Journal of Molecular Catalysis B: Enzymatic 68 (2011) 181–186

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Improvement of aldehyde tolerance and sequential aldol condensation activity of deoxyriboaldolase via immobilization on interparticle pore type mesoporous silica

Takayuki Y. Nara [a], Hideaki Togashi [a,1], Seigo Ono [a], Miki Egami [b], Chisato Sekikawa [a], Yo-hei Suzuki [a], Isao Masuda [a], Jun Ogawa [c], Nobuyuki Horinouchi [c], Sakayu Shimizu [c], Fujio Mizukami [a], Tatsuo Tsunoda [a,*]

[a] Research Center for Compact Chemical Process, AIST, Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan
[b] JGC Catalysis and Chemicals Ltd., 13-2 Kitaminato, Wakamatsu, Kitakyushu 808-0027, Japan
[c] Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

ARTICLE INFO

Article history:
Received 12 July 2010
Received in revised form 26 October 2010
Accepted 30 October 2010
Available online 9 November 2010

Keywords:
Deoxyriboaldolase
Interparticle type mesoporous silica
Immobilized enzyme
Aldehyde tolerance
Aldol condensation

ABSTRACT

Deoxyriboaldolase from Klebsiella pneumonia (KpDERA) was immobilized on interparticle pore type mesoporous silica (IMS), which consists of aggregates of hexagonally close-packed silica nanoparticles. Incubation of the free KpDERA in 300 mM acetaldehyde resulted in a decrease in the free enzyme activity to 20% of the initial activity after incubation for 30 min. No activity was observed after 90 min incubation. In contrast, the IMS-immobilized KpDERA retained more than 50% of its activity after 30 min and 11% activity after 90 min incubation. The product yield of a sequential aldol condensation reaction, which is central to the production of pharmaceutically important intermediates, was found to be higher for IMS-immobilized KpDERA than for the free enzyme. These results suggest the potential utility of IMS as an immobilization support for DERA.

1. Introduction

An enzyme is a biocatalyst with several advantageous properties, including extremely high catalytic activity, substrate-, stereo-, regio-, and reaction-specificity, and a low environmental load [1,2]. Enzymes have emerged as important catalysts for the industrial synthesis of bulk chemicals, pharmaceutical intermediates, food ingredients, and agricultural chemicals [2–4].

Deoxyriboaldolase (DERA; EC 4.1.2.4) catalyzes a reversible aldol reaction that generates 2-deoxy-D-ribose-5-phosphate (DR5P) from D-glyceraldehyde 3-phosphate (G3P) and acetaldehyde [5]. DERA is unique in catalyzing aldol condensations between two or more aldehydes, and its broad substrate specificity confers considerable utility as a biocatalyst [6]. Gijsen and Wong [7] reported the double aldol condensation between three acetaldehyde molecules catalyzed by DERA from *Escherichia coli* (EcDERA). The reaction began with the stereospecific addition of acetaldehyde to a substituted acetaldehyde to form 3-hydroxy-4-substituted butyraldehyde, followed by reaction with a third acetaldehyde. The obtained 2,4,6-trideoxyhexoses are valuable chiral intermediates for the production of pharmaceutical products, for example a cholesterol-lowering drug [7–10]. DERA-catalyzed reactions may provide an alternative to chemical methodologies used for the synthesis of these intermediates. However, DERA is labile at high aldehyde concentrations, and a large quantity of enzyme is required to obtain useful product yields [10]. Unless the tolerance for aldehydes is improved, DERA may not be practical for commercial industrial applications.

Enzyme immobilization on a solid support offers several advantages over catalysis methods that employ free enzymes. Solid supports can enhance the enzyme stability, facilitate separation, may be reused, and permit continuous operation. Many immobilization techniques, such as physical adsorption, covalent attachment, and encapsulation, as well as organic and inorganic supports for enzyme immobilization have been studied [2,11–13]. No single technique or support has emerged as a universal standard, meaning that the technique and support must be optimized for each application.

Interparticle pore type mesoporous silica (IMS) consists of hexagonally close-packed silica nanoparticle aggregates. The aggregated nanoparticles form micron-size particles. IMS parti-

Abbreviations: DERA, deoxyriboaldolase; IMS, interparticle pore type mesoporous silica; DR5P, 2-deoxyribose 5-phosphate.

* Corresponding author. Tel.: +81 29 861 4633; fax: +81 29 861 4633.
E-mail address: t.tsunoda@aist.go.jp (T. Tsunoda).
[1] Present address: JGC Corporation, 2205 Narita, Oarai, Ibaraki 311-1313, Japan.

