HIGH PRESSURE IN ENZYME CATALYZED ORGANIC REACTIONS

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Abstract - Adenosine deaminase and lipases catalyzed hydrolysis under high pressure were described. The hydrolysis by both enzymes was facilitated by high pressures. This technique was used for the kinetical resolution of racemic highly modified nucleosides having biological activities.

1. INTRODUCTION

High pressure technique has been widely used for the organic synthesis, and has become a strong tool in the field of organic chemistry.[1-8] The relation between reaction rate and pressure is shown by formula (1), in which $\Delta V^{\ddagger}$ shows activation volume.[9] According to the formula, the reaction rate increases with increasing of negative activation volume. As typical reactions, pericyclic reactions, reactions through polar transition states, and reactions with steric hindrance are accerelated by high pressure and all these reactions have large negative activation volume. For example, the activation volume of Diels-Alder reaction is between -25 - -50 cm^3/mol. If it is -30 cm^3/mol, the reaction is accerelated 7.7 $X10^7$ times under 1.5 GPa.

High Pressure Mediated Reaction

$$\frac{d \ln k}{dp} = - \frac{\Delta V^{\ddagger}}{RT} \quad \text{------} \quad (1)$$

k : rate constant, p : pressure,
$\Delta V^{\ddagger}$: activation volume
Diels-Alder reaction : $\Delta V^{\ddagger}$ = - 25 ~ - 50 cm^3/ mol

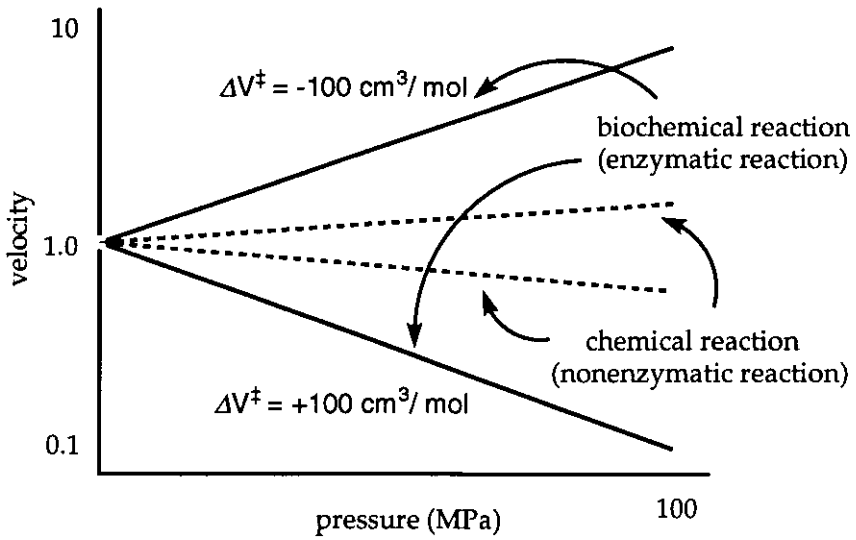
- pericyclic reactions
- reactions through polar transition states
- reactions with steric hindrance

$\Delta V^{\ddagger}$ (cm^3 / mol)	k_p / k_1		
	0.5 GPa	1.0 GPa	1.5 GPa
- 30	430	1.8×10^5	7.7×10^7

It is reported that a biochemical reaction (enzymatic reaction) has a large activation volume ($\Delta V^{\ddagger} = \pm 100$ cm^3/mol) compared with a chemical reaction.[10] It would be

due to the three-dimensional variation of huge molecule structure (enzyme). This means that we can expect effective promotion of biochemical reactions under high pressure. However, few references are available concerning the enzymatic reaction under high pressure.[11]

Comparison between Bichemical and Chemical Reactions under High Pressure

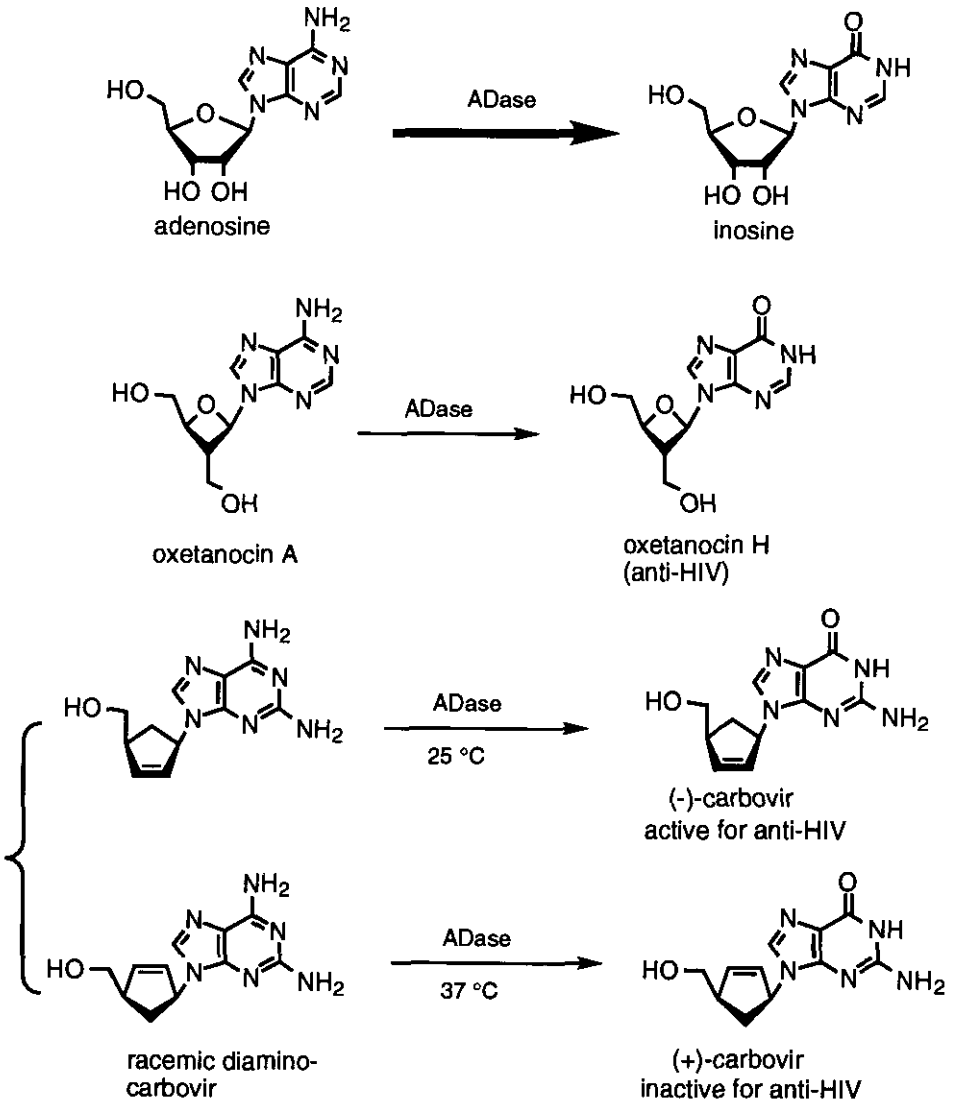


Enzyme catalyzed reactions have been recently used very often in the field of organic synthesis from the following reasons: 1) the reaction proceeds under mild conditions (room temperature and atmospheric pressure); 2) the reaction proceeds with highly regio-, enantio-, and stereo-selectivity; 3) the reaction does not use hazardous chemicals.[12] Therefore, the enzyme catalyzed organic reaction is attractive in the practical synthesis of optically active biological substances. On the contrary, the enzymatic reaction is often quite specific to substrates compared with a common chemical reaction and hence its application is restricted. Recently, we have found that the deamination by adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4: ADase) is remarkably facilitated by high pressure.[13] This is the first instance to show that the enzymatic reaction which hardly proceeds under normal condition has been greatly promoted by high pressure. This methodology has possibility to broaden the scope of the enzymatic reactions. In this review, the deamination and deacylation of highly modified nucleosides having antiviral activities under high pressure are described, which would provide an efficient method for the kinetical resolution of biologically active nucleosides.

