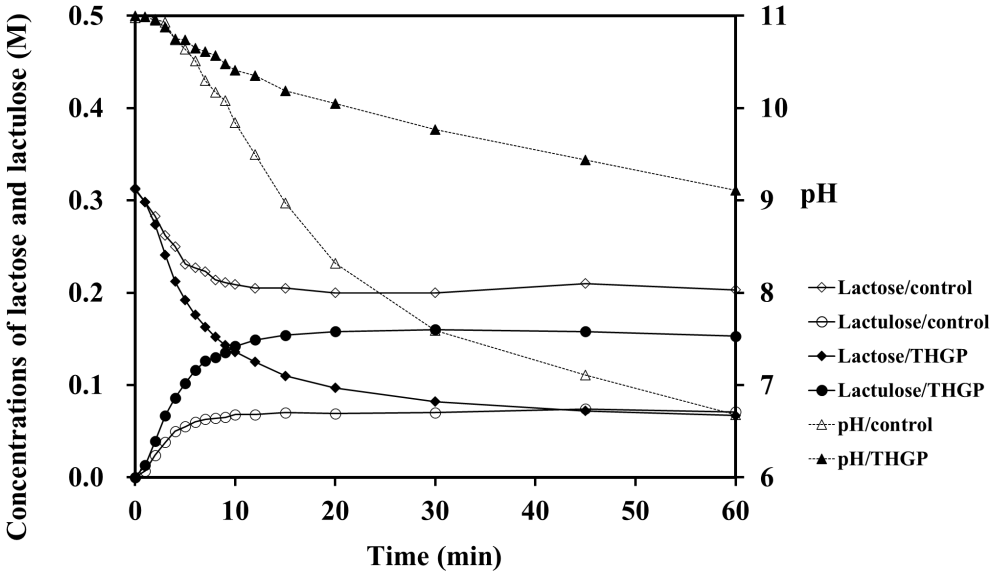

Fig. 3. Effects of the organogermanium compound THGP on alkaline isomerization from lactose to lactulose at 0.3 M, 80 °C, and pH 11.

Time course of the remaining lactose content, the amount of lactulose formed, and the pH during alkaline isomerization with and without THGP.

Table 1. Comparison of the initial ketose-formation rates during the aldose-ketose alkaline isomerization with and without THGP

Substrate (Experiment No.)		Reaction time (min)	Total saccharide ratio (%)	ΔpH	Ketose formation rate (M/min)	Coefficient of determination (R^2)
Glucose-fructose						
(Experiment 1−1)	control 1	0−5	≧ 98	≦ 0.2	0.021	0.994
	THGP 1		≧ 97	≦ 0.1	0.048	0.994
(Experiment 1−2)	control 2	0−6	≧ 96	≦ 0.1	0.019	0.992
	THGP 2		≧ 95	≦ 0.1	0.041	0.987
Lactose-lactulose						
(Experiment 2)	control	0−7	≧ 97	≦ 0.2	0.0022	0.988
	THGP		≧ 98	≦ 0.1	0.0047	0.997
Lactose-lactulose						
(Experiment 3)	control	0−4	≧ 96	≦ 0.2	0.012	0.983
	THGP		≧ 95	≦ 0.3	0.021	0.982

Total saccharide ratio (%) = 100 × (remaining aldose + formed ketose) / initial aldose amount.
ΔpH, the difference in pH from the initial pH at each reaction time.