1381-1177/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2010.10.008

cles contain mesopores with tetrahedral symmetry defined by the interstitial spaces between nanoparticles. IMS particles possess excellent features as a support for enzyme immobilization. The pore size (2–50 nm) can be controlled via the size of the silica nanoparticles used, the particles are inexpensive to synthesize, they are chemically inert, and they exhibit a high mechanical stability. The surfaces of IMS particles display silanol groups to which biomolecules may bind via electrostatic interactions. The surface silanol groups can be modified using a variety of organosilanes to alter the surface charge and/or form covalent bonds with the immobilized biomolecules [14].

In the present study, DERA from *Klebsiella pneumonia* (KpDERA) [15] was overexpressed and immobilized on IMS, and the applicability of IMS as an enzyme immobilization support was tested. The IMS-immobilized KpDERA showed improved tolerance toward high concentrations of acetaldehydes. The aldol condensation product yield was higher than that obtained using free enzymes.

2. Experimental

2.1. Reagents

IMS particles with pore size 20 nm (MACS P-20H) were developed by JGC Catalysts and Chemicals Ltd., Kanagawa, Japan. Acetaldehyde, DR5P, DL-G3P, α-glycerophosphate dehydrogenase-triose phosphate isomerase from rabbit muscle (TPI/GDH), and triethanolamine (TEA) were purchased from Sigma–Aldrich Japan K.K. (Tokyo, Japan), the *p*-anisaldehyde (containing acetic acid and H_2SO_4) ethanol solution was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). β-D-Thiogalactopyranoside (IPTG) and nicotinamide adenine dinucleotide phosphate in the reduced form (NADH) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All chemicals used in this study were high-quality and analytical grade.

2.2. Subcloning and purification of KpDERA

The *KpDERA* gene [16] was amplified using the polymerase chain reaction (PCR) using the primers: forward, 5′-GACATATGACTGATTTATCTGCAAGCAGCCTG-3′ and reverse, 5′-AGACTCGAGTTAGTAGCTGCTGGCGCTCTTACC-3′. The PCR product was ligated to the pBluescript II (SK+) plasmid (Agilent Technologies, Inc., California, USA) and sequenced to confirm its veracity using the dideoxynucleotide chain termination method. N-terminal hexahistidine-tagged KpDERA was produced by inserting the appropriate clone into the *Nde*I–*Xho*I sites of the pET28b vector (EMD Chemicals, Inc., California, USA).

E. coli strain BL21 Star™ (DE3) (Invitrogen, California, USA) was transformed with the *KpDERA* expression vector and grown in Turbo broth (Athena Environmental Sciences, Inc., Maryland, USA) at 37 °C. The expression of KpDERA was induced by the addition of IPTG to a final concentration of 1 mM. After incubation for a further 4 h at 37 °C, the cells were harvested by centrifugation at 7500 × *g* for 5 min. The cell pellet was resuspended in 20 mM Tris–HCl (pH 7.5), 500 mM NaCl containing 10 mM 2-mercaptoethanol, snap frozen in liquid N_2, and stored at −80 °C.

To purify KpDERA, the cells were thawed and 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL pepstatin A, 0.5 mg/mL lysozyme, 1 mM $MgCl_2$, 100 units DNase1, and 10 mM imidazole were added. After incubation at 4 °C for 30 min, the cells were disrupted by sonication and were centrifuged at 100,000 × *g* for 1 h at 4 °C. The supernatant was then filtered through a 0.45 μm filter and loaded onto a HisTrap HP column (GE Healthcare UK Ltd., Buckinghamshire, England) on the ÄKTA Explorer liquid chromatography system (GE Healthcare UK Ltd.). The protein was eluted using a concentration gradient of 10–500 mM imidazole in 20 mM Tris–HCl (pH 7.5), 500 mM NaCl. The peak fractions were collected and dialyzed against 50 mM TEA (pH 7.5). The purity of the enzyme was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.3. Characterization

Nitrogen adsorption/desorption isotherms were measured at the temperature of liquid N_2 (77 K) using the AUTOSORB-1 analyzer (Quantachrome Instruments, FL, USA). Prior to measurement, the samples were degassed at 200 °C for 3 h under vacuum. The specific surface area was calculated by the Brunaur–Emmett–Teller (BET) method using adsorption data over the relative pressure range of P/P_0 = 0.05–0.30. The total pore volume was estimated from the quantity of N_2 adsorbed at the maximum relative pressure. The pore size distribution was determined by the Barrett–Joyner–Halenda (BJH) method using the adsorption or desorption branches. To study the changes in the nitrogen adsorption isotherms and pore size distributions by KpDERA immobilization, IMS was incubated with KpDERA (3 mg/mL) or buffer (50 mM TEA, pH 7.5), washed 4 times with buffer and once with ddH_2O, and dried at 60 °C. The samples were degassed at 60 °C for 24 h under vacuum.