2. DEAMINATION OF HIGHLY MODIFIED NUCLEOSIDES BY ADASE UNDER HIGH PRESSURE

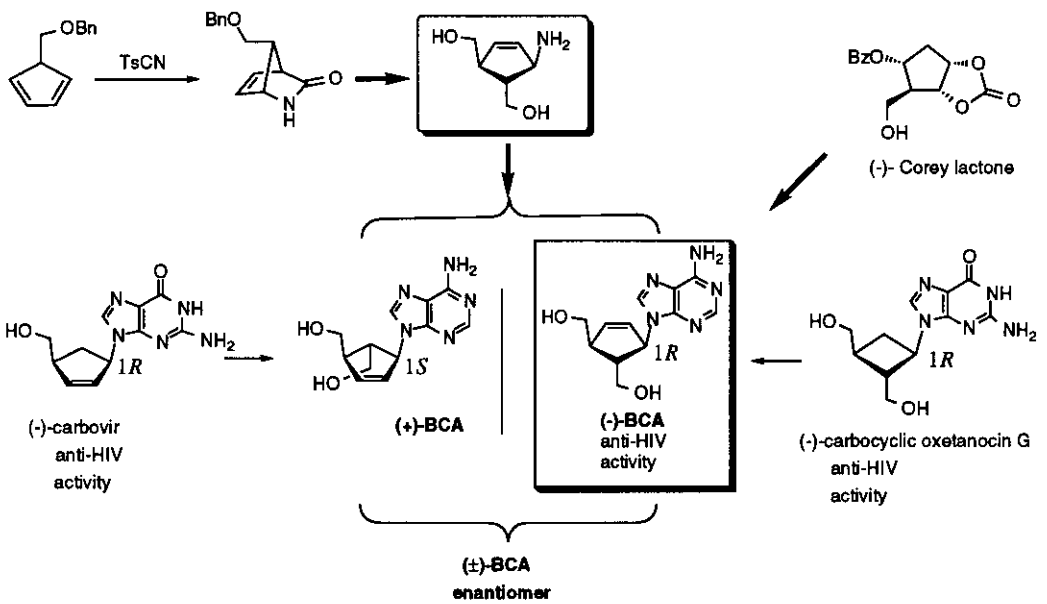
Adenosine deaminase (ADase), one of the most important enzyme in purine metabolism, catalyzes the hydrolysis of adenosine to inosine and ammonia.[14] Lack of ADase causes severe combined immunodeficiency desease (SCID).[15] It is also known

that the levels of ADase change in the patients of acquired immunodeficiency syndrome (AIDS), anemia, various lymphomas, and leukemias.[16] Recently, Quiocho and his coworkers[17] have proposed an elegant mechanism for the deamination by ADase on the basis of the X-Ray crystallographical analysis of the complex with 6*R*-hydroxy-1,6-dihydropurine ribonucleoside (HDPR). ADase has recently been used for the preparation of pharmacologically active substances. For example, natural oxetanocin A is hydrolyzed to oxetanocin H having anti-HIV activity.[18] The same enzymatic hydrolysis was applied to the resolution of (±)-carbovir to each enantiomers by Vince and coworker.[19] Thus, (±)-diamino-carbovir was deaminated by ADase (0.3 units/μmol) at 25°C for 72 h to give selectively (-)-carbovir having anti-HIV activity whereas the unreacted enantiomer was treated with excess of the same enzyme (20 units/μmol) at 37°C for 48 h to give (+) carbovir showing no activity.



2.1. Deamination of BCA having anti-HIV activity

9-[*c*-4,*t*-5-Bis(hydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine (BCA) having significant anti-HIV activity was developed in our laboratory.[20] The racemic BCA synthesized from a cyclopentenylamine corresponds to the hybrid nucleoside of carbovir[21,22] and carbocyclic oxetanocin,[23,24] both of which are potent anti-HIV reagents. Chemical resolution of racemic BCA and biological evaluation of each enantiomer revealed that only (-)-BCA showed anti-HIV activity.[25] The absolute structure of (-)-BCA was determined to be (1*R*, 4*S*, 5*R*) by its total synthesis from (-)-Corey lactone.[26,27]

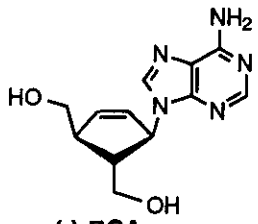


In order to provide an economical preparation of (-)-BCA, we investigated the deamination of (±)-BCA by ADase to the corresponding hypoxanthine derivative and found that, though the reaction did not proceed at atmospheric pressure, the reaction under high-pressure resulted in the formation of (-)-BCH (H designates hypoxanthine base) in nearly 100% *ee*. The findings that the optimum pressure for the reaction was *ca*. 400 MPa (which can be achieved in a large-scale apparatus), and that BCH can be chemically converted to BCA, provided the basis for an extremely economical route to (-)-BCA.

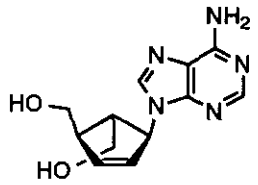
Before investigating the deamination reaction, we examined the binding[28,29] of racemic and enantiomeric BCA to ADase by competitive experiments. It was found that BCA inhibited the deamination of adenosine, and that the binding ability of (+)-BCA to the enzyme was much stronger than that of (-)-BCA.

Although (-)-BCA and (+)-BCA bound to ADase, they were not deaminated at ambient conditions. Then, we investigated the deamination under high-pressures, for the following two reasons. 1) A well-known advantage of the high-pressure technique over reactions carried out under atmospheric pressure in the field of synthetic organic chemistry is an increase of the reaction rate when the reaction has a large negative activation volume ($\Delta V^{\ddagger}$). 2) The high pressure investigation in the

Inhibition of the Deamination of Adenosine with ADase by BCA



(-)-BCA



(+)-BCA

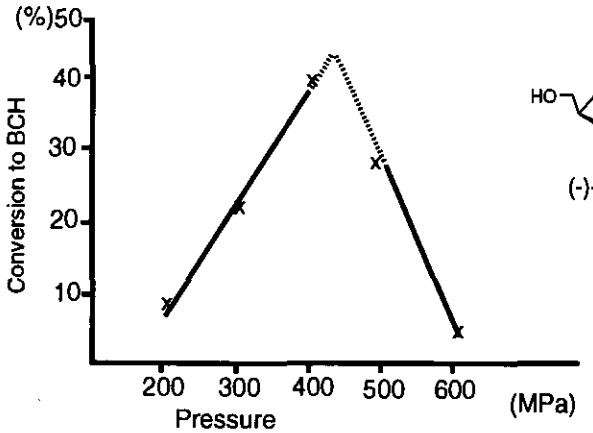
	Substrate	Inhibitor	Ki (μM)
(-)-BCA	-	+	265
(+)-BCA	-	+++	82
(±)-BCA	-	++	90

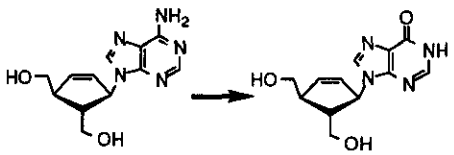
+ : acted as; -: did not act as

area of enzymatic organic synthesis might be useful not only for the preparation of pharmacologically active substances or their intermediates but also for elucidation of the catalytic mechanism of enzymes.

