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Solubility-dependent melting temperature depression of binary substrate mixtures: A model study

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ABSTRACT

In formulating eutectic media, the role of solvent adjuvant is not well understood in depressing the melting temperature of a binary substrate mixture (T_{MB}). Here, we demonstrated the effect of solubility on the depression of T_{MB} by using combinatorial model mixtures of *N*-benzyloxycarbonyl-L-Asp (CBZ-L-Asp) with either an L-amino acid or a D-sugar in different solvent adjuvants. When CBZ-L-Asp was mixed with an individual L-amino acid at an equi-molar ratio, T_{MB} values varied approximately from 34 to 113 °C according to the hydrophobicity of adjuvants. Similarly, CBZ-L-Asp/D-sugar mixtures showed large variations of T_{MB} values approximately from 43 to 112 °C according to the hydrophobicity of adjuvants. In the water adjuvant, T_{MB} values in both CBZ-L-Asp/L-amino acid and CBZ-L-Asp/D-sugar showed strong correlations with the water solubility of either L-amino acids or D-sugars. In contrast, the hydrophobicity of individual compounds did not show any correspondence with T_{MB} depression. The peak height of X-ray diffractions in the mixture of either CBZ-L-Asp/L-Lys or CBZ-L-Asp/L-Tyr was significantly reduced only when either L-Lys or L-Tyr was soluble in the adjuvant. These results show that the solubilization of a substrate component to an adjuvant contributed to the T_{MB} depression, which may be caused by the enhanced structural homogeneity of a binary mixture.

1. Introduction

Over the past few decades, technical breakthroughs for the industrial biocatalysis have been progressed in terms of biocatalyst engineering [1–3], reaction engineering [4,5], media engineering [6–8], and process engineering [9,10]. However, successful applications of the biocatalysis in the industrial scale biocatalysis still remain challenging because the concentration level of reaction mixtures does not satisfy the industrial demands regardless of the high conversion yield. Accordingly development of reaction media with sufficiently high concentrations is recognized as a crucial key for the successful applications in the industrial biocatalysis.

In terms of media engineering, several approaches have been proposed for the possible applications of media engineering with fairly high concentrations, named 'solid-to-solid' [11], 'heterogeneous eutectic substrate' [12,13], and 'eutectic media' [14–19]. Among those methods, 'eutectic media' provide a simple methodology by heating the binary substrate mixture including a solvent adjuvant, resulting in ultra-high concentrations of a substrate mixture beyond 2–3 M. This eutectic media is potentially applicable for the esterification, interesterification, acylation, or other condensation reactions because it requires two or more components of substrate for the binary melting.

Terminology 'eutectic media' came from the 'eutectic mixture' [16], but it should be discriminated to each other. The 'eutectic mixture' was defined as an equilibrated melt of a binary mixture when the lowest melting temperature was attained at a particular mole fraction, i.e., eutectic composition, without any adjuvants [13,16]. In contrast, 'eutectic media' was as an equilibrated melt of an equi-molar binary mixture with an appropriate solvent adjuvant for the applications in biocatalysis [16]. This eutectic medium has been investigated with respect to the formation mechanism via computer simulations [17], physicochemical and thermodynamic characterizations [18], and applications to enzyme reactions [16,19]. However, the role of solvent adjuvant in depressing the melting temperature of a binary substrate mixture (T_{MB}) was not well understood in formulating eutectic media.

Here, we demonstrate the combinatorial model of binary substrate mixtures comprising *N*-benzyloxycarbonyl-L-Asp as a component for melting stimulation and either an L-amino acid or a D-sugar as the counter component. Profiles of T_{MB} depression in an equi-molar model mixture may provide a useful guideline for selecting appropriate adjuvants in preparation of the eutectic media. Based on the T_{MB} depression profile of model mixtures depending on the adjuvant, we claimed which adjuvant is effi-

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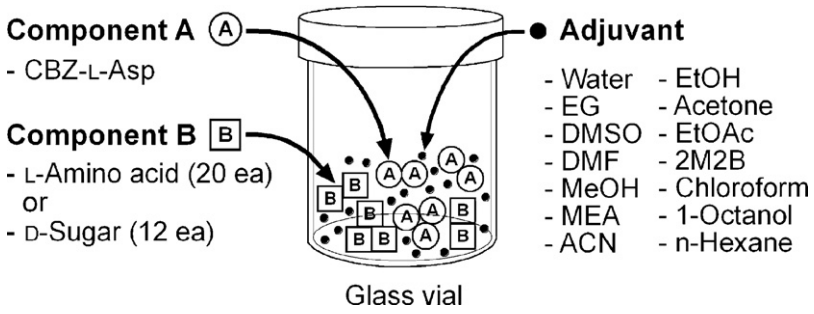

