Development of Biocatalysts for Production of Fine Chemicals (Asymmetric Bioreduction Systems)

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Biocatalysis has matured into the standard technology for synthesizing industrially-important chemicals such as pharmaceuticals and agrochemicals. The principal advantage of biocatalysts is their ability to catalyze reactions with high specificity (often enantio- or regio-selectively). Furthermore, biocatalysts have the advantage of operating under mild conditions, typically at ambient temperature and pressure. Here, I present recent industrial applications of biocatalysts to perform asymmetric reduction synthesis.

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Introduction

In recent years efficient production with little impact on the environment has been demanded from chemical manufacturers. At Sumitomo Chemical, environmentally friendly green processes such as a vapor phase Beckmann rearrangement process[1] and a hydrochloric acid oxidation process[2] have been developed. According to OECD validations in 2002, it has been reported that with application of biotechnology, processes have become more environmentally friendly than they were. As a method for reducing environmental impact, biotechnology is substance production technology that makes use of enzymes and microorganisms, so-called biocatalysts. In the cells of living organisms, high reactivity and substance conversion with strict configuration recognition are put into practice under very mild ambient temperature and pressure, conditions and aqueous solutions. What catalyze these reactions in the cells are proteins that are known as enzymes. Production of industrial chemical products using these enzymes (or microorganisms that contain these enzymes) is attracting attention as technology that holds the possibility of contributing to a sustainable future.

Up until now at Sumitomo Chemical, processes have been developed as useful substance production methods for intermediates of synthetic pyrethroids using asymmetric hydrolysis techniques that use biocatalytic methods.[3]

In this paper, the author will introduce biocatalysts that can be used in the synthesis of optically active alcohols, which are important key compounds because they are intermediates for pharmaceuticals and agricultural chemicals, and in particular, he will introduce the development of techniques for asymmetric reduction enzymes and catalysts. In addition, a work on the engineering of enzymes to make industrial use of enzymes possible will be introduced.

Asymmetric Reduction Biocatalysts Developed by Sumitomo Chemical

Optically active alcohols are important chemical compounds for use as intermediates for the manufacturing of useful substances such as pharmaceuticals and agricultural chemicals. With ketone reduction using the normal methods of synthetic organic chemistry, racemic alcohols are often synthesized as so-called mixtures in which optical purity is not high. As methods for obtaining optically active alcohols with high purity, there have been reports of methods such as optical resolution of the racemic alcohols obtained and asymmetric synthesis[4] using asymmetric catalysts such as BINAP. Asymmetric reduction using biocatalysts has been known for a long time, and there have been reports of asymmetric reduction of β-keto esters and other compounds using baker's yeast.[5]

For most enzymes to exhibit catalytic activity, organic molecule cofactors known as coenzymes are necessary

to supplement the work of the enzymes. In oxidation-reduction reactions in vivo, coenzymes function as oxidizing agents or reducing agents, and typically ones such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) are used. These coenzymes have complex structures, so enzymes can be distinguished as coenzymes. Thus, in asymmetric reduction that uses biocatalysts, expensive coenzymes are necessary in mole equivalent amounts to the substrates, and up until now, industrial use has been difficult. Now it has become possible to synthesize the target optically active alcohols inexpensively and in high concentrations by coupling a recycling reaction that returns the oxidized coenzyme (NAD^+ or $NADP^+$) that has been used in the reduction to the reduced form (**Fig. 1**). **Table 1** shows some of the enzymes that catalyze the asymmetric reductions developed by Sumitomo Chemical and representative reducing substrates and the selectivity for them. All of them have been screened from natural microorganisms, and SCR-1 is an enzyme which is able to catalyze the reduction of β-keto esters with high optical purity. In addition, SCR-2–4 are enzymes that can selectively reduce α-keto esters, and SCR-3 and 4 can reduce enantioselectively carbonyl compounds even if there are large substituents in the ortho position (R_2).

SRC-5 and 6 have a broad substrate range, and can catalyze asymmetric reduction of acetophenone type compounds and β-keto esters. All of them have catalytic activity that can be used industrially, and since they work on a variety of substrates, they are used as Sumitomo Chemical's biocatalyst libraries. In addition, engineered enzymes in which the thermostability and solvent tolerance of these wild-type enzymes has been improved are used. In consideration of space in this paper, the author will limit himself to introducing the history of development focusing on SRC-1.