Scanning electron microscope (SEM) images were obtained using S-800 (Hitachi High-Technologies Corporation, Tokyo, Japan).

2.4. Adsorption of KpDERA onto IMS

To study the adsorption of KpDERA onto IMS particles, a quantity of IMS particles (50 mg) was incubated with 1 mL KpDERA solution (2 mg/mL) at 4 °C for 24 h on a Rotator RT-50 (Taitec Corporation, Saitama, Japan) to establish the adsorption equilibrium. The IMS was removed by centrifugation at 20,800 × *g* for 2 min at 4 °C, and the protein concentration in the supernatant was measured using the BCA Protein Assay Reagent (Thermo Fisher Scientific Inc., Massachusetts, USA). The quantity of protein adsorbed was calculated by subtracting the amount of protein in the supernatant after centrifugation from the amount of KpDERA present before adsorption.

For the enzyme activity studies, 200 mg of IMS was incubated with 3.5 mg KpDERA (1.4 mg/mL) in 50 mM TEA buffer (pH 7.5) at 4 °C on a Rotator RT-50. The IMS-immobilized KpDERA was collected by centrifugation at 2150 × *g* for 5 min at 4 °C. The IMS-immobilized KpDERA was washed five times then resuspended in the buffer to a protein concentration of 1 mg/mL.

2.5. Enzyme activity

The DR5P cleavage activity was determined by measuring the oxidation of NADH in a coupled assay using glycerol-3-phosphate dehydrogenase and triose phosphate isomerase [17]. The assay mixture (2 mL) contained 50 mM TEA (pH 7.5), 0.2 mM NADH, 1 mM DR5P, 3 μL TPI/GDH, and 1.05 μg free or IMS-immobilized KpDERA. The reduction in absorbance at 340 nm was monitored using a Shimazu UV-2450 spectrophotometer (Shimazu, Kyoto, Japan). The extinction coefficient of NADH was taken to be 6.22 mM^{-1} cm^{-1}.

The DR5P production activity was determined using a modification of the method reported by Chen et al. [18]. The reaction solution (350 μL) contained 100 mM TEA (pH 7.5), 300 mM acetaldehyde, 100 mM DL-G3P, and 3 μg/mL free or IMS-immobilized KpDERA. The solution was incubated at 25 °C for 20 min with shaking. Forty microliters of these mixtures were removed and centrifuged at 20,800 × *g* for 5 min at 4 °C. A 20 μL aliquot of the supernatant was quenched by addition of 8 μL 60% perchloric acid and was incubated on ice for 10 min. This solution was neutralized with 13.4 μL 4 M NaOH and 179 μL 1 M TEA, pH 7.5. DR5P in the resulting supernatant was measured by the cysteine-sulfate method [19]. One unit

of enzyme activity refers to the activity required to produce or degrade 1 μmole DR5P per minute.

2.6. Acetaldehyde tolerance of free and IMS-immobilized KpDERA

To investigate the enzyme aldehyde tolerance, 0.2 units of IMS-immobilized KpDERA were incubated in a 50 mM TEA, pH 7.5, solution containing 300 mM acetaldehyde (200 μL) at 25 °C for the indicated times. The samples were washed with 50 mM TEA, pH 7.5, and the DR5P cleavage activity was measured. In the case of the free enzyme, acetaldehyde was removed by filtration through a Zeba™ Desalt Spin Column (Thermo Fisher Scientific Inc, Massachusetts, USA).

2.7. Time course of DR5P production

The reaction mixture containing 0.2 units of free or IMS-immobilized enzyme was incubated at 25 °C. Aliquots (25 μL) of mixtures were removed at the indicated time points and centrifuged at 20,800 × *g* for 5 min at 4 °C. Twenty microliters of the supernatant were quenched by addition of 8 μL 60% perchloric acid and neutralized with 13.4 μL 4 M NaOH and 179 μL 1 M TEA, pH 7.5.

2.8. Sequential aldol condensation

The sequential aldol condensation reaction was analyzed by thin-layer chromatography (TLC) using a modification of the method described by Sakuraba et al. [20]. The reaction mixture (100 μL) containing 100 mM TEA (pH 7.5), 500 mM acetaldehyde, and 0.5 mg/mL free or IMS-immobilized KpDERA was incubated at 25 °C for 24 h with shaking. An aliquot (4 μL) of the sample was spotted onto a TLC plate (Silica gel 60 F_{254}, Merck Ltd., Tokyo, Japan) and developed with a running solvent consisting of 1-butanol, acetic acid, and H_2O at a ratio of 4:1:1 (v/v/v). The reaction products were localized using a *p*-anisaldehyde ethanol solution.