Fig. 1. A schematic drawing of the eutectic media formulation. Component A is the compound inducing melting stimulation. In this study, *N*-benzyloxycarbonyl-L-Asp (CBZ-L-Asp) was used as a melting stimulant. Component B is the compound as a partner substrate in a substrate mixture. In this study, a single compound was added in a substrate mixture among either L-amino acids or D-sugars. Adjuvant is the solvent enhancing eutectic melting in a substrate mixture. In this study, a single solvent was added in a substrate mixture depending on the experimental setup.

cient to induce the T_{MB} depression and how the adjuvant effectively reduces the T_{MB} value of substrate mixture.

2. Experimental

2.1. Chemicals

Among L-amino acids and D-sugars, L-Ala, L-Cys, L-Gln, L-His, L-Ile, L-Leu, L-Phe, L-Pro, L-Ser L-Thr, D-inositol (Ino), and D-lactose (Lac) were products of Junsei Chemical Co., Ltd. (Tokyo, Japan). L-Gly, L-Met, L-Trp, L-Val, D-fructose (Fru), and D-maltose (Mal) were purchased from Yakuri Pure Chemicals Co., Ltd. (Osaka, Japan). L-Arg, L-Asn, L-Asp, L-Glu, L-Lys, L-Tyr, D-gluconic acid sodium salt (GAS), D-gluconic acid lactone (GAL), and D-xylitol (Xyl) were obtained from Sigma Co. (St. Louis, USA). D-Glucose (Glu), D-sucrose (Suc), D-sorbitol (Sor), and D-mannitol (Man) were purchased from Duksan Pure Chemicals Co., Ltd. (Ansan, Korea). *N*-CBZ-L-Asp was purchased from Novabiochem Co. (Darmstadt, Germany). Silicone oil (KF-96) was a product of Shin-Etsu Silicone Korea Co., Ltd. (Eumsung, Korea).

Among solvents, acetone, acetonitrile (ACN), chloroform, dimethyl sulfoxide (DMSO), and *N*,*N*-dimethylformamide (DMF) were products of Sigma Co. (St. Louis, USA). 2-Methyl 2-butanol (2M2B) and 2-methoxyethyl acetate (MEA) were obtained from Fluka Chemika (Buchs, Switzerland) and Aldrich (Milwaukee, USA), respectively. Ethanol (EtOH), ethylene glycol (EG), ethyl acetate (EtOAc), *n*-hexane, and methanol (MeOH) were products of Duksan Pure Chemicals Co., Ltd. (Ansan, Korea) and 1-octanol was a product of Yakuri Pure Chemicals Co., Ltd. (Osaka, Japan). All chemicals and solvents were of reagent grade.

2.2. Measurement of the melting temperature

Experimental setup is provided in Fig. 1. A binary mixture of either CBZ-L-Asp/L-amino acid or CBZ-L-Asp/D-sugar was prepared in a 10-mL screw-top glass vial. In the mixture comprising equi-molar ratio of each component, an appropriate solvent was added at 10% (weight of adjuvant/weight of a mixture) as an adjuvant to each vial, then the mixture was uniformly mixed by a vortex for 1 min. The melting temperature of a mixture was measured in an oil bath containing silicone oil (KF-96). In case of an oil bath, the heating rate of the oil was adjusted approximately at $5\,^{\circ}C\,min^{-1}$ until the temperature reaches to 200 °C. For the phase equilibration of a model mixture, the liquefaction and the solidification were repeated by the heating and cooling processes prior to the analysis. Then, melting temperature of equilibrated mixtures was measured three times, and averaged T_{MB} values were denoted.