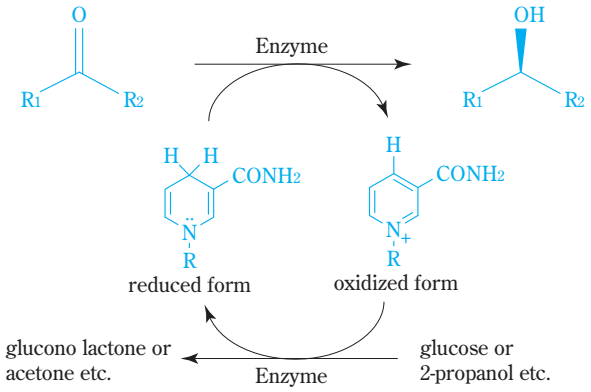
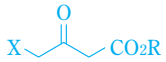
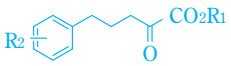
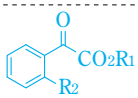
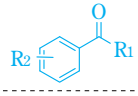
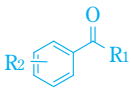


Fig. 1 Asymmetric reduction and recycling of the coenzyme

Table 1 Reductase libraries

Enzyme	Substrate	
SCR-1		99%*e.e.* (*S*)
SCR-2		99%*e.e.* (*R*)
SCR-3 SCR-4		> 99%*e.e.* (*R*)
SCR-5		> 99%*e.e.* (*R*)
SCR-6		> 99%*e.e.* (*S*)

Progression of Biocatalyst Development

Fig. 2 shows his strategy for the development of biocatalysts. First, microorganism that catalyzes the target reaction among microorganisms are searched for in nature. After finding a microorganism with the highest selectivity and reactivity, screening is carried out for the gene encoding enzyme (protein) that catalyzes the target reaction. By overproducing the target enzyme into a microorganism (host), such as *Escherichia coli*, which can serve as a recipient, a recombinant microorganism is constructed. Furthermore, if necessary, the performances of the enzyme are improved. Finally, the microorganism culture method for producing the biocatalyst in large quantities is established. Thus, gene recombination technology is indispensable for the development of biocatalysts. Since the amount of the target enzyme in

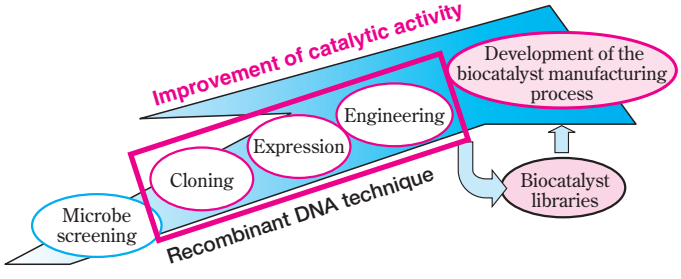


Fig. 2 Strategy for biocatalyst development

the wild-type microorganisms is small and the activity is low even when microorganisms that catalyze a target reaction are found, industrialization using the wild-type microorganisms is often difficult. By constructing recombinant organisms, the target enzyme can be produced in large amounts, and the enzymatic performances can be improved freely. In particular, gene recombination techniques have been established for *E. coli*, which is often used as a host, and since there is a large accumulation of the technology for culturing it, biocatalysts that can be used industrially can be developed with comparative ease. Currently, approximately 90% of the enzymes used worldwide are produced using such recombination techniques.

Development of SCR-1 Biocatalyst

1. Screening of Microorganisms for SCR-1 Production

The SCR-1 target compounds, 4-halo-3-hydroxybutyrates, are important compounds because they are key intermediates for pharmaceutical agents such as those for hyperlipidemia. Up until now, there have been many reports of methods for synthesizing 4-halo-3-hydroxybutyrates using biocatalysts, but at Sumitomo Chemical, development of a biocatalyst that can synthesize 4-bromo-3-hydroxybutyrate and that also has higher reactivity and can easily be used in the chemical procedures for the processes that follow has been attempted.