Since (-)-BCA is readily available from (-)-Corey lactone, we first investigated the deamination of (-)-BCA to (-)-BCH {(1*R*, 4*S*, 5*R*)-9-[4,5-bis(hydroxymethyl)-cyclopent-2-en-1-yl]-1*H*,9*H*-hypoxanthine} under various pressures at 22 °C for 12 h. As expected, the rate of deamination increased with increasing pressure. It reached a maximum at *ca.* 400 MPa, and then decreased dramatically at 600 MPa.

Deamination of (-)-BCA by ADase under Various Pressures at 22 °C for 12 h





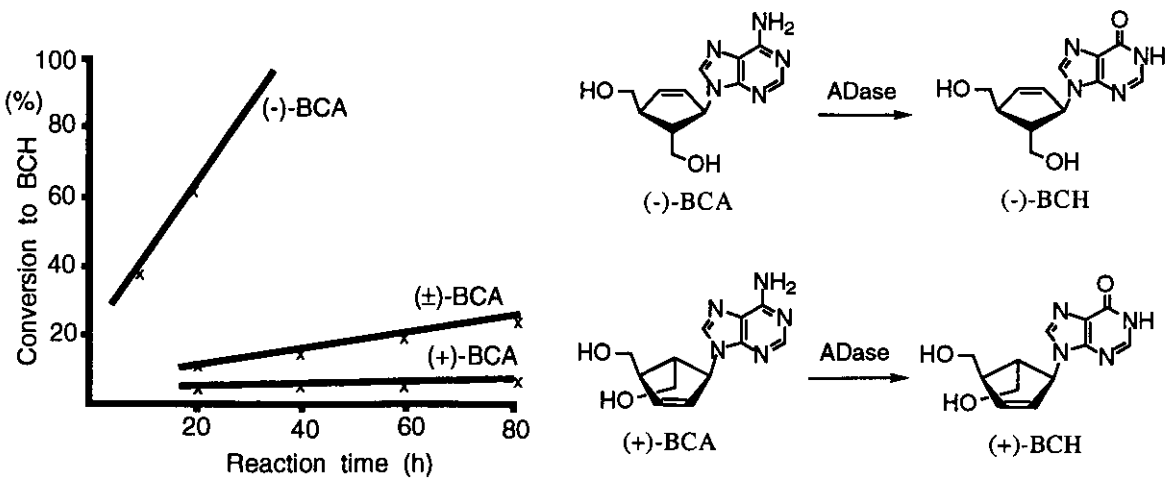
(-)-BCA (-)-BCH

ADase
1 unit/ μmol
phosphate buffer(2 mL) pH. 7.0

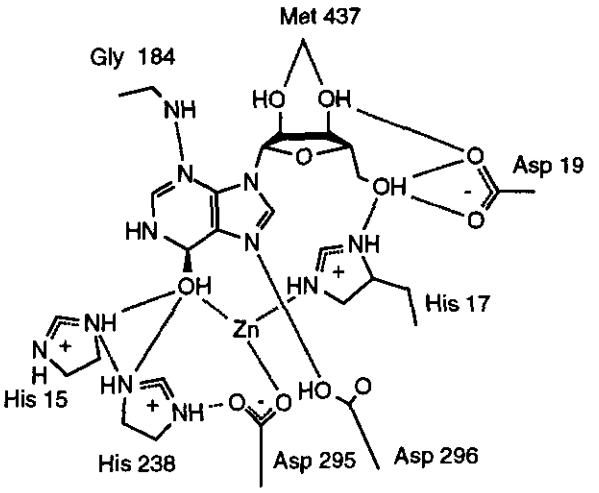
Conversion (%) represents BCH formed / total BCA initially used and was estimated by high-performance liquid chromatography (HPLC) (Waters, column: μ-Poracil C_{18}, solvent, dioxane-water = 1 : 7, detection, UV 254 nm, retention times, BCH = 3 min and BCA = 5.5 min).

This means that inactivation of ADase occurs between 500 MPa and 600 MPa. The inactivation was irreversible, because the enzyme pressurized at 600 MPa no longer catalyzed the deamination of BCA after the pressure was decreased again.
On the basis of these observations, we conducted the deamination of (±)-BCA and both of its enantiomers at 400 MPa at 22 °C in order not only to resolve the racemic BCA into each enantiomer with high enantiomeric excess (*ee*), but also to clarify the difference between the deamination rate of (-)-BCA and that of (+)-BCA. Thus, (-)-BCA was converted to (-)-BCH in quantitative yield within 40 h, whereas the conversion yield of (+)-BCA to (+)-BCH was only 5 % even at 80 h. Moreover, the deamination of (±)-BCA for 80 h provided (-)-BCH in 31% conversion yield. For determination of the *ee*, the (-)-BCH thus obtained was transformed to its (*S*)-(-)-2-methoxy-2-(trifluoromethyl)phenylacetic acid (MTPA-OH) diester, whose 500 MHz ^{1}H-NMR examination revealed that the *ee* of (-)-BCH was more than 99%.

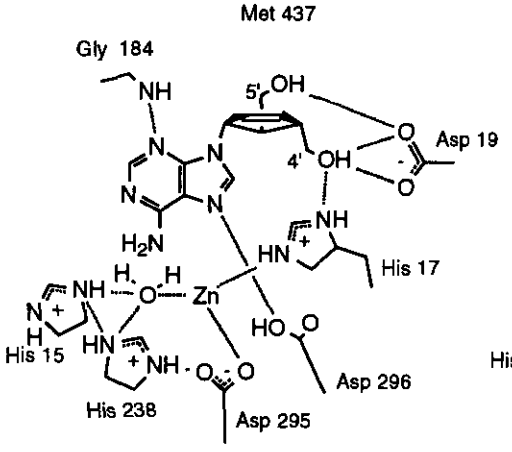
Deamination of (-)-BCA, (+)-BCA, and (±)-BCA by ADase under 400 MPa at 22 °C



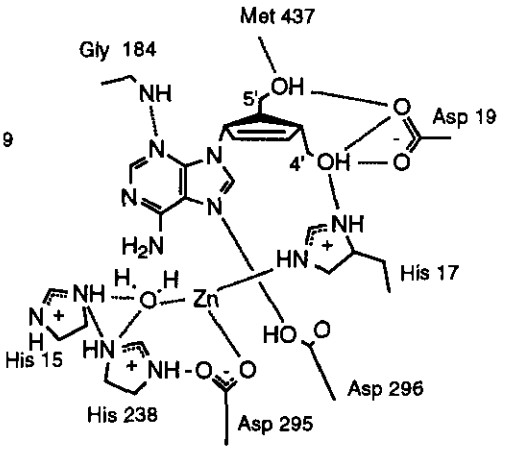
The binding structure of HDPR with ADase is shown below, which was proposed on the basis of X-Ray crystallographical analysis by Quiocho *et al.*[17] In this structure, His^{238}, Asp^{295}, and zinc ion are situated in the α-site whereas Met^{437}, Asp^{19}, and His^{17} are located in the β-site which would play important roles in the enantioselectivity. If this binding model is applied to the complex of (+)-BCA and (-)-BCA, (-)-BCA appears to bind more strongly with Met^{437} than (+)-BCA *via* 5'-hydroxy group. This may be the reason why (-)-BCA is deaminated selectively to give (-)-BCH.
Finally, the conversion of (-)-BCH to (-)-BCA was carried out essentially in the same manner as reported previously for the conversion of hypoxanthine to adenine.[30] (-)-BCH was converted to the diacetate, whose chlorination by thionyl chloride - *N,N*-dimethylformamide afforded the 6-chloropurine derivative. Amination of the latter gave the desired (-)-BCA. All reactions proceeded in nearly quantitative yields. We



6*R*-hydroxy-1,6-dihydropurine ribonucleoside (HDPR)