For the preparation of a mixture CBZ-L-Asp/L-amino acid, a single L-amino acid among 20 different L-amino acids (L-Arg, L-Lys, L-Asn, L-Gln, L-Asp, L-Glu, L-His, L-Pro, L-Tyr, L-Trp, L-Ser, L-Thr, L-Gly, L-Ala, L-Met, L-Cys, L-Phe, L-Leu, L-Val, and L-Ile; in order of hydropathy index [20]) was mixed with CBZ-L-Asp at an equi-molar ratio. Then, a single solvent among 14 different solvents (water, EG, DMSO, DMF, MeOH, MEA, ACN, EtOH, acetone, EtOAc, 2M2B, chloroform, 1-octanol, and *n*-hexane; in order of logarithmic partition coefficient, $\log P_{ow}$) was added to a binary mixture as an adjuvant.

For the preparation of a mixture CBZ-L-Asp/D-sugar, a single D-sugar among 12 different sugars (GAS, Suc, Mal, Lac, Sor, Man, Ino, Xyl, Glu, Gal, Fru, and GAL; in order of $\log P_{ow}$ value) was mixed with CBZ-L-Asp at an equi-molar ratio. Then, a single solvent among 6 different solvents (water, DMSO, DMF, MeOH, EtOH, and 2M2B; in order of $\log P_{ow}$ value) was added as an adjuvant. The $\log P_{ow}$ values of solvents and $\log S_w$ values of solutes were obtained from the literature [21–25].

2.3. Analysis of X-ray diffraction patterns

The X-ray diffraction (XRD) analysis (Rigaku, D/max; Rint 2000) was performed on an equi-molar mixture of either CBZ-L-Asp/L-Lys or CBZ-L-Asp/L-Tyr, which mixture was respectively analyzed before and after thermal treatment. In a 10-mL glass vial containing an appropriate mixture, either water or sodium hydroxide solution (3 M) was added as an adjuvant, then incubated at 95 °C for 30 min. For the XRD analysis, the liquefied mixture was re-solidified at room temperature for 48 h. The XRD pattern was obtained at 30 kV and 25 mA with a scan speed of $4^{\circ}\,min^{-1}$. The intensity of XRD peaks was plotted in Figs. 4 and 5 as a function of the corresponding 2θ diffraction angles.

3. Results and discussion

3.1. Melting temperature depression of a binary mixture depending on the adjuvant

It has been known that amino acids containing a protecting group such as acetyl (Ac), benzyloxycarbonyl (CBZ), *tert*-butyloxycarbonyl (Boc), or 9-fluorenylmethyloxycarbonyl (Fmoc) can favorably form binary eutectic melts with a counter compound [13,15]. We preliminarily confirmed that CBZ-containing compounds were most effective for lowering the binary melting temperature (data not shown). In this model study, CBZ-L-Asp was chosen as a melting stimulant and either an L-amino acid or a D-sugar was combinatorially mixed as a counter substrate. Melting temperatures of binary mixture (T_{MB}) were determined by adding 14 different adjuvants, respectively. Eventually, total number of

combinatorial setups of either CBZ-L-Asp/L-amino acid or CBZ-L-Asp/D-sugar reached either 280 or 72, respectively.

First, the T_{MB} value of CBZ-L-Asp/L-amino acid mixtures was measured under the presences or the absence of a solvent adjuvant. Table 1 shows the T_{MB} values of CBZ-L-Asp/L-amino acid mixtures containing 10% (w/w) solvent as an adjuvant. Table 1 was organized as the increasing order of the hydropathy index (i.e., hydrophobicity) of amino acids in the column, and as the increasing order of the hydrophobicity of a solvent adjuvant in the row. While most of T_{MB} value was higher than 100 °C without any adjuvant, the T_{MB} was highly depressed when relatively hydrophilic adjuvant was added in a mixture. When hydrophilic adjuvant, such as EG or DMSO, was added to the mixture the T_{MB} depression was occurred at the range of 50–60 °C. In contrast, when relatively hydrophobic adjuvant, such as chloroform, 1-octane, or *n*-hexane, was used, the T_{MB} depression was mere and the temperature showed the range of 95–105 °C. However, there was no clear correlation between the T_{MB} value and hydropathy index values of amino acids.

Next, a D-sugar was tested as a partner component. The adjuvant was added to an equi-molar mixture of CBZ-L-Asp/D-sugar at 10% (w/w). When the adjuvant such as water, DMSO, or DMF was added to the CBZ-L-Asp/D-sugar mixture, T_{MB} was depressed in an approximate range from 45 to 70 °C (Table 2). On the contrary, when the relatively hydrophobic adjuvants than DMSO or DMF were added, the T_{MB} values reached approximately 90 °C or higher. The hydrophilic adjuvant is supposed to be important to the T_{MB} depression, but the hydrophobicity of D-sugars did not display any strong correlations with the T_{MB} depression.