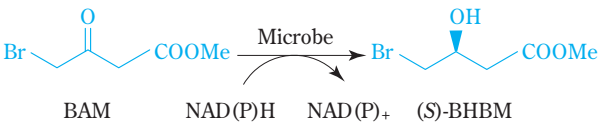


Fig. 3 Asymmetric reduction of BAM to BHBM

Since a biocatalytic method that could synthesize methyl 4-bromo-3-hydroxybutyrate (BHBM) using methyl 4-bromo-3-oxobutyrate (BAM) has hardly been investigated, the author investigated the distribution of BAM-reducing strains in natural microorganisms (**Fig. 3**). As a result, the author found that Penicillium citrinum produced optically active (*S*)-BHBM. In addition, the author found that Bacillus alvei produced the (*R*)-form (**Table 2**). The enantiomeric excess of BHBM was 98.1% *e.e.* and 95.5% *e.e.*, respectively, indicating a high degree of optical purity. In addition, the coenzyme requirements differed and were NADPH and NADH, respectively.

2. Cloning of the SCR-1 Gene

Next, the (*S*)-form selective BAM asymmetric reducing enzyme (SCR-1) from P. citrinum was purified and a cDNA clone encoding SCR-1 on the basis of partial amino acid sequences was isolated.[6] In homology searches using the presumed amino acid sequence from the gene sequence information, the primary structure of SCR-1 showed 85% identity to Aspergillus nidulans glycerol dehydrogenase, but SCR-1 did not catalyze the dehydrogenation of glycerol. In addition, SCR-1 had strong similarity with the proteins of the aldo-keto reductases (AKR), and since the amino acid residues that contribute to the catalytic activity are conserved, SCR-1 was classified as a new enzyme member of the yeast AKR (AKR3E1) according to the updated AKR nomenclature system.

3. Expression of the SCR-1 Gene in *E. coli* and Characterization of SCR-1

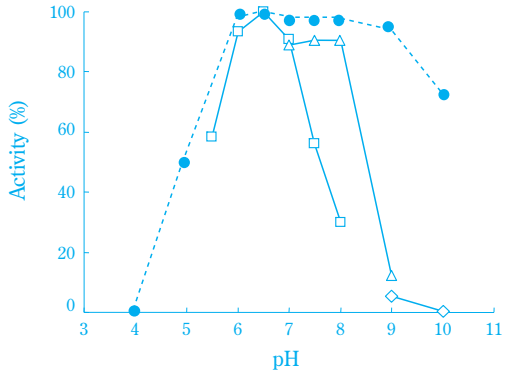
The cloned SCR-1 gene was overexpressed in *E. coli*, and recombinant *E. coli* cells with high productivity for SCR-1 were constructed. SCR-1 was purified from the cell-free extract of the recombinant *E. coli* cells, and as

Table 2 Biotransformation of BAM by acetone-dried microorganisms

Microorganisms	Coenzyme availability					
	NADPH			NADH		
	Molar yield (%)	Stereo- selectivity	*e.e.* (%)	Molar yield (%)	Stereo- selectivity	*e.e.* (%)
Arthrobacter paraffineus	4.2	*S*	54.7	4.1	*S*	27.7
Bacillus alvei	44.1	*R*	71.6	30.0	*R*	95.5
Rhodotorula minuta	10.1	*S*	82.1	1.0	N.T.[1)]	N.T.
Cryptococcus humicolus	12.9	*S*	88.0	0.4	N.T.	N.T.
Penicillium citrinum	36.0	*S*	98.1	N.D.[2)]	N.T.	N.T.

1) N.T.: not tested, 2) N.D.: not detected.

a result of various investigations, the recombinant SCR-1 was found to have an approximately 37 kDa molecular weight that was the same as the wild SCR-1 isolated and purified from *P. citrinum*, and the ability for asymmetric reduction of BAM selectively to the (*S*)-form was confirmed. In addition, the coenzyme requirement was NADPH. The enzyme showed maximum activity at pH 6.0–6.5, and the enzyme was stable in the range of pH 6–10 (**Fig. 4**). With heat treatment at 40°C for 20 minutes, 75% of the initial activity remained, but with heat treatment at 50°C for 20 minutes activity was lost.[7] In addition, as a result of examinations of the substrate specificity of this enzyme, strong reduction activity was observed with 4-halo-3-oxobutyrate derivatives, and besides these, reduction activity was found for dihydroxyacetone, 1,1-dichloroacetone and 1-bromo-1-indanone (**Table 3**).