(+)-BCA

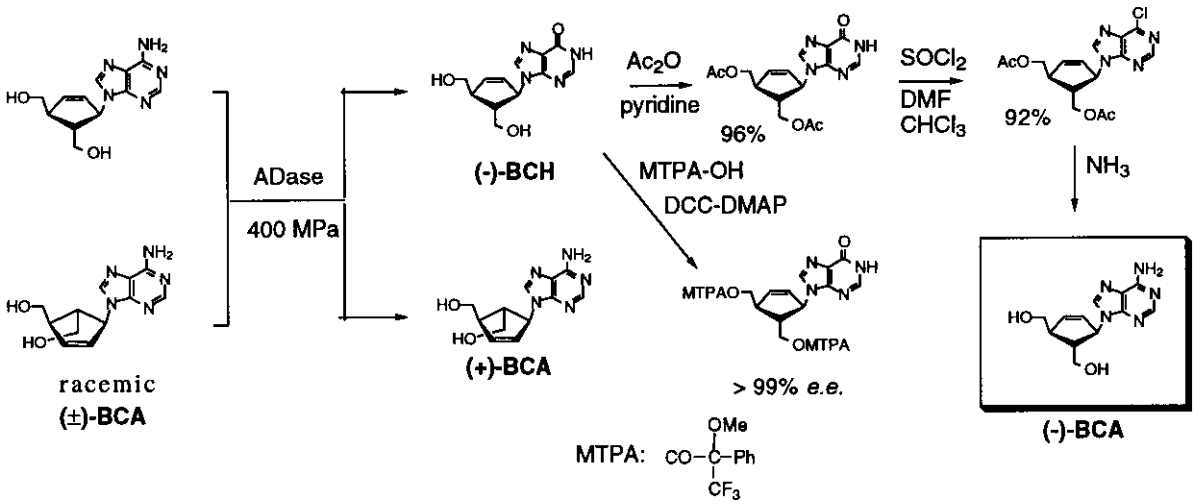


(-)-BCA

have therefore achieved the enzymatic resolution of (±)-BCA into (-)-BCA with a high enantiomeric excess. Moreover, it is noteworthy that the 6-chloropurine derivative would be a versatile intermediate for the synthesis of (-)-BCA analogues because the chlorine atom can be substituted by various groups such as alkylamino, alkoxyl, and mercapto groups.

In summary, we have found that (-)-BCA binds less strongly to ADase but are much more susceptible to deamination under high pressure than (+)-BCA. Though the mechanism of the acceleration of deamination under high-pressure remains obscure, it could involve conformational change of the enzyme under high pressure. The irreversible inactivation of the adenosine deaminase at rather higher pressure (600 MPa), as mentioned above, supports this mechanism.

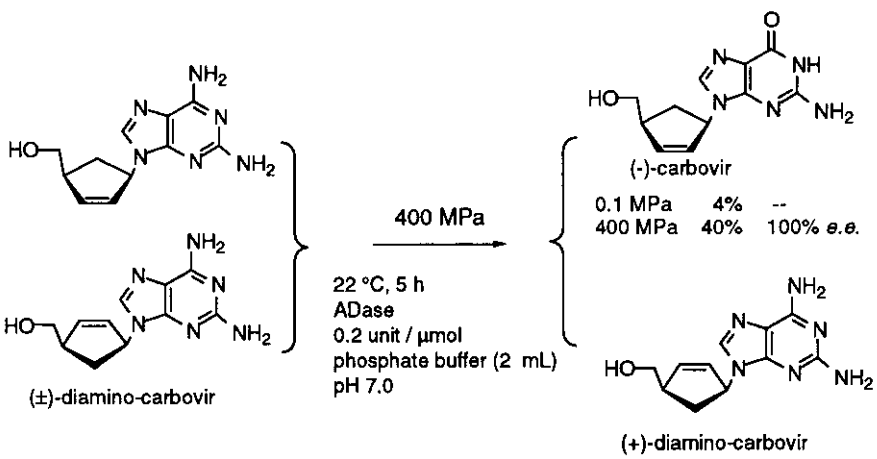
Enzymatic and Chemical Conversion of Racemic BCA to (+)- and (-)-BCA Enantiomers



2.2. Deamination of diamino-carbovir, adenine, and adenosine

Next, we applied this high-pressure technique to the preparation of (-)-carbovir having anti-HIV activity from racemic diamino-carbovir (**6**). The resolution was previously conducted by Vince and his coworker[19] at ambient pressure. The reaction proceeded within 5 h under 400 MPa to give (-)-carbovir with high enantiomeric excess (>99%) in 40% yield. This method is superior to the deamination at atmospheric pressure, which requires a much longer reaction time (72 h).[19]

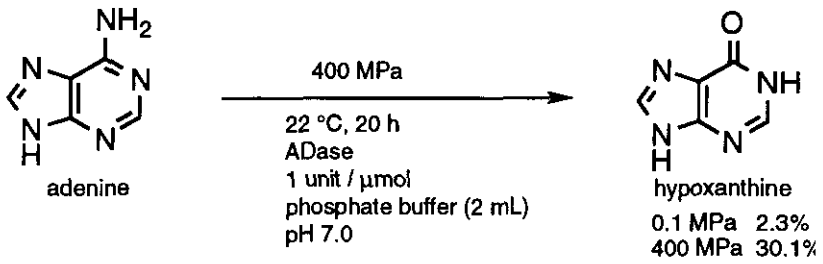
Deamination of (±)-Diamino-carbovir by ADase at 400 MPa



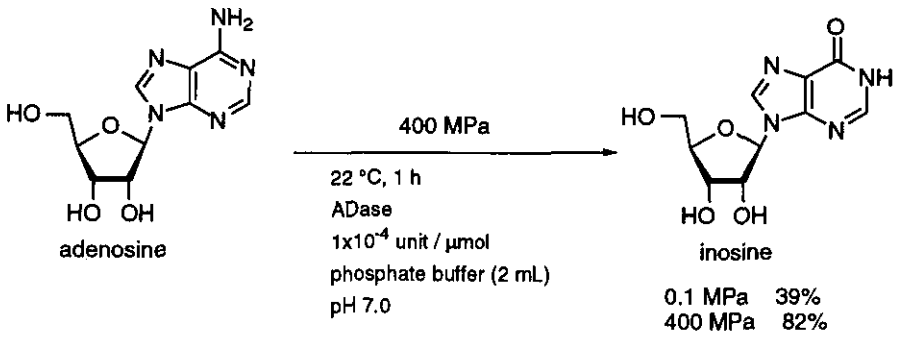
It is well known that adenine is a strong inhibitor of ADase and does not act as a substrate for ADase. However, we found that adenine was also hydrolyzed by ADase under high pressure to give hypoxanthine. The deamination rate of adenine at 400

MPa was ten times faster than that at atmospheric pressure. We also found that the deamination of adenosine was definitely accelerated by high pressure and the rate at 400 MPa was twice faster than that at atmospheric pressure.