3.2. Melting temperature depression depending on the water solubility of a component

In Tables 1 and 2, hydrophilic adjuvants such as EG, DMSO, and DMF effectively induced the T_{MB} depression in a mixture of either CBZ-L-Asp/L-amino acid or CBZ-L-Asp/D-sugar. However in water adjuvant, T_{MB} varied with a wide range from 35 to 106 °C regardless of the hydrophobicity of either amino acids or sugars. For instance, a mixture with either L-Lys, L-Pro, L-Ser, or Gly showed the T_{MB} value from 33 to 41 °C in the water adjuvant, whereas a mixture with either L-Asp, L-Tyr, or L-Phe showed over 100 °C (Table 1). Interestingly, L-Lys, L-Pro, L-Ser, and Gly are highly soluble in water, whereas L-Asp, L-Tyr, and L-Phe are quite insoluble in water.

In this context, the T_{MB} values of mixtures were rearranged according to the water solubility of the amino acids, then plotted (Fig. 2). As the water solubility ($\log S_w$) of L-amino acids decreased, the T_{MB} values of the mixture increased. The lowest T_{MB} value was obtained in the mixture CBZ-L-Asp/L-Pro/water, wherein the L-Pro has the highest water solubility. On the contrary, the CBZ-L-Asp/L-Asp/water or CBZ-L-Asp/L-Tyr/water mixtures showed the highest T_{MB} values. As expected, the amino acids such as L-Asp and L-Tyr are almost insoluble in water. However, no correlation between the hydropathy index (hydrophobicity) of L-amino acids and the corresponding T_{MB} value was observed. The T_{MB} value of the CBZ-L-Asp/D-sugar mixture showed the similar profile with L-amino acid mixtures that T_{MB} decreased as increasing the water solubility ($\log S_w$) of D-sugars (Fig. 3). However there was no significant correlation between the mixture T_{MB} values and the hydrophobicity ($\log P_{ow}$) of the D-sugars.

3.3. The effect of adjuvants on the homogeneity enhancement in a binary mixture

In case of water adjuvant, the mixture CBZ-L-Asp/L-Lys/water displayed the lowest T_{MB} value of 35 °C, whereas the mixture CBZ-L-Asp/L-Tyr/water showed the highest T_{MB} value of 104 °C. We

Table 1
The melting temperature of the binary mixture CBZ-L-Asp/L-amino acid with different adjuvants.

Amino acid	Hydropathy index[a]	w/o Adjuvant	Water	EG	DMSO	DMF	MeOH	MEA	ACN	EtOH	Acetone	EtOAc	2M2B	Chloroform	1-Octanol	*n*-Hexane
L-Arg	−4.5	n.d.[b]	65	49	70	65	78	86	75	83	106	103	101	105	87	102
L-Lys	−3.9	n.d.	35	48	67	56	65	93	83	92	92	98	78	104	91	98
L-Asn	−3.5	101	94	54	52	55	60	78	100	98	106	109	90	100	91	97
L-Gln	−3.5	108	99	65	55	47	98	97	107	105	95	108	96	103	103	101
L-Asp	−3.5	107	105	62	48	57	100	83	107	104	104	99	101	100	99	99
L-Glu	−3.5	104	99	64	48	50	97	86	102	97	92	105	98	96	98	96
L-His	−3.2	106	89	43	62	63	76	92	105	95	106	109	94	104	104	99
L-Pro	−1.6	98	34	47	83	92	81	84	93	82	100	98	100	100	91	99
L-Tyr	−1.3	110	104	68	78	79	112	106	115	96	94	104	103	105	115	103
L-Trp	−0.9	n.d.	92	54	85	95	105	106	103	107	107	105	108	111	104	108
L-Ser	−0.8	n.d.	41	54	54	60	105	98	103	99	104	107	105	101	100	104
L-Thr	−0.7	105	70	60	54	58	101	103	98	101	99	104	102	101	93	102
L-Gly	−0.4	106	41	32	54	61	95	84	100	90	104	105	102	104	105	104
L-Ala	1.8	101	56	63	58	62	98	105	99	104	95	101	100	101	93	103
L-Met	1.9	n.d.	91	59	45	61	111	102	102	87	105	105	98	100	104	109
L-Cys	2.5	102	50	57	55	66	87	101	102	93	98	102	103	102	84	106
L-Phe	2.8	n.d.	101	74	65	50	97	101	113	104	106	112	90	112	103	101
L-Leu	3.8	n.d.	96	54	74	81	105	99	103	90	101	103	96	98	93	100
L-Val	4.2	102	81	65	71	84	93	103	97	102	102	98	93	103	97	103
L-Ile	4.5	n.d.	84	82	69	69	84	94	82	109	102	106	103	111	92	101