SCR-1 activity (solid lines) and stability (dashed lines) as a function of pH. The activity was measured in the following buffers (0.1 M): KPB (pH 5.5–8.0; open squares), Tris-HCl buffer (pH 7.0–9.0; open triangles), and Tris-glycine buffer(pH 9.0–10.0; open diamonds). The remaining activity of SCR-1 (filled circles) was also measured after incubation in the following buffers at 20°C for 1 h: citrate-K_2HPO_4 buffer (pH 4.0–5.0), KPB (pH 6.0–8.0), Tris-HCl buffer (pH 8.0–9.0) and Tris-glycine buffer (pH 10.0).

Fig. 4 Optimum pH and pH stability of SCR-1

Table 3 Substrate specificity of SCR-1

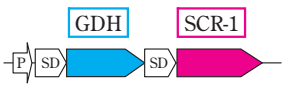
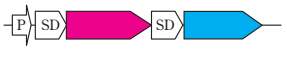
Substrate	Relative activity (%)
Aldehydes	
Acetaldehyde	2.2
n-Butylaldehyde	3.4
n-Valeraldehyde	2.8
DL-glyceraldehyde	3.2
Pyridine-3-aldehyde	2.0
Ketones	
Ethyl 4-Chloro-3-oxobutyrate	100
BAM	139
Ethyl 4-bromo-3-oxobutyrate	667
Isopropyl 4-bromo-3-oxobutyrate	125
Octyl 4-bromo-3-oxobutyrate	24
1,1-Dichloroacetone	42
Chloroacetone	2.5
3-Chloro-2-butanone	2.4
Methyl 3-oxopentanoate	1.2
2-Bromo-1-indanone	20
Dihydroxyacetone	56

4. Construction of Recombinant *E. coli* Cells Containing SCR-1 and a Cofactor-regeneration Enzyme

Next, in addition to the SCR-1 gene, a glucose dehydrogenase (GDH) gene was overexpressed into *E. coli* cells for in situ regeneration of NADPH, and recombinant *E. coli* cells that expressed simultaneously the SCR-1 and GDH genes was constructed. First, circular DNA (plasmid DNA) producing SCR-1 and GDH was constructed. Plasmid DNA (**Table 4** a)) arranged in the order of the GDH gene then the SCR-1 gene downstream of the DNA region (promoter) for accommodating gene expression, plasmid DNA (**Table 4** b)) substituted in the order of the SCR-1 gene then the GDH gene, and plasmid DNA (**Table 4** c)) substituted in the order of the GDH gene then the SCR-1 gene and in which the last base (A) of the GDH gene and the first base (A) of the SCR-1 gene were coupled were each constructed. BAM reduction activity and GDH activity in the cell-free extract of recombinant *E. coli* cells harboring each plasmid were evaluated. As a result, *E. coli* cells with the plasmid DNA in **Table 4** c) were comparatively good and balanced regarding the proportion of BAM reduction activity to GDH activity (**Table 4**).

Therefore, *E. coli* cells harboring the plasmid DNA in **Table 4** c) were used for further (*S*)-BHBM production experiments.

Table 4 Activities of SCR-1 and GDH of recombinant *E. coli* cells

Plasmid	SCR-1 activity (units/mL of culture)	GDH activity (units/mL of culture)
a) 	2.5	26.1
b) 	11.7	0.8
c) 	6.6	29.0

P: Promoter, SD: Shine-Dalgarno sequence

The reaction was carried out in a two-phase system of *n*-butyl acetate and phosphate buffer solution (pH 6.5). The reaction mixtures contained recombinant *E. coli* cells, $NADP^+$ and glucose in aqueous solution, to which BAM dissolved in *n*-butyl acetate was added. The reaction was carried out at 30°C with pH controlled to 6.5. As a result, (*S*)-BHBM was produced with a chemical yield of 100% and an optical purity of 97.9% *e.e.* after a two-hour reaction.