3. Results and discussion

3.1. Expression and purification of KpDERA

The expression vector containing the N-terminal hexahistidine-tagged *KpDERA* gene was transformed into the *E. coli* strain BL21 Star™ (DE3), and expression of KpDERA was induced. The protein was purified through a HisTrap HP column. The protein was purified to near homogeneity and was found to be suitable for subsequent studies (Supplementary Fig. 1). The final yield of the purified KpDERA was 60 mg/L *E. coli* culture. Gel filtration analysis (Supplementary Fig. 2) revealed that the molecular mass of KpDERA was 58 kDa. The molecular mass of KpDERA, determined by SDS-PAGE analysis (Supplementary Fig. 1) and predicted from the amino acid sequence [16], was 28 kDa. Therefore, KpDERA was confirmed to be present as a dimer. It has been reported that *E. coli* DERA (EcDERA) consists of two identical 28 kDa subunits with unit cell dimensions 49 Å × 42 Å × 145 Å [21]. The amino acid sequence of KpDERA is similar to that of EcDERA (86% identity) [16]. The unit cell dimensions of KpDERA may also be similar to those of EcDERA.

3.2. Characterization of IMS

As shown in Fig. 1, the IMS particles were characterized as well-ordered spheres. Dynamic light scattering analysis revealed that the mean diameter was 3 μm (data not shown). A magnified image (Fig. 1 inset) shows that the IMS particles contained pores formed from the voids between the aggregated silica nanoparticles.

Fig. 2 shows the nitrogen adsorption/desorption isotherms and the pore size distributions of the IMS particles. The isotherm curves showed that IMS was a type IV mesoporous material according to the International Union of Pure and Applied Chemistry (IUPAC) classification system [22]. McBain described a "bottle-neck" theory, which proposed that adsorption isotherms are determined by the diameter of the inner pores, and desorption isotherms are determined by the pore openings [23]. According to this theory, the pore diameters of IMS, calculated from the adsorption and desorption isotherms, were 33 and 21 nm, respectively.

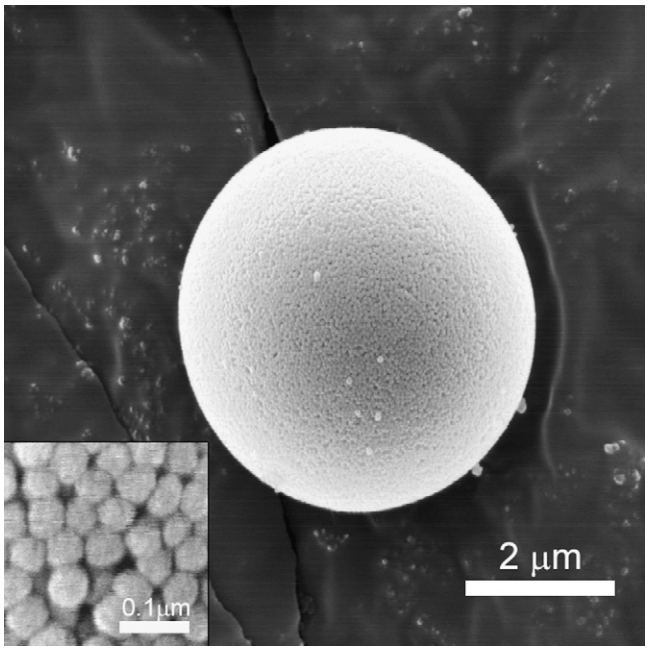

Fig. 1. SEM image of IMS particle. A high magnification image is shown in the inset.

3.3. Immobilization of KpDERA on IMS

Fig. 3 shows the adsorption isotherm for KpDERA on IMS. The isotherm curve indicated that adsorption increased sharply then reached a plateau. This type of isotherm can be modeled using the Langmuir equation [24]. The maximum adsorption capacity,

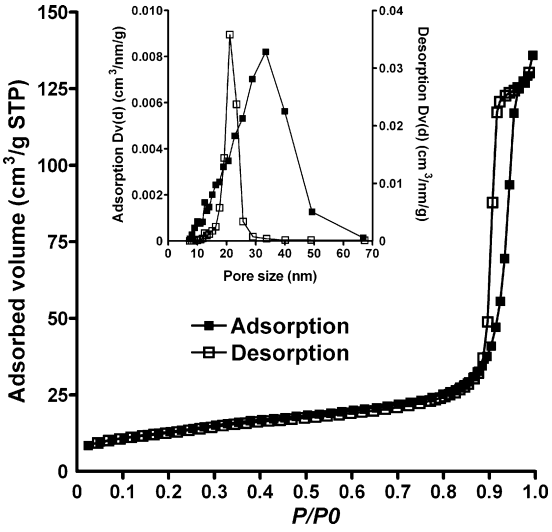

Fig. 2. Nitrogen adsorption/desorption isotherms and pore size distributions of the IMS particles. Nitrogen adsorption (■) and desorption (□) isotherms. (Inset) Pore size distributions: adsorption (■), desorption (□). The isotherms were measured using an AUTOSORB-1 analyzer. The pore size distributions were determined by the BJH method using the adsorption and desorption branches.