*All T_{MB} values were measured in triplicate, and averaged values were denoted with standard deviations of ±0.5 except water adjuvant.
[a] Hydropathy index values were obtained from the prior literature [20].
[b] n.d.: not detected.

Table 2
The melting temperature of the binary mixture CBZ-L-Asp/D-sugar with different adjuvants.

Sugars	log P_{ow}	w/o Adjuvant	Water	DMSO	DMF	MeOH	EtOH	2M2B
D-Gluconic acid[a]	−5.99	104	43	44	38	86	99	99
D-Lactose	−5.12	108	106	96	80	110	105	103
D-Maltose	−5.03	106	62	85	89	103	101	100
D-Galactose	−5.03	107	58	64	65	112	102	103
D-Sucrose	−4.27	105	60	55	52	94	99	106
D-Sorbitol	−3.01	88	49	52	60	91	82	84
D-Mannitol	−3.01	104	104	68	64	111	99	97
D-Glucose	−2.89	108	102	70	61	105	104	102
D-Xylitol	−2.56	76	53	46	36	81	80	80
D-Inositol	−2.08	96	99	64	58	102	102	96
D-Gluconic acid[b]	−1.98	101	70	52	46	106	93	95
D-Fructose	−1.55	91	65	57	60	101	106	101

*All T_{MB} values were measured in triplicate, and averaged values were denoted with standard deviations of ±0.5 except water adjuvant.
[a] D-Gluconic acid sodium salt form.
[b] D-Gluconic acid lactone form.

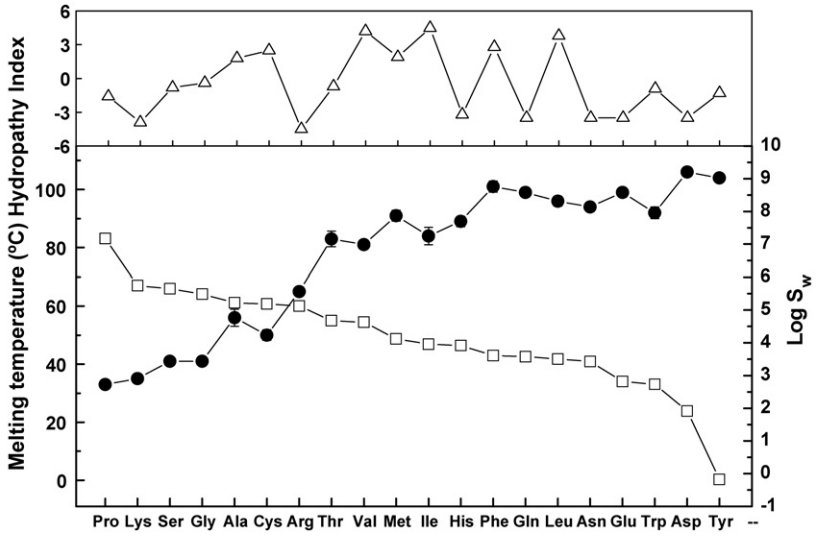

Fig. 2. A profile of the melting temperature depression in a binary mixture of CBZ-L-Asp/L-amino acid depending on the water solubility of L-amino acid. Deionized water was added at 10% (weight of water/weight of a mixture) as an adjuvant to an individual mixture. (△) Hydropathy index; (●) melting temperature; (□) log S_w.