Deamination of Adenine by ADase at 400 MPa

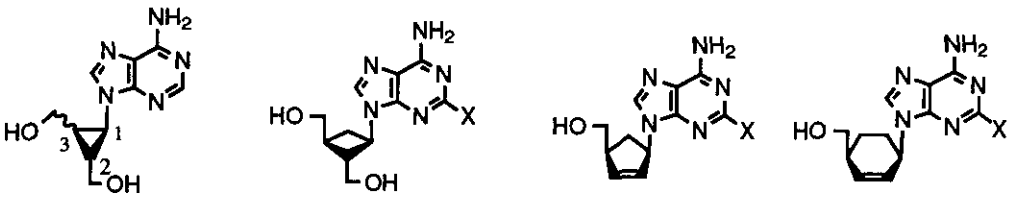


Deamination of Adenosine by ADase at 400 MPa



2.3. Deamination of various carbocyclic adenine nucleosides: Effect of high pressure for the reaction rate and enantioselectivity

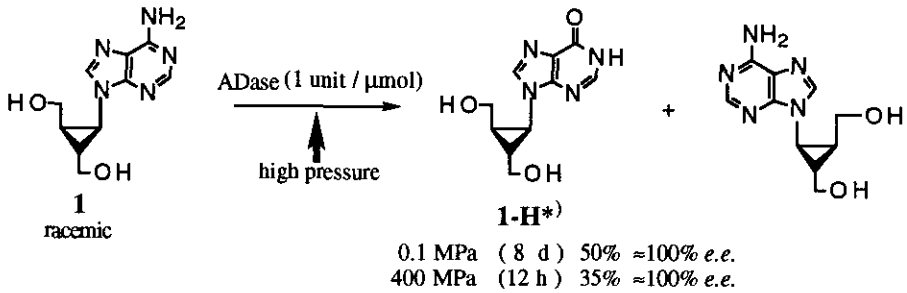
In oder to clarify the generality and limitation of deamination by ADase under high pressure, the deamination of 9-(hydroxymethylated cycloalkyl)-9*H*-adenines and their 2-amino derivatives (carbocyclic adenine nucleosides) was examined. The substrates used for the deamination are eight kinds of carbocyclic nucleosides (**1**-**8**).



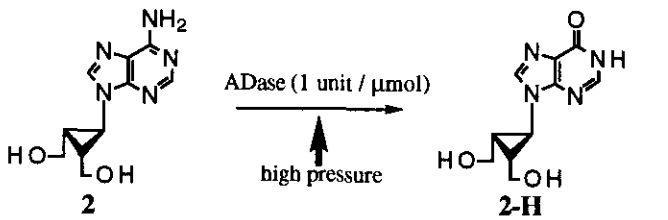
1 : 2,3-*trans* **3** : X = H (carbocyclic oxetanocin A) **5** : X = H **7** : X = H
2 : 2,3-*cis* **4** : X = NH_2 (carbocyclic oxetanocin D) **6** : X = NH_2 **8** : X = NH_2

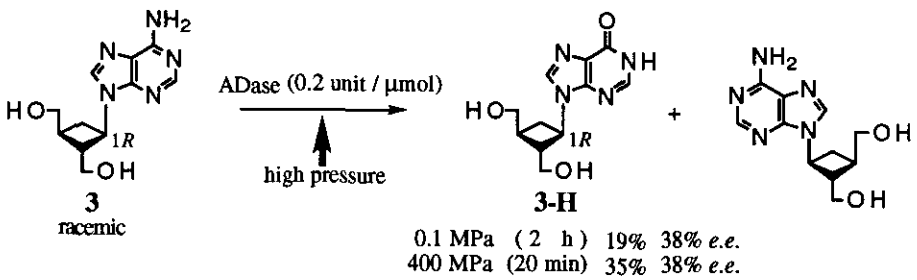
Deamination of the carbocyclic nucleosides (**1** - **8**) by ADase was carried out under high pressure and the result was compared with that obtained from the reaction at atmospheric pressure. Since it was clarified that the optimum pressure was 400 MPa for the deamination of (-)-BCA,[13] this pressure was used for all deamination reactions under high pressure. As a reference, the deamination of (-)-BCA was also included.

First, we examined the deamination of 9-[*t*-2,*c*-3-(bishydroxymethyl)cycloprop-*r*-1-yl)]-9*H*-adenine (**1**)[31] corresponding to a lower methylene homolog of carbocyclic oxetanocin A. Compound (**1**) was treated with ADase (1 unit/μmol) at 25 °C under 0.1 MPa (*ca.* 1 atm) for 8 d to give the hypoxanthine derivative (**1-H**) in 50% yield. The optical purity (enantio excess: *e.e.*) of **1-H** was determined to be more than 99% by HPLC analysis. Although the absolute structure of **1-H** has not been determined as yet, it would be considered to be 1*R*,2*R*,3*R* on the basis of the deamination of (±)-BCA.[13] This deamination was remarkably facilitated by high pressure (400 MPa) to give **1-H** having highly optical purity in 35% conversion yield in 12 h.



*) H designates a hypoxanthine derivative

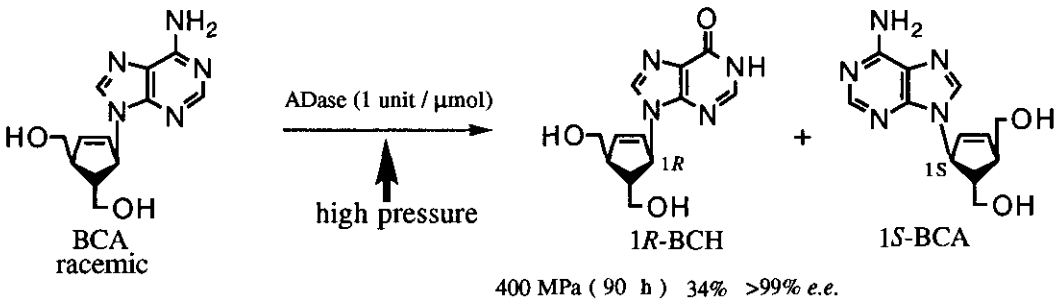


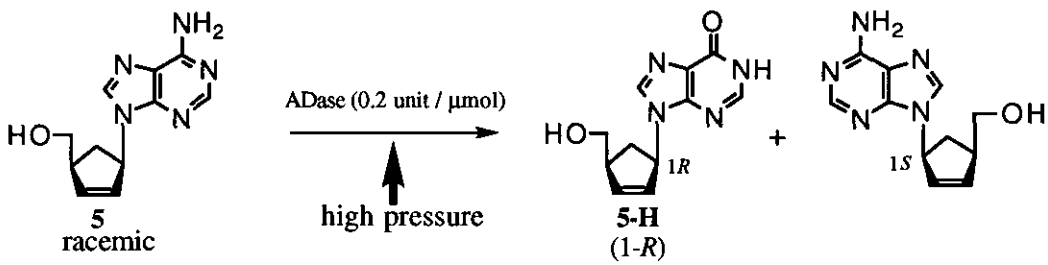


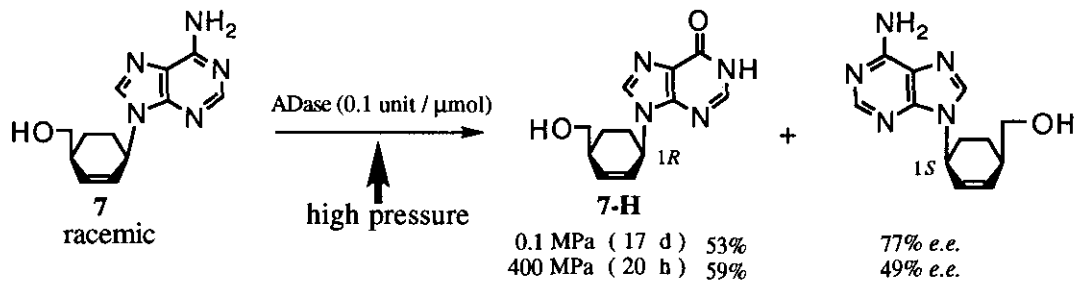
The deamination of meso compound, 9-[*t*-2,*t*-3-(bishydroxymethyl)cycloprop-*r*-1-yl]-9*H*-adenine (**2**)[31] having anti-BLV (bovine leukemia virus) was faster than that of **1**. Thus, **2** was hydrolized to **2-H** in 46% by treatment with ADase (1 unit/μmol) at 25 °C under 0.1 MPa for 5 d whereas under 400 MPa the reaction was completed in 7 h to give **2-H** in quantitative yield.