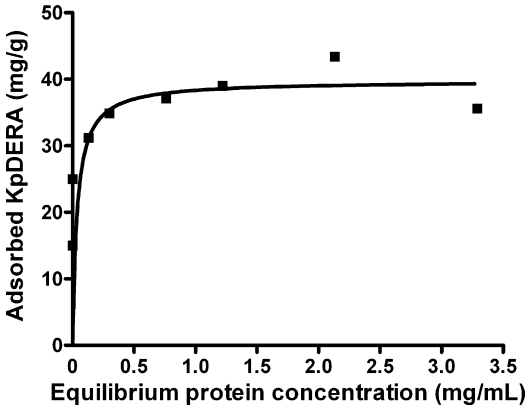

Fig. 3. Adsorption isotherm of KpDERA on IMS. KpDERA (1 mL, varying concentrations) was permitted to adsorb onto 20 mg IMS particles for 24 h at 4 °C. The protein concentration in the supernatant was subsequently measured.

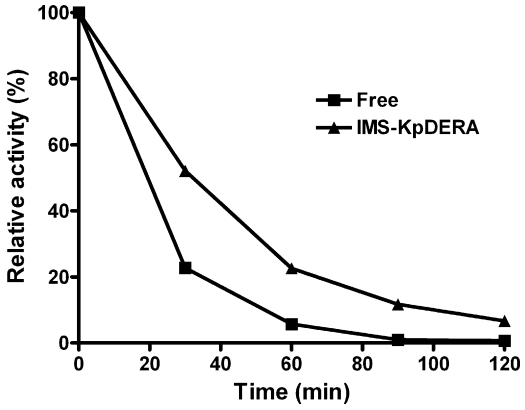

Fig. 5. Acetaldehyde tolerance of free and IMS-immobilized KpDERA. Free (■) or IMS-immobilized (▲) KpDERA (0.2 units) were incubated with 300 mM acetaldehyde at 4 °C for the indicated times, and DR5P cleavage activity was subsequently measured. The relative activity was calculated with respect to the activity of each sample at 0 min.

calculated by curve fitting, was 39.7 mg/g support. Because the pore openings in the IMS particles were sufficiently larger than the unit cell dimensions of KpDERA (estimated from the EcDERA structure), it was possible that KpDERA was immobilized within the pores of IMS. To confirm that KpDERA was present within the pores of IMS, the nitrogen adsorption before and after KpDERA loading was investigated. As shown in Fig. 4, the magnitudes of the nitrogen adsorption and pore size distribution curves decreased after adsorption of KpDERA. The surface area and total pore volume also decreased (41.4–29.9 m^2/g and 0.211–0.171 cm^3/g, respectively). These results suggested that the IMS pores were occupied by KpDERA [25].

3.4. Activities of free and IMS-immobilized KpDERA

The specific activities of free and IMS-immobilized KpDERA for the DR5P cleavage reactions were 119 and 18.6 units/mg, respectively. Indeed, the specific activity decreased to about 16% by immobilization on IMS. Several factors may account for the reduction in the specific activity of IMS-immobilized KpDERA. Enzyme leaching was improbable because the proteins were not observed in the supernatant of the wash solutions during the immobilization step or in the reaction mixtures (data not shown). Enzyme conformational changes and/or blocking of the active site may have been induced by the enzyme–support interaction [26]. Limited substrate diffusion and steric hindrance may also have decreased the activity of the immobilized KpDERA relative to that of the free enzyme [27].

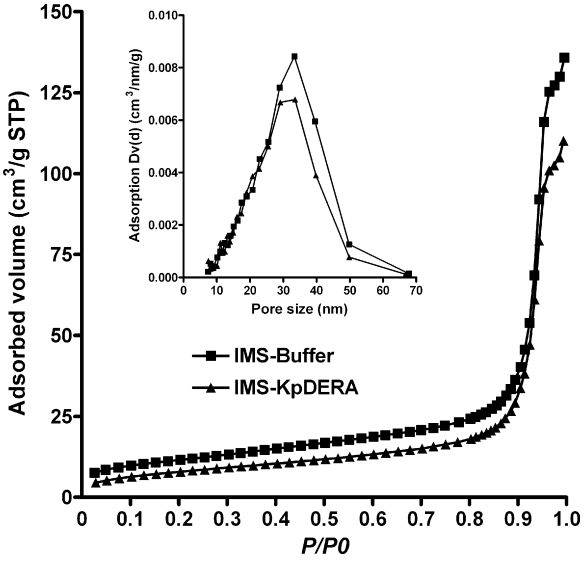

Fig. 4. Changes in the nitrogen adsorption isotherms and pore size distributions of the IMS particles before and after immobilization of KpDERA. The IMS particles were incubated in buffer (■) or KpDERA in buffer (▲). The isotherms were then measured using an AUTOSORB-1 analyzer. The pore size distributions were determined by the BJH method using the adsorption branches.