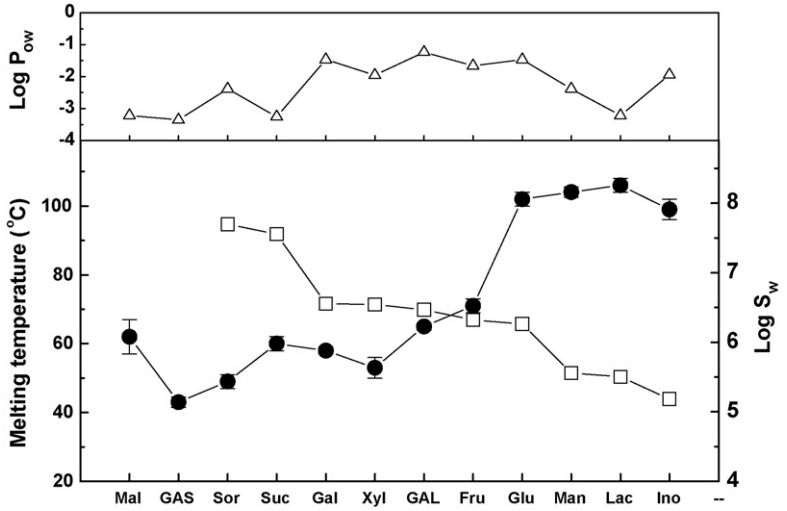

Fig. 3. A profile of the melting temperature depression in a binary mixture of CBZ-L-Asp/D-sugar depending on the water solubility of D-sugars. Deionized water was added at 10% (weight of water/weight of a mixture) as an adjuvant to each mixture. Mal: maltose; GAS: D-gluconic acid (sodium salt); Sor: D-sorbitol; Suc: D-sucrose; Gal: D-galactose; Xyl: D-xylitol; GAL: D-gluconic acid (lactone form); Fru: D-fructose; Glu: D-glucose; Man: D-mannitol; Lac: D-lactose; Ino: D-inositol; (△) log P_{ow}; (●) melting temperature; (□) log S_w.

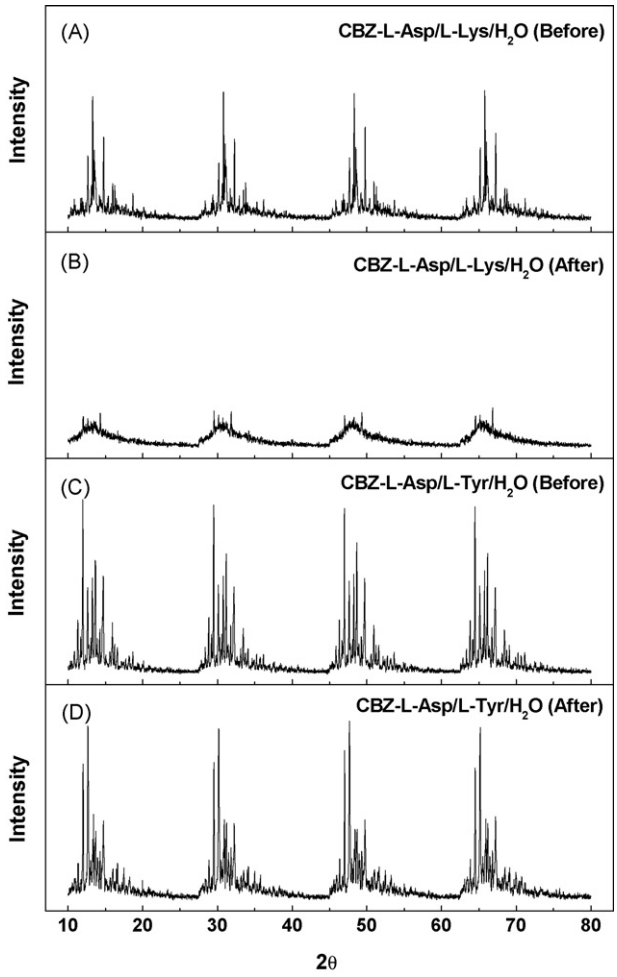

Fig. 4. The X-ray diffraction pattern of a mixture either CBZ-L-Asp/L-Lys/H_2O or CBZ-L-Asp/L-Tyr/H_2O. The XRD intensity of CBZ-L-Asp/L-Lys/H_2O was obtained from either before (A) or after (B) the heat treatment. The XRD intensity of CBZ-L-Asp/L-Tyr/H_2O was obtained from either before (C) or after (D) the heat treatment. Water was added at 10% (weight of water/weight of a mixture) as an adjuvant to each mixture.