(±)-Carbocyclic oxetanocin A (**3**)[32] itself was rapidly deaminated to (-)-carbocyclic oxetanocin H (**3-H**) by ADase (0.2 unit/mmol) under high pressure. Thus, the deamination of **3** under 0.1 MPa for 2 h gave **3-H** with (1*R*)-configuration in only 19% yield whereas the reaction under 400 MPa gave **3-H** in 35% yield within 20 min. However, the enantioselectivity was low (38% *e.e.*) both under ambient and high pressure.

The cyclopentenyladenine (**5**),[33] on treatment with ADase under atmospheric pressure for 6 h, was hydrolysed to the cyclopentenylhypoxanthine (**5-H**) in 46% yield, whose *e.e.* was determined to be more than 99%. The reaction was accelerated by high pressure (400 MPa) to give 48% yield of **5-H** having ≥99 % *e.e.* in 20 min. The absolute structure of **5-H** should be 1*R* by analogy with that of carbovir (**6-G**) as described before.

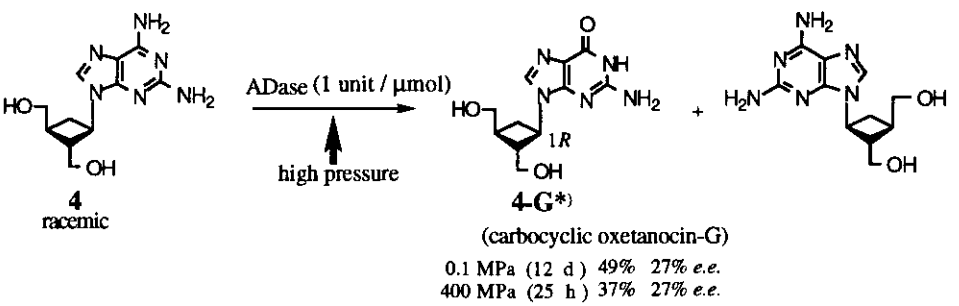


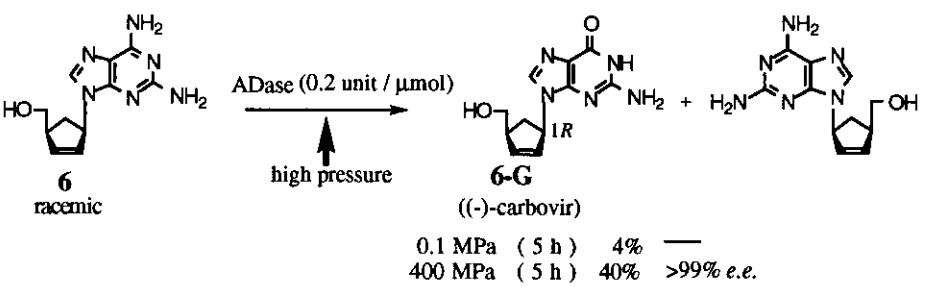


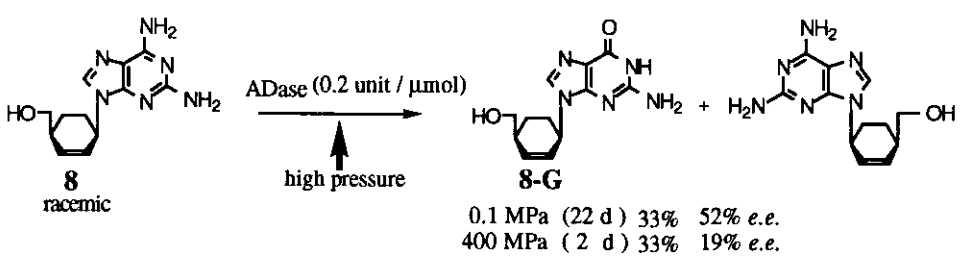


Deamination of 9-[*c*-4-(hydroxymethyl)cyclohex-2-en-*r*-1-yl]-9*H*-adenine (**7**)[34] with ADase under 0.1 MPa for 17 d gave **7-H** in 53% yield. The enantiomeric excess of the unreacted **7** was determined to be 77%. The deamination under 400 MPa for 20 h gave **7-H** in 59% yield. In this case, the *e.e.* of unreacted **7** was 49%. Although high pressure remarkably accelerates the deamination, it decreases the enantioselectivity.

Next, we examined the deamination of carbocyclic 2,6-diaminopurine nucleosides (**4**) and (**8**) under high pressure, and compared with that of diamino-carbovir (**6**). Carbocyclic oxetanocin G (**4-G**) is an interesting carbocyclic nucleoside, because the 1*R*-isomer of **4-G** has significant anti-viral activities such as anti-HIV and anti-HSV.[24] If we can use deamination of carbocyclic oxetanocin D (**4**)[35]for the resolution, it would provide an efficient method for the preparation of 1*R*-**4-G**. The deamination of **4** proceeded in 12 d at 0.1 MPa to give **4-G** in 49% yield while the reaction under high pressure gave a 37% yield of **4-G** within 25 h. Compound (**4-G**) thus obtained showed negative optical rotation and hence corresponded to carbocyclic oxetanocin G having anti-viral activities. However, in both reactions the enantioselectivity was low (27%). Diamino-carbovir (**6**) was more susceptible to the deamination than **4**, and the enantioselectivity was remarkably higher.





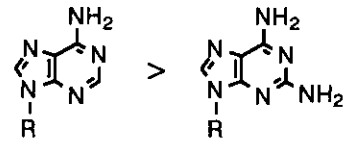


*) G designates a guanine derivative.

The diamino derivative (**8**) resisted to the deamination, and 3 weeks were needed for its conversion of 33% to **8-G** under 0.1 MPa giving 52% *e.e.* of **8-G**. The similar reaction under 400 MPa for 2 d gave **8-G** in 33% yield, whose *e.e.* was only 19%. The absolute structure of major product of **8-G** has not been determined as yet.

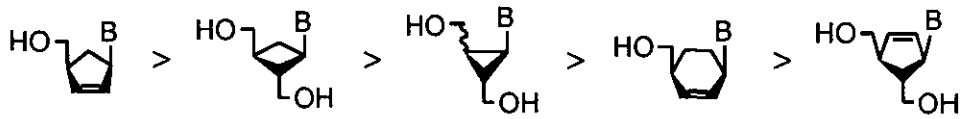
In conclusion, the deamination of all carbocyclic aminopurine nucleosides irrespective of their ring size was facilitated remarkably by high-pressure.[3,4] However, their enantioselectivities were lowered except for three and five membered carbocyclic nucleosides (**1**, **5** and **6**). This means that **5** and **6** behave as the better substrates for ADase because their structures resemble more to normal nucleosides having pentose moiety than other carbocyclic nucleosides. However, it is not clear why **1**, on the deamination, gives **1-H** with very high enantioselectivity. The deamination of carbocyclic nucleosides by ADase under high-pressure is summarized as follows:

1) The reaction rate of the adenine nucleosides is faster than that of the 2,6-diaminopurine derivatives.