5. Engineering of SCR-1 (Improvement of Thermostability)

The stability of biocatalysts is important for using them industrially. Therefore we attempted to improve the thermostability of SCR-1. A variety of methods have been reported for improving the properties of enzymes, and artificially changing (substituting, deleting, adding, etc.) the arrangement of the amino acids, which are the constituent elements of the enzyme, is known as one of these methods. Rational design requires detailed knowledge of protein structure, function and catalytic properties, and relies on molecular modeling to predict relevant amino acid changes in the protein. However, SCR-1 is a new enzyme, and since the three-dimensional structure could not be determined, engineering of SCR-1 was made by a method of randomly introducing amino acid substitutions into the enzyme and selecting from the mutant libraries using the target properties and characteristics as indices.

As a result of screening SCR-1 mutants in which the thermostability for retained reduction activity was improved even with heat treatment at 40°C for two hours from bacterial clones of approximately 4000, three engineered enzymes, A3-49, A8-39 and T1-99, were found (**Table 5**). It was clear that, in A3-49, the amino acid position 245 had arginine substituted for lysine (K245R). In A8-39, the amino acid position 271 had aspartic acid substituted for asparagine (N271D), and in T1-99, there were two amino acid substitutions such that the amino acid position 54 had glutamine substituted for leucine (L54Q) and the amino acid position 104 had cysteine substituted for arginine (R104C).

T1-99 not only enhanced thermostability, but also improved the enantioselectivity for BAM. Next, to further improve the thermostability, the author reengineered the four amino acid substitutions that clearly contributed to thermostability. As a result, the highest level of thermostability was exhibited by a triple mutant containing L54Q, K245R, and N271D (**Table 6**). In addition, the engineered L54Q variant of SCR-1 clearly had improved thermostability and enantioselectivity. Therefore, leucine at amino acid position 54 was substituted with the other 19 amino acids, and the effects on the thermostability and enantioselectivity were examined. As a result, the thermostability was improved when amino acid position 54 had an amino acid having a positive charge or negative charge (lysine (K), arginine (R), histidine (H), aspartic acid (D) and glutamic acid (E)) substituted for the leucine. These results indicate that electrostatic interactions or hydrogen bonds contribute favorably to protein stability at elevated temperatures. In addition, most of the L54 mutants, with the exception of phenylalanine and tryptophan, exhibited significantly increased enantioselectivity for BAM and 4-chloro-3-oxobutyrate (CAE) (**Table 7**).

The engineered L54Q variant of SCR-1 (SCR-1 (L54Q)) was purified and the enzymatic properties examined. The Km value, which is an index showing the affinity for a substrate, was equivalent to that of the wild-type enzyme (SCR-1). To characterize the thermostability of SCR-1 (L54Q) in more detail, the enzyme was incubated at various temperatures for 30 minutes,

Table 5 Thermostability and enantioselectivity of SCR-1 mutants generated by random mutagenesis

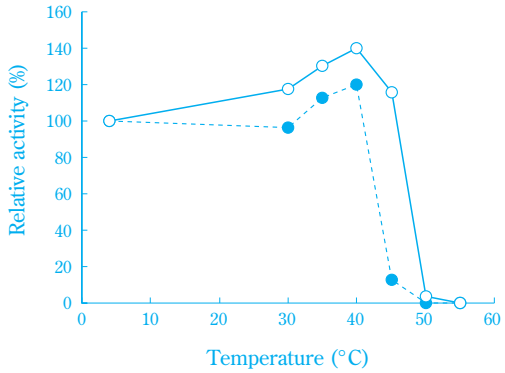
Clone	Mutation site(s)	Remaining activity at 40°C for 2 h (%)	(*S*)-BHBM *e.e.* (%)
SCR-1	None	1.7	97.1
A3-49	K245R	3.8	97.0
A8-39	N271D	10.1	96.8
T1-99	L54Q/R104C	74.9	99.0

Table 6 Thermostability and enantioselectivity of SCR-1 mutants generated by site-directed mutagenesis