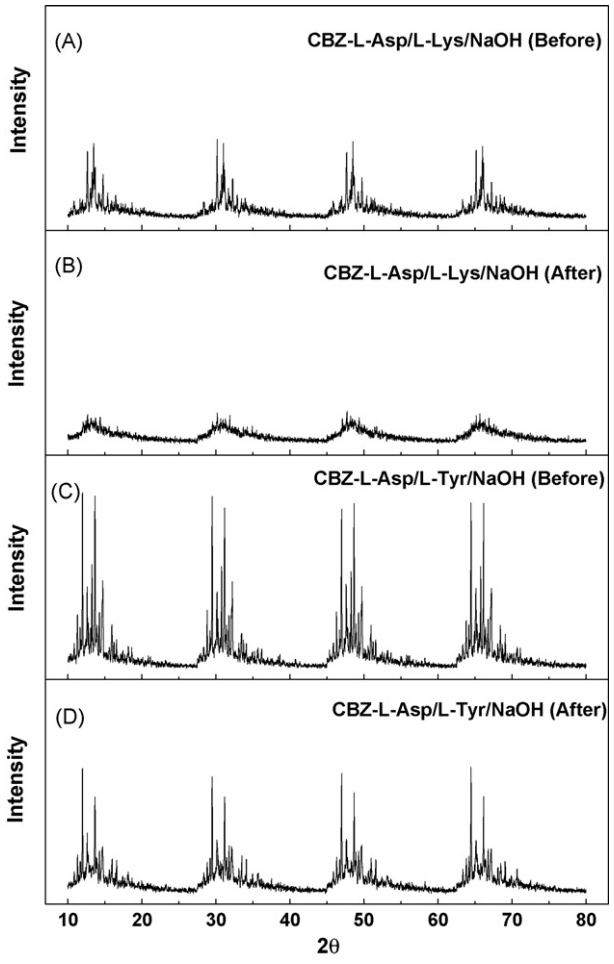

Fig. 5. The X-ray diffraction pattern of a mixture either CBZ-L-Asp/L-Lys/NaOH or CBZ-L-Asp/L-Tyr/NaOH. The XRD intensity of CBZ-L-Asp/L-Lys/NaOH obtained from either before (A) or after (B) heat treatment. The XRD intensity of CBZ-L-Asp/L-Tyr/NaOH obtained from either before (C) or after (D) heat treatment. Sodium hydroxide solution (NaOH, 3 M) was added at 10% (weight of NaOH solution/weight of a mixture) as an adjuvant to each mixture.

hypothesized that if solvent adjuvant can solubilize the counter component, T_{MB} value may be further decreased because it has been known that the eutectic melting is accommodated by the extension of homogeneously liquefied regions at the molecular level [26–28]. Furthermore, the molecular structure of equilibrated melts was much more homogeneous than the molecular structure of mixtures before melting. In this viewpoint, the sodium hydroxide solution (NaOH, 3 M) was selected an alternative adjuvant for the model mixture of CBZ-L-Asp/L-Tyr/water because L-Tyr showed high solubility in alkaline solutions such as NaOH solution.

It has been experimentally shown that the molecular homogeneity can be feasibly evaluated by examining the X-ray diffraction (XRD) patterns of mixtures before and after melting [16,18]. Thus, we compared the homogeneity of both mixtures, CBZ-L-Asp/L-Lys and CBZ-L-Asp/L-Tyr, by adding an adjuvant of either water or NaOH solution via XRD patterns. In XRD patterns of CBZ-L-Asp/L-Lys/water mixture, irregular sharp peaks and noisy baseline dramatically disappeared after the heat treatment (Fig. 4A and B). On the other hand, major large peaks and noisy base line were still remained except only some peaks even after the heat treatment (Fig. 4C and D). It was clearly shown that the enhanced homogeneity in a CBZ-L-Asp/L-Lys/water model was much distinguished before and after the heat treatment, whereas the structural homogeneity of CBZ-L-Asp/L-Tyr/water model was not. As a contrast to the water adjuvant, NaOH solution (3 M) was selected because it shows high solubility to both L-Lys and L-Tyr. When the NaOH solution was used as an adjuvant, the XRD peaks and base line noises of the CBZ-L-Asp/L-Lys/NaOH model were significantly decreased (Fig. 5A and B) as similar to the results in Fig. 4. Interestingly, in case of the CBZ-L-Asp/L-Tyr/NaOH model mixture, the XRD peaks were dramatically shorten to the half size of the original one (Fig. 5C and D). The enhanced homogeneity triggered by NaOH adjuvant was confirmed by the reduced height and intensity of XRD peaks (Figs. 4 and 5) because L-Tyr was highly soluble to the NaOH solution, otherwise nearly insoluble in pure water. These results verified our hypothesis that solubilization of a component in the binary mixture enables to decrease the T_{MB} values regardless of the hydrophobicity of the mixture component. Furthermore, solubilization of the mixture component to the adjuvant may increase the homogeneity and liquefaction area. This enhanced homogeneity of a mixture is supposed to be a driving force to formulate the eutectic media. Previous studies support the enhancement of homogeneity is the typical feature of eutectic melting [13,16,18,29].