3.5. Acetaldehyde tolerance of free and IMS-immobilized KpDERA

DERA has shown poor tolerance to high aldehyde concentrations, and large quantities of enzyme are required to obtain useful product yields [10]. This may limit its practicality for industrial applications. The resistance of DERA to aldehydes is, therefore, required. To investigate the effects of immobilization on acetaldehyde tolerance, free or IMS-immobilized KpDERA was incubated in a buffered solution containing 300 mM acetaldehyde, and the DR5P cleavage activity was measured. As shown in Fig. 5, the activity of the free enzyme decreased to 20% after incubation for 30 min, and the activity disappeared completely after 90 min. In contrast, IMS-immobilized KpDERA retained more than 50% of its activity after 30 min, and showed 11% activity after 90 min. This result indicated that the acetaldehyde tolerance was improved by immobilization within the pores of IMS.

Enzyme inactivation by aldehydes arises mainly from Schiff base formation between the surface lysine residues and the aldehydes [28,29]. Because the silica support surface is negatively charged at pH > 2 [30], positively charged lysine residues of KpDERA may be masked from acetaldehyde attack by interacting with the IMS. It is also suggest that the enzyme structure was stabilized by confinement in a narrow space, even if the aldehyde modified the enzyme surface.

Chemical modification of protein surface is also a useful technique for stabilizing enzymes [31–33]. Chemical modification with formaldehyde causes the transformation of primary amine groups on the enzyme surface into secondary amino groups [34,35]. This modification might prevent deleterious modification effect of acetaldehyde on KpDERA surface. Furthermore, when penicillin G acylase was immobilized through multipoint attachment prior to chemical modification, the enzyme showed superior resistance to the effect of chemical modification with formaldehyde [34]. Multipoint covalent attachment, in which the enzyme is immobilized by contact with the support through multipoint covalent bonds, highly stabilizes the enzyme by rigidification of enzyme structure [31,36–38]. Silica surface contains very dense silanol groups (4.3–6.7 OH/nm^2) [39] that can be easily functionalized with the

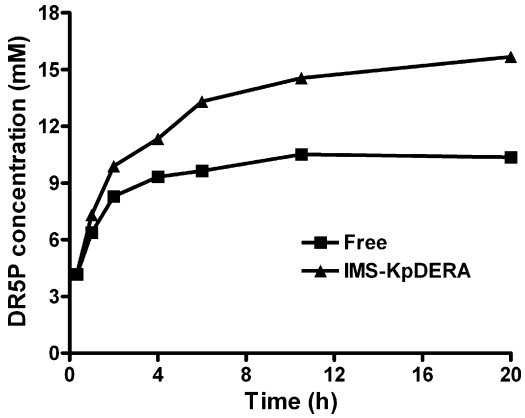

Fig. 6. Time course of DR5P production. Free (■) or IMS-immobilized (▲) KpDERA (0.2 units) was incubated in a reaction mixture containing 300 mM acetaldehyde and 100 mM DL-G3P at 23 °C for the indicated times. The concentration of DR5P was subsequently measured.

functional groups for covalent immobilization of enzymes. Therefore, the functionalized IMS could also be used to stabilize the enzyme through multipoint attachment.

3.6. Aldol condensation using a natural substrate of DERA

The aldol condensation reaction catalyzed by DERA is important for organic synthesis. However, quantification of the sequential aldol condensation products is difficult. We evaluated these products by analyzing the production of DR5P, which is the natural product of DERA, from G3P in the buffered solution containing 300 mM acetaldehyde.

The specific activity of the IMS-immobilized KpDERA, surprisingly, displayed 74% activity relative to that of the free enzyme (75.4 versus 102 units/mg). Fig. 6 shows the time course of DR5P production for a fixed initial quantity of enzyme units. The DR5P concentration reached a plateau after 10.5 h reaction with the free enzyme, whereas the IMS-immobilized KpDERA retained activity after 20 h. The enhanced activity of the immobilized enzyme may indicate improved acetaldehyde tolerance. It is also possible that the equilibrium of the enzyme reaction shifted to favor the aldol condensation product.