Mutation site(s)	Remaining activity at 45°C for 7 h (%)	(*S*)-BHBM *e.e.* (%)
None (wild-type)	0	97.1
L54Q	54.1	98.7
R104C	0	97.7
L54Q/R104C	9.6	99.0
N271D	0	96.8
L54Q/N271D	53.1	98.6
L54Q/R104C/N271D	41.7	98.8
K245R	0	97.0
L54Q/K245R	42.3	98.6
L54Q/K245R/N271D	69.9	98.3
L54Q/R104C/K245R/N271D	18.1	98.7

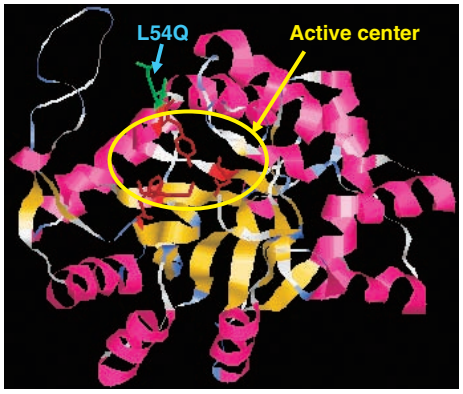
Table 7 Thermostability and enantioselectivity for BAM and CAE of SCR-1(L54) mutants generated by saturation mutagenesis

Mutation	Remaining activity at 45°C for 2 h (%)	BAM		CAE	
		Relative activity (%)	(*S*)-BHBM *e.e.* (%)	Relative activity (%)	(*S*)-CHBE *e.e.* (%)
None (wild-type)	0	100	97.1	100	63.1
L54G	64.0	113	98.3	127	89.1
L54S	7.7	101	98.8	151	91.2
L54T	2.2	122	97.7	131	70.4
L54C	5.0	67	97.5	123	76.9
L54Y	0	114	98.4	285	86.7
L54N	49.6	90	98.3	102	88.7
L54Q	53.8	153	98.8	245	89.7
L54A	23.5	125	98.7	175	92.6
L54V	0	69	98.8	59	84.8
L54I	0	84	98.6	128	78.1
L54M	9.9	107	98.2	138	83.7
L54P	0	119	97.4	190	80.9
L54F	0	152	95.5	206	67.2
L54W	0	52	96.9	128	74.8
L54K	49.2	100	98.1	74	82.8
L54R	41.3	147	98.6	123	88.6
L54H	51.4	121	97.4	173	82.2
L54D	38.3	97	98.4	124	90.4
L54E	12.9	110	98.9	97	90.3



Purified enzyme was incubated for 30 min at the indicated temperature, and activity was determined

Fig. 5 Effect of temperature on the stability of SCR-1 (L54Q) (open circle) and wild-type SCR-1 (filled circle)



This model based on the crystal structure of AKR4C9 from *Arabidopsis thaliana* (PDB code: 3h7u)

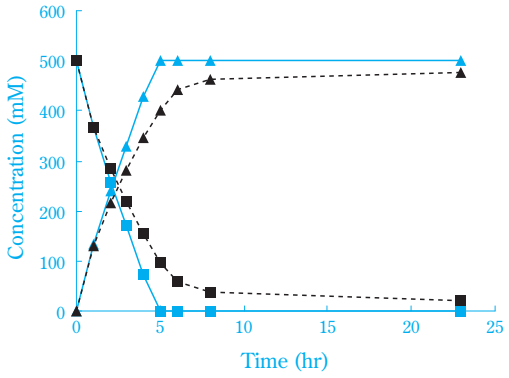
Fig. 6 Homology modeling of SCR-1

and residual BAM reduction activity was measured. SCR-1 (L54Q) retained its full activity after heat treatment at 45°C for 30 minutes, whereas only slight activity of wild-type SCR-1 was observed after the same treatment (**Fig. 5**). From the results of homology modeling (**Fig. 6**), the amino acid position 54 in SCR-1 is located near the active site in the primary sequence; therefore, amino acid substitution of L54 of the protein may not only improve the thermostability, but also increase the enantioselectivity.