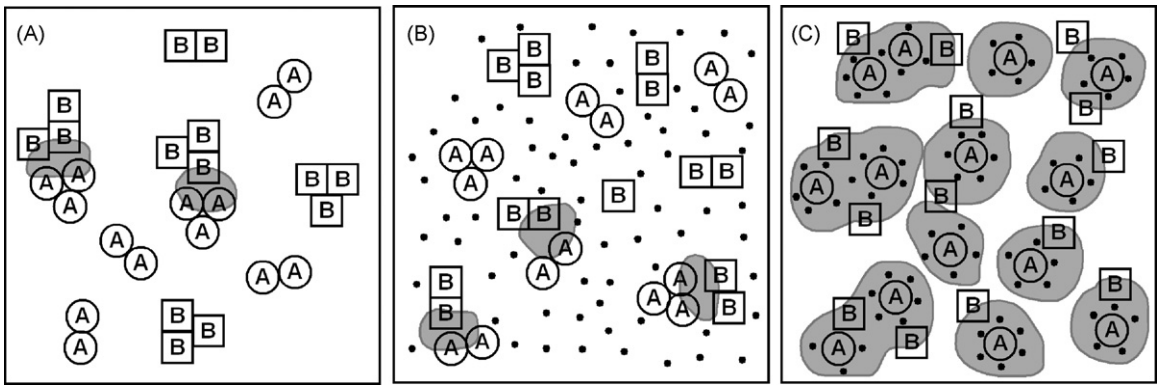

Fig. 6. The hypothetical schematic of the eutectic melting in a binary substrate mixture. (A) Eutectic melting of a binary substrate mixture comprising component A and B without any adjuvants. (B) Eutectic melting of a binary substrate mixture with an adjuvant, which is not soluble to either component A or B. (C) Eutectic melting of a binary substrate mixture with an adjuvant, which is highly soluble to substrate A. A circle containing 'A' demonstrates the individual molecule of component A, and a square containing 'B' demonstrates the individual molecule of component B. A black solid dot represents the individual molecule of an adjuvant solvent. A gray area represents a liquefied region.

Here, we classified three different situations of molecular interactions during the eutectic media formulation. The hypothetical process of eutectic melting was illustrated in Fig. 6. When the components A and B are mixed without any adjuvants (Fig. 6A), typical eutectic melting is triggered by the direct contact of solid components at higher temperature at a certain molar ratio of components A and B. However, most of powdery components may exist as a granular cluster before the eutectic melting. Even though the solvent is added as an adjuvant on the mixture of component A and B, melting point depression may not be clearly occurred as long as the component is insoluble to the adjuvant (Fig. 6B). This feature was experimentally confirmed in Fig. 4C and D. In contrast, if the component in a mixture is soluble to the adjuvant, solvation of component increase the area of liquefied region (gray region, Fig. 6C), resulting in the facilitated T_{MB} depression in a binary mixture (Fig. 6C). This schematic illustration supports the strong correlation between the melting point depression and the solubility of a substrate component on the adjuvant. Moreover, the schematic in Fig. 6B suggests a possible reason why some solvents do not work to reduce the T_{MB} value as an adjuvant.

4. Conclusion

As an alternative method of media engineering in biocatalysis, eutectic media has been newly defined and confirmed for the wide applicability. However, people have not known which solvent effectively promotes the eutectic media formulation as an adjuvant and how those solvents contribute to reduce the binary melting temperature. Based on our combinatorial databases, we found that the dissolution of substrate component to the adjuvant plays a crucial role to reduce the binary melting temperature. Our XRD results support that the dissolution of substrate component by adding adjuvants can initiate a dramatic enhancement of molecular homogeneity, which substantially contribute to formulate the eutectic media. Conclusively, our results enable to clarify which solvent should be selected as an adjuvant for the eutectic media preparation.

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