3.7. Sequential aldol condensation

Finally, the sequential aldol condensation reaction with acetaldehyde was analyzed by TLC analysis. Fig. 7 showed that the TLC spot intensity corresponding to the aldol condensation products produced from 500 mM acetaldehyde was higher for the IMS-immobilized KpDERA than for the free enzyme. This result suggested that the efficiency of DERA as a catalyst for sequential aldol condensation was also improved by immobilization on IMS. It should be noted that it was not possible to identify the physical properties of this product, although the TLC pattern was similar to that reported by Sakuraba et al. [20]. Further studies are required to determine whether this product was a chiral trideoxyhexose. Previous attempts to improve the yield of the industrially useful chiral trideoxyhexoses have used DERA from a variety of organisms [37,38,40,41] or environmental DNA [9], or modified structures of EcDERA by molecular evolution [10,17]. A combination of the immobilization of DERA on IMS and these techniques may enable the practical application of DERA for the production of pharmaceutically important intermediates.

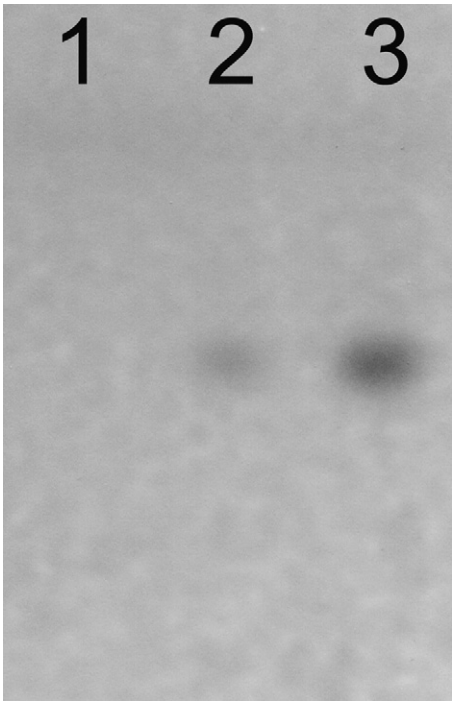

Fig. 7. TLC analysis of the sequential aldol condensation product. Lane 1, buffer control; Lane 2, free; Lane 3, IMS-immobilized KpDERA. The reaction mixture containing 500 mM acetaldehyde was incubated with 0.5 mg/mL free or IMS-immobilized KpDERA at 25 °C for 24 h. The products were developed on TLC plates and were stained using a *p*-anisaldehyde ethanol solution.

4. Conclusions

KpDERA was successfully immobilized on IMS particles, which are aggregate mesostructures formed from the hexagonal close packing of silica nanoparticles. The immobilized enzyme exhibited superior aldehyde tolerance relative to the free enzyme. The equilibrium concentration of the sequential aldol condensation product was higher when produced by the IMS-immobilized KpDERA. These results suggest the potential use of IMS as an immobilization support for DERA for the production of pharmaceutically important intermediates.

Acknowledgments

The authors thank Mr. Shuzo Kojima and Dr. Naoki Tahara and (JGC Corporation) for their helpful discussions concerning the experimental design and results. This work was performed under a collaboration with the JGC Corporation and JGC Catalysis and Chemicals Ltd. entitled “Development of enzyme reaction systems using the new immobilization support”.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2010.10.008.

References

[1] A.M. Klibanov, Science 219 (1983) 722–727.
[2] General Production Methods, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2007.
[3] A. Schmid, J.S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, Nature 409 (2001) 258–268.
[4] H.E. Schoemaker, D. Mink, M.G. Wubbolts, Science 299 (2003) 1694–1697.
[5] A. Heine, G. DeSantis, J.G. Luz, M. Mitchell, C.H. Wong, I.A. Wilson, Science 294 (2001) 369–374.