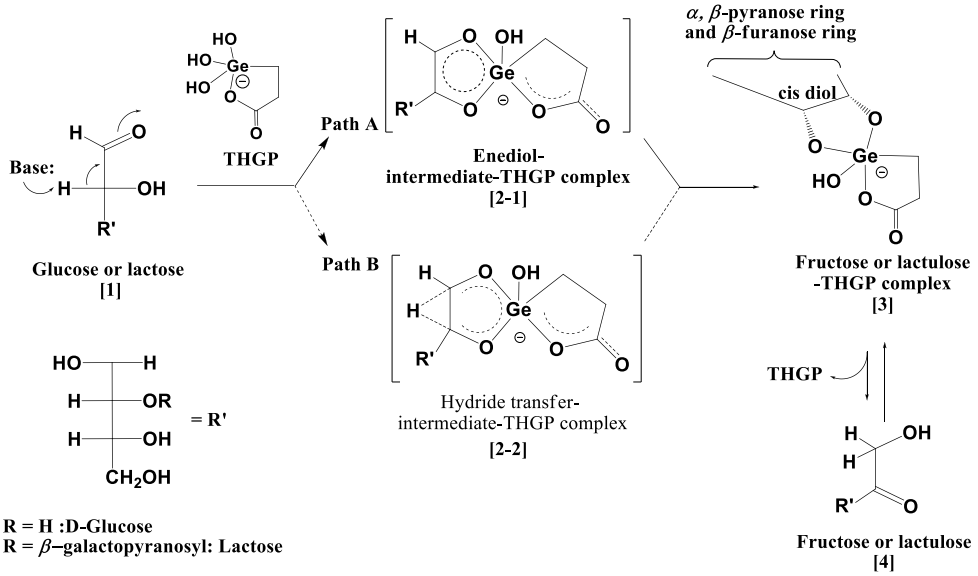

Fig. 4. The stabilization mechanism of 1,2-enediol intermediates (transition states) with THGP in the glucose (lactose)-into-fructose (lactulose) alkaline isomerization reaction in aqueous solutions.

The coordination number of the THGP germanium atom varies between 5 and 6 depending on the solution pH.[1)] In the case of fructose, THGP can form complexes with pyranose-type *cis*-diols at positions 2, 3 and 4, 5 and with furanose-type *cis*-diols at positions 2, 3 [3]. In the case of lactulose, THGP can form a complex with the *cis*-diol at positions 2, 3 of the reducing terminal fructofuranose or fructopyranose [3].

obtained from Table 1 were 2.3, 2.2, 2.1, and 1.8, respectively (average 2.1). The results showed that THGP enhanced the fructose or lactulose isomerization per unit time by approximately 2-fold compared to the control.

The figures show that after the initial reaction, THGP suppresses the production of organic acids by the alkaline decomposition of saccharides.[1)2)] However, as shown in Table 1, in the initial reaction region, this mechanism could not be applied because the pH differences from the initial values were quite negligible in both the presence and absence of THGP. In addition, the ketose yield in the group with added THGP is approximately twice that in the control group, even though the alkaline decomposition rate is less than 5 % in the control group, as shown in Table 1. Therefore, an increase in the ketose-formation rates during the initial reaction indicate another mechanism, namely, that THGP contributed to stabilizing the *cis*-enediol intermediate (transition state) of the alkaline isomerization reaction. In other words, THGP could be an artificial enzyme-like ligand.

The transition state of the aldose-ketose alkaline isomerization reaction in aqueous solutions proceeds through both 1,2-enediol and hydride migration from the C-2 position to the C-1 position. In addition, zinc salts catalyze this reaction because zinc coordinates to the *cis*-enediol.[5)] The electron acceptability (Lewis acidity) of the Ge atomic orbital using (p−d)π conjugation[9)10)] would allow extension of the conjugate system in both pathways *via* a 5-coordinated THGP structure (Fig. 4). These conjugations stabilize the transition state of this reaction. At present, considering the high complex-forming ability of THGP with catechol, a reasonable mechanism would be path A in Fig. 4. The enhanced initial kinetics due to the enzymatic function and the subsequent ketose protection against alkaline conditions by THGP complexation both favor ketose production. The increase in the initial reaction rate by THGP could probably occur in the aldose-ketose (where the *cis*-diol structure is more dominant) isomerization reaction under weakly alkaline conditions, thus shortening the reaction time and increasing the yield. Moreover, whether THGP can promote other aldose-ketose alkaline isomerizations (e.g., mannose-fructose, xylose-xylulose, arabinose-ribulose, and ribose-ribulose) through its *cis*-diol affinity is an interesting question.

CONFLICTS OF INTEREST

The authors, KS and TN, are employees of Asai Germanium Research Institute Co., Ltd., a Ge-132 manufacturing, research and sales company.

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