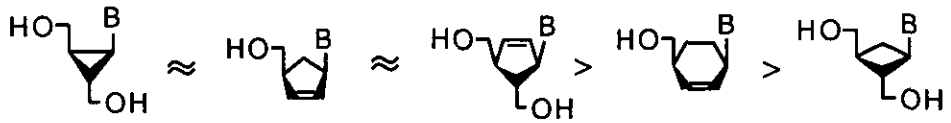


adenine 2,6-diaminopurine

The reaction rate decreases in order of:



2) The enantioselectivity decreases in order of:

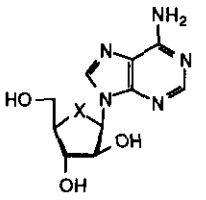


2.4. Deamination of cyclaradine having anti-HSV activity

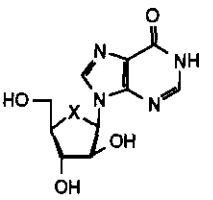
Ara-A (9-β-D-arabinofuranosyladenine) has a broad spectrum of activity against DNA viruses. However, a major drawback in the clinical use of ara-A lies in the fact that the nucleoside is rapidly deaminated by adenosine deaminase (ADase) to the much less active ara-H (9-β-D-arabinofuranosyl-hypoxanthine).[36-38] To overcome the problem, cyclaradine (carbocyclic arabinosyladenine) was developed by Vince and his coworkers as an ADase resistant ara-A derivative.[39,40]

Therefore, we examined the deamination of (+)-, (-)-, and (±)-cyclaradine by ADase under various pressure and achieved the resolution. First, we examined the deamination of (+)-cyclaradine by ADase (type IV, Sigma) under various pressure. One unit ADase was used for the deamination of one μ mol (+)-cyclaradine. The deamination under atmospheric pressure (0.1 MPa) was very slow, and more than

14 h was required for the complete deamination of cyclaradine to carbocyclic Ara-H. However, the reaction proceeded quantitatively within about 8 h and 2 h under 50 MPa and 400 MPa, respectively.

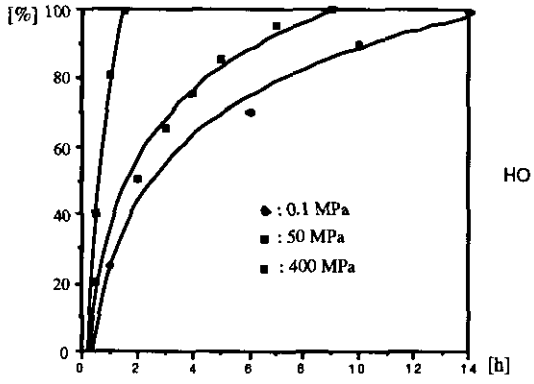


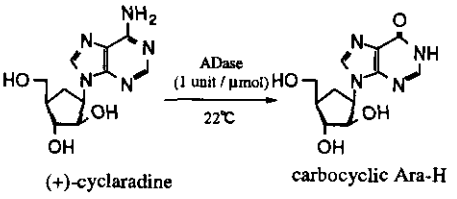
X = O : Ara-A
X = CH_2 : cyclaradine



X = O : Ara-H
X = CH_2 : carbocyclic Ara-H

Deamination of (+)-Cyclaradine by ADase (typ IV, Sigma) under Various Pressure

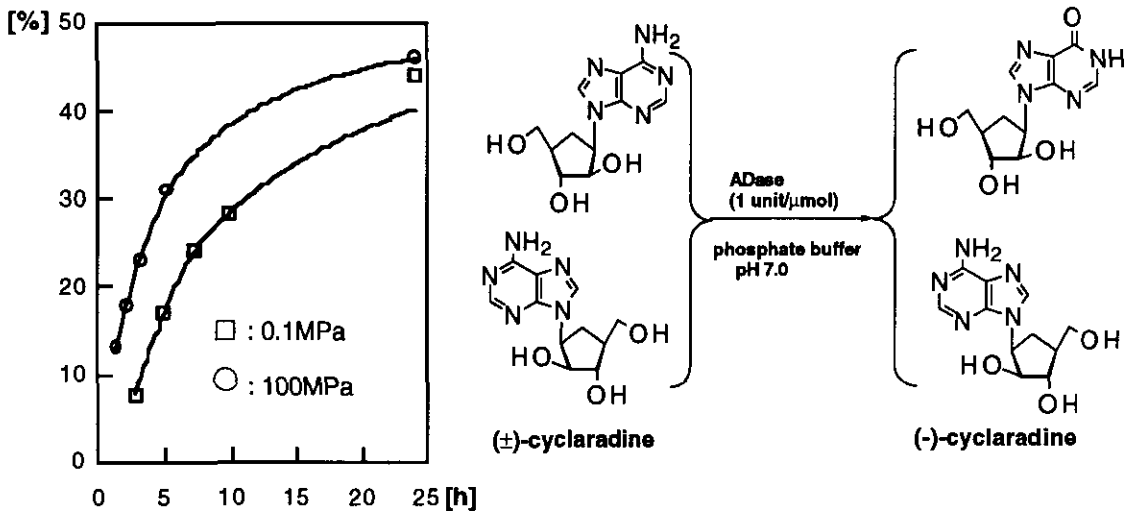




(+)-cyclaradine carbocyclic Ara-H

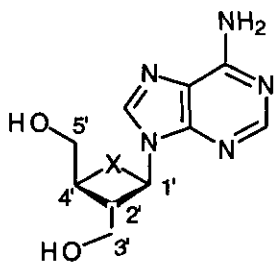
Next, we carried out the deamination of racemic cyclaradine by ADase at 0.1 MPa and 100 MPa. The deamination rate was much less than the half of the (+)-cyclaradine reaction. The phenomena mean that (-)-cyclaradine inhibits the deamination of (+)-cyclaradine. Furthermore, the formation of carbocyclic Ara-H under 0.1 MPa was observed in about 40% yield for 25 h whereas the yield of carbocyclic Ara-H was *ca.* 45% under 100 MPa after the same time. The yield of carbocyclic Ara-H did not exceed 50% even for longer reaction period. Carbocyclic ara-H obtained from the deamination showed the high optical purity. (-)-Cyclaradine was not deaminated by ADase even under 100 MPa for 3 d. Thus, the high pressure mediated deamination can be applied for the resolution of (±)-cyclaradine.[41]

Deamination of Racemic Cyclaradine by ADase

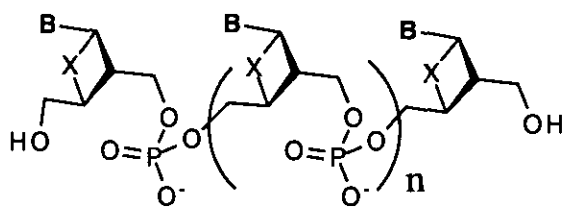


3. DEACYLATION OF 3',5'-DI-*O*-ACYLOXETANOCINS BY LIPASES UNDER HIGH PRESSURE

Oxetanocin A, isolated from *Bacillus megaterium*, is a constitutional isomer of 2'-deoxyadenosine bearing an oxetane ring as the sugar moiety. It shows significant biological activity.[23,44] Recently, the carbocyclic analogue of oxetanocin A has attracted much attention as entities with potential antibacterial and antiviral activities.[44,45]