[6] C.F. Barbas, Y.F. Wang, C.H. Wong, J. Am. Chem. Soc. 112 (1990) 2013–2014.
[7] H.J.M. Gijsen, C.-H. Wong, J. Am. Chem. Soc. 116 (1994) 8422–8423.
[8] H.J.M. Gijsen, C.-H. Wong, J. Am. Chem. Soc. 117 (1995) 7585–7591.
[9] W.A. Greenberg, A. Varvak, S.R. Hanson, K. Wong, H. Huang, P. Chen, M.J. Burk, Proc. Nat. Acad. Sci. U.S.A. 101 (2004) 5788–5793.
[10] J. Stefan, S.M. Martin, W. Michael, H. Iris, L. Ruud, W. Marcel, M. Daniel, Biotech. J. 1 (2006) 537–548.
[11] K. Buchholz, J. Klein, M. Klaus, in: K. Mosbach (Ed.), Characterization of Immobilized Biocatalysts, Meth. Enzymol., Academic Press, London, 1987, pp. 3–30.
[12] Immobilization of Enzymes and Cells, Humana Press Inc., Totowa, NJ, 2006.
[13] U. Hanefeld, L. Gardossi, E. Magner, Chem. Soc. Rev. 38 (2009) 453–468.
[14] C.-H. Lee, T.-S. Lin, C.-Y. Mou, Nano Today 4 (2009) 165–179.
[15] J. Ogawa, K. Saito, T. Sakai, N. Horinouchi, T. Kawano, S. Matsumoto, M. Sasaki, Y. Mikami, S. Shimizu, Biosci. Biotechnol. Biochem. 67 (2003) 933–936.
[16] N. Horinouchi, J. Ogawa, T. Sakai, T. Kawano, S. Matsumoto, M. Sasaki, Y. Mikami, S. Shimizu, Appl. Environ. Microbiol. 69 (2003) 3791–3797.
[17] G. DeSantis, J.J. Liu, D.P. Clark, A. Heine, I.A. Wilson, C.H. Wong, Bioorg. Med. Chem. 11 (2003) 43–52.
[18] L. Chen, D.P. Dumas, C.H. Wong, J. Am. Chem. Soc. 114 (1992) 741–748.
[19] P.K. Stumpf, J. Biol. Chem. 169 (1947) 367–371.
[20] H. Sakuraba, K. Yoneda, K. Yoshihara, K. Satoh, R. Kawakami, Y. Uto, H. Tsuge, K. Takahashi, H. Hori, T. Ohshima, Appl. Environ. Microbiol. 73 (2007) 7427–7434.
[21] A. Heine, J.G. Luz, C.H. Wong, I.A. Wilson, J. Mol. Biol. 343 (2004) 1019–1034.
[22] F. Rouquerol, J. Rouquerol, K. Sing, in: J. Rouquerol, F. Rouquerol, K. Sing (Eds.), Adsorption by Powders and Porous Solids, Academic Press, London, 1999, pp. 1–26.
[23] J.E.S.S. Lowell, Powder Surface Area and Porosity, 2nd ed., John Wiley & Sons, New York, 1984.
[24] M. Miyahara, A. Vinu, K. Ariga, Mater. Sci. Eng. C: Biomim. Supramol. Syst. 27 (2007) 232–236.
[25] A. Vinu, V. Murugesan, M. Hartmann, J. Phys. Chem. B 108 (2004) 7323–7330.
[26] M. Tortajada, N. Ramon, D. Beltran, P. Amoros, J. Mater. Chem. 15 (2005) 3859–3868.
[27] G. Ozyilmaz, S.S. Tukel, O. Alptekin, J. Mol. Catal. B: Enzym. 35 (2005) 154–160.
[28] A.F.S.A. Habeeb, R. Hiramoto, Arch. Biochem. Biophys. 126 (1968) 16–26.
[29] I. Matsumura, J.B. Wallingford, N.K. Surana, P.D. Vize, A.D. Ellington, Nat. Biotech. 17 (1999) 696–701.
[30] D. Moelans, P. Cool, J. Baeyens, E.F. Vansant, Catal. Commun. 6 (2005) 307–311.
[31] A.M. Klibanov, Anal. Biochem. 93 (1979) 1–25.
[32] R. Schmid, Adv. Biochem. Eng. 12 (1979) 41–118.
[33] P.V. Iyer, L. Ananthanarayan, Process Biochem. 43 (2008) 1019–1032.
[34] R. Fernandez-Lafuente, C.M. Rosell, G. Alvaro, J.M. Guisan, Enzyme Microb. Technol. 14 (1992) 489–495.
[35] R.C. Rodrigues, J.M. Bolivar, G. Volpato, M. Filice, C. Godoy, R. Fernandez-Lafuente, J.M. Guisan, J. Biotechnol. 144 (2009) 113–119.
[36] K. Martinek, A.M. Klibanov, V.S. Goldmacher, I.V. Berezin, Biochim. Biophys. Acta 485 (1977) 1–12.
[37] S. Ichikawa, K. Takano, T. Kuroiwa, O. Hiruta, S. Sato, S. Mukataka, J. Biosci. Bioeng. 93 (2002) 201–206.
[38] C. Mateo, J.M. Palomo, G. Fernandez-Lorente, J.M. Guisan, R. Fernandez-Lafuente, Enzyme Microb. Technol. 40 (2007) 1451–1463.
[39] R.F. de Farias, C. Airoldi, J. Therm. Anal. Calorim. Calorimetry 53 (1998) 751–756.
[40] H. Sakuraba, H. Tsuge, I. Shimoya, R. Kawakami, S. Goda, Y. Kawarabayasi, N. Katunuma, H. Ago, M. Miyano, T. Ohshima, J. Biol. Chem. 278 (2003) 10799–10806.
[41] Y.-M. Kim, Y.-H. Chang, N.-S. Choi, Y. Kim, J.J. Song, J.S. Kim, Protein Expr. Purif. 68 (2009) 196–200.