6. Construction of Recombinant *E. coli* Cells Containing the Engineered SCR-1 (L54Q) and Cofactor-regeneration Enzyme

E. coli cells were transformed with the plasmid DNA which had undergone recombination of the SCR-1 gene part for the plasmid DNA shown in **Table 4** c) to the SCR-1 (L54Q) gene with its improved thermostability and enantioselectivity. As a result of comparisons with recombinant *E. coli* cells in which both the wild SCR-1 and GDH were overproduced together and the recom-

binant obtained was used, the BAM reduction activity (initial reaction rate) and GDH activity (initial reaction rate) were substantially the same. When a BAM asymmetric reduction was carried out, (*S*)-BHBM could be synthesized with a chemical yield of 100% and optical purity of 98% *e.e.* in a five-hour reaction with SCR-1 (L54Q), and with the wild SCR-1 BAM conversion substantially stopped at eight hours of reaction, and the chemical yield was 93% with optical purity of 97% *e.e.* (**Fig. 7**). With SCR-1 (L54Q) the thermostability was improved over the wild SCR-1, and it can be assumed that because it was stabilized, the productivity improved.[8)]



BAM (filled square), (*S*)-BHBM (filled triangle)

Fig. 7 Time-course of BAM reduction to (*S*)-BHBM by recombinant *E. coli* expressing wild-type SCR-1 (*dashed line*) and SCR-1 (L54Q) (*solid line*)

7. Comparison with BAM Asymmetric Reduction Using SCR-5 and SCR-6

Besides SCR-1, S form selective asymmetric reduction of BAM is also possible with SCR-5 shown in **Table 1**. SCR-5 is used for a variety of ketone compounds. It is possible to make use of the 2-propanol dehydrogenation activity that SCR-5 itself has in a regeneration system for coenzymes, and it depends on NADH which is less expensive than NADPH (**Fig. 8**).[9)] In addition, asymmetric reduction of BAM with *R* form selectivity is possible with SCR-6. As with SCR-5, the substrate selectivity is broad, and it is possible to use the 2-propanol dehydrogenation activity that SCR-6 itself has in a regeneration system for coenzymes. It also depends on NADH (**Fig. 8**).[10)]

Therefore the author conducted a comparative examination of the BAM asymmetric reduction with that of SCR-1. Moreover, the engineered enzyme (SCR-1 (L54Q)) with improved thermostability was used for SCR-1, and an engineered enzyme (SCR-6 (HAR1))[11)] was also used for SCR-6. All of the reactions were carried out in a two-phase system in which *n*-butyl acetate and a buffer solution were added at a proportion of 1:1. To recycle the coenzyme with SCR-1 (L54Q), glucose was added and the reaction was carried out with the pH controlled to 6.5. 2-propanol was added and the reaction carried out to recycle the coenzyme with SCR-5 and SCR-6 (HAR1). As a result, when *E. coli* cells that overproduced both SCR-1 (L54Q) and GDH were used, (*S*)-BHBM was synthesized with a chemical yield of 86% (589 mM) and an optical purity of 99% *e.e.* or greater in a four hour reaction time (**Table 8**). When SCR-5 was used, (*S*)-BHBM was synthesized with a chemical yield of 97% (413 mM) and an optical purity of 99% *e.e.* or greater, but 53 hours were required until BAM conversion was completed. In addition, when SCR-6 (HAR1) was used, substantially 100% of the BAM was converted in 10 hours, and (*R*)-BHBM was synthesized with a chemical yield of 80% (176 mM) and an optical purity of 99% *e.e.* or greater, but productivity was low and a large amount of biocatalyst was required. From the results above, the SCR-1 (L54Q) system was the most suitable for BAM asymmetric reduction, and this can be thought of as industrially implementable. It can be assumed that because SCR-1 (L54Q) has a higher degree of specific

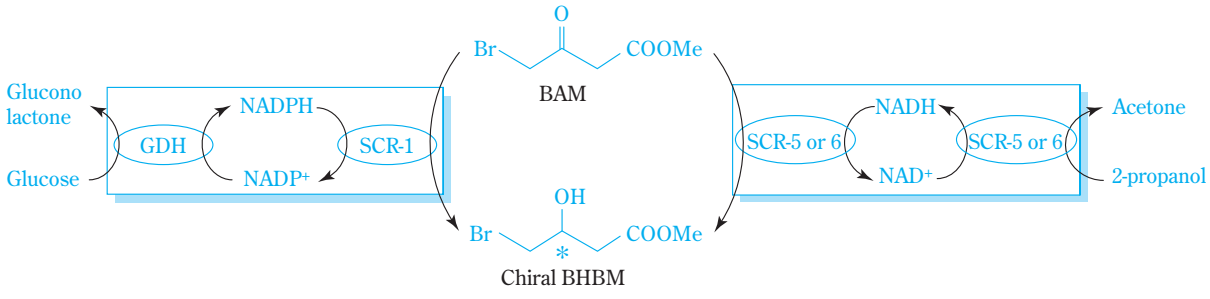


Fig. 8 Asymmetric reduction of BAM and recycling of the coenzyme

Table 8 Biocatalytic reduction of BAM

Enzyme	Coenzyme	Wet cells (w/w-BAM)	Reaction time (h)	Final concn. of BHBM (mM)	Yield (%)	*e.e.* (%)
SCR-1(L54Q)+GDH	NADPH	0.23	4	589	86	> 99 (*S*)
SCR-5	NADH	0.55	53	413	97	> 99 (*S*)
SCR-6(HAR1)	NADH	6.6	10	176	80	> 99 (*R*)

Table 9 Properties of BAM reduction enzymes

Enzyme	Specific activity for BAM (units/mg protein)	Activity in *E. coli* cells (units/g wet cells)	Half-life in the two-phase system (h)
SCR-1(L54Q) +GDH	144	179	17
SCR-5	9.1	13	3
SCR-6(HAR1)	0.5	0.4	0.5

activity for BAM and stability in two-phase systems of *n*-butyl acetate and a buffer solution than SCR-5 and SCR-6 (HAR1), the BHBM productivity was higher (**Table 9**).[12]

However, SCR-5 and SCR-6 (HAR1) can effectively regenerate NADH by transferring hydrogen from 2-propanol. Acetone, which is a by-product of the cofactor-regeneration reaction, does not generate a pH change during the enzymatic reaction. Furthermore, they prefer NADH as a cofactor, which is more stable and inexpensive than NADPH. Therefore, they are advantageous in terms of process and cost, and it is possible that processes suitable for BAM asymmetric reduction can be established through further investigations of reaction conditions. In addition, application to the production of other useful compounds can be expected in the future.

Conclusion

Industrially useful chemical products must be synthesized from inexpensive starting compounds through multiple processes, and it is important to construct production processes with consideration given to the connections to the processes that follow. At the Sumitomo Chemical Organic Synthesis Research Laboratory we have developed hybrid processes combining organic synthesis and bioprocesses which are carried out in environmentally friendly total process development. The production methods using the asymmetric reduction enzymes introduced above are a unit bioprocess for one process. However, most organisms have conventionally carried out strict reaction control under mild conditions. The energy production and substance production to continue life are magnificently carried out inside the organism (one pot) regardless of the many stages of chemical processes. Fermentation methods skillfully use the functions for metabolizing substances that are carried out by these organisms, and this is technology for producing useful compounds necessary for mankind from inexpensive raw materials in a single pot. Enzymes are often used in organic solvents. Furthermore, there have also been reports of efficient production techniques for target products with combined use of metal catalysts.[13] If such techniques are integrated, the author assumes that many useful substances can be efficiently produced by bioprocesses.

On the other hand, the current situation is still one in which many enzymes that catalyze target reactions are discovered by screening from microorganisms that occur naturally. These microorganism screening methods are useful, but because it is said that the microorganisms that humans can culture are only around 0.1 to several percent[14] of all of the microorganisms, there are limitations, and time and techniques (screening sense) are required. These problems can be conquered by the recent remarkable progress in biotechnology, such as complete analysis from gene and protein databases, search techniques, direct acquisition of DNA without culturing microorganisms, application techniques and high-speed analysis techniques for genome DNA using next-generation sequencers. In addition, progress is being made in developing enzymes with improved performance by artificially engineering enzymes obtained from the natural world as introduced in this paper. It can be assumed that it will take still more time for human beings to be able to construct their own target enzymes freely. But if the three-dimensional structures for various enzymes and the mechanisms operating in reactions become clear, more precise enzyme improvement will become possible. Moving forward, the author wants to establish environmentally friendly production methods for useful substances using these biocatalysts.

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