X=O : oxetanocin A
X=CH_2 : carbocyclic oxetanocin A

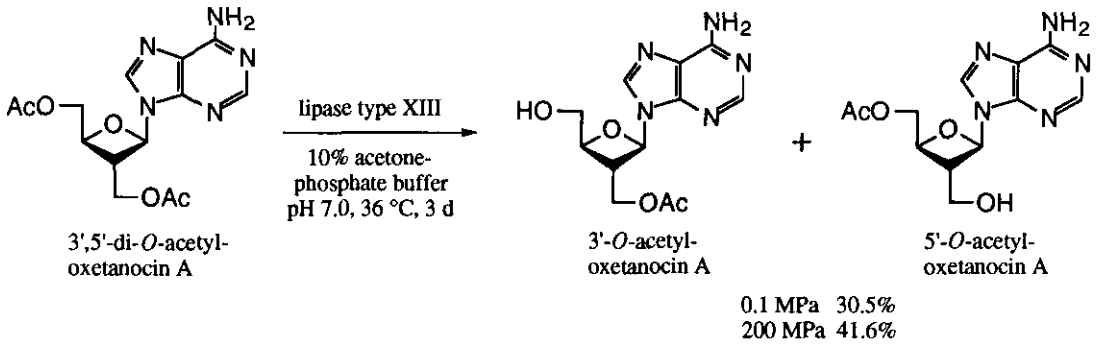


X=O or CH_2

oligonucleotides

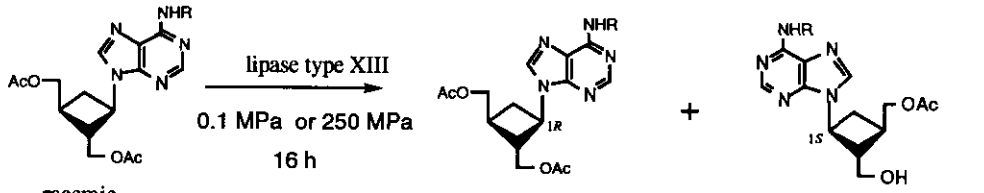
B=thymine, cytosine, adenine, guanine

We have been interested in the structures and functions of oligonucleotides containing oxetanocin A or its carbocyclic analogue from the viewpoint of antisense strategy[46,47] and examined the selective protection of two primary hydroxyl groups required for the synthesis of the corresponding oligonucleotides. In this Section, the regio- and enantio-selective deacylation of 3',5'-di-*O*-acyloxetanocins by lipases under high pressure was described, comparing the deacylation under atmospheric pressure. The methodology provided an effective protection for one of the two hydroxyl groups of oxetanocins. The deacetylation of 3',5'-di-*O*-acetyloxetanocin A derived from oxetanocin A was examined by using various lipases such as lipase PS, MY, type I, II, VII, XI, and XIII. Among them, only lipase type XIII was effective for the deacetylation. Thus, 3',5'-di-*O*-acetyloxetanocin A was treated with lipase type XIII at 36°C for 3 d to give 3'-*O*-acetyloxetanocin A and its regio-isomer with the ratio of 6.6 : 1 in 30.5% totally yield. When the reaction was carried out at 200 MPa, the yield increased to 41.6% with the same ratio of each regioisomer. This means that high pressure facilitates the deacetylation by lipase but to the less extent compared with ADase.[48]



During our recent studies on the novel artificial DNA, we needed mono-protected carbocyclic oxetanocin A, and selective deacylation was examined for diacyl derivative. Racemic carbocyclic 3',5'-di-*O*-acetyloxetanocin A[49] was hydrolyzed by lipase type XIII under atmospheric pressure for 16 h to give [1*S*]-5'-*O*-acetyl-oxetanocin A with high regio (100%) - and enantio (90%) -selectivity in 22% yield. The *N*-acetyl derivative was more susceptible to the hydrolysis, and the yield was 38%. Both reactions were again slightly accerelated by high pressure (250 MPa) to give the corresponding 5'-*O*-acetyl derivatives in 30 and 45% yields, respectively. The regio- and enantio-selectivity did not change.

Deacetylation of Carbocyclic Di-*O*-acetyloxetanocin A by Lipase Type XIII under 0.1 MPa and 250 MPa



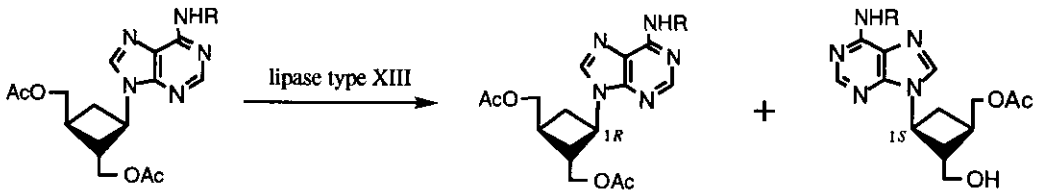
racemic
R = H or Ac

R=H: 0.1 MPa; 22.3 % 90% *e.e.*
250 MPa; 30.0 % 90% *e.e.*

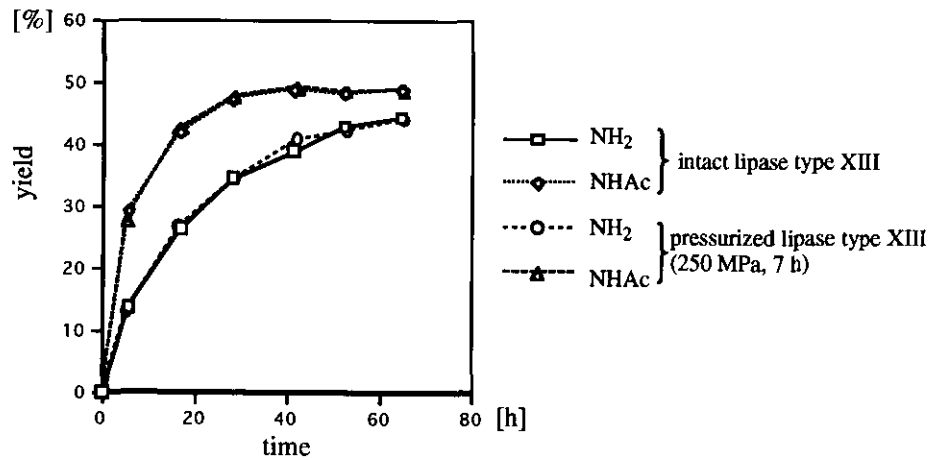
R=Ac: 0.1 MPa; 37.9 % 89% *e.e.*
250 MPa; 45.3 % 89% *e.e.*

In order to clarify mechanism for the accerelation of deacetylation, lipase type XIII was initially pressurized under 250 MPa at room temperature for 7 h. The enzyme thus obtained was used for the hydrolysis. The results are shown in the Figure, and compared with the deacetylation by the intact enzyme. As a result, no difference was

Deacetylation of Carbocyclic Di-*O*-acetyloxetanocin A by Pressurized Lipase Type XIII



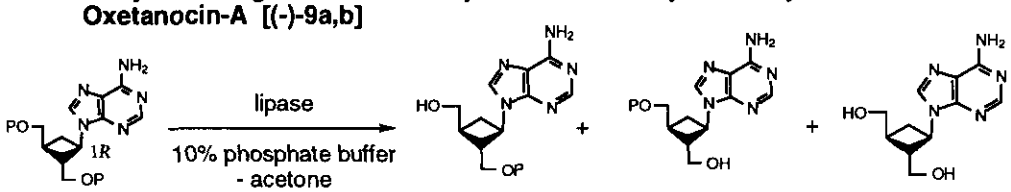
racemic
R = H or Ac



observed between both deacetylations. This means that the enzyme does not cause the irreversible variation in the conformation. Therefore, the acceleration of the deacetylation by high pressure would be due to the reversible conformational change of the enzyme or due to the transition state of hydrolysis accompanying charge, which has negative activation volume ($\Delta V\ddagger$).[9]

We also found that (1*R*)-carbocyclic 3',5'-di-*O*-benzoyloxetanocin A was hydrolyzed with lipase MY to give 3'-*O*-benzoylated compound selectively.[50] While lipase type XIII hydrolyzed 3'-acetyl group, lipase MY attacked at 5'-*O*-benzoyl group. Furthermore, the latter enzyme hydrolyzed benzoyl ester more rapidly than acetate. (Table 1). To accelerate the reactions, high pressure technique was applied. Lipase type XIII as well as lipase MY catalyzed the hydrolysis under high pressure to give 3'-*O*-benzoyloxetanocin exclusively. (1*R*)-3',5'-Di-*O*-benzoyl-oxetanocin U was also hydrolyzed by lipase type XIII under high pressure to give carbocyclic 3'-*O*-benzoylated oxetanocin U.[51]

Table 1. Enzymatic Regioselective Deacylation of Carbocyclic Diacylated Oxetanocin-A [(-)-9a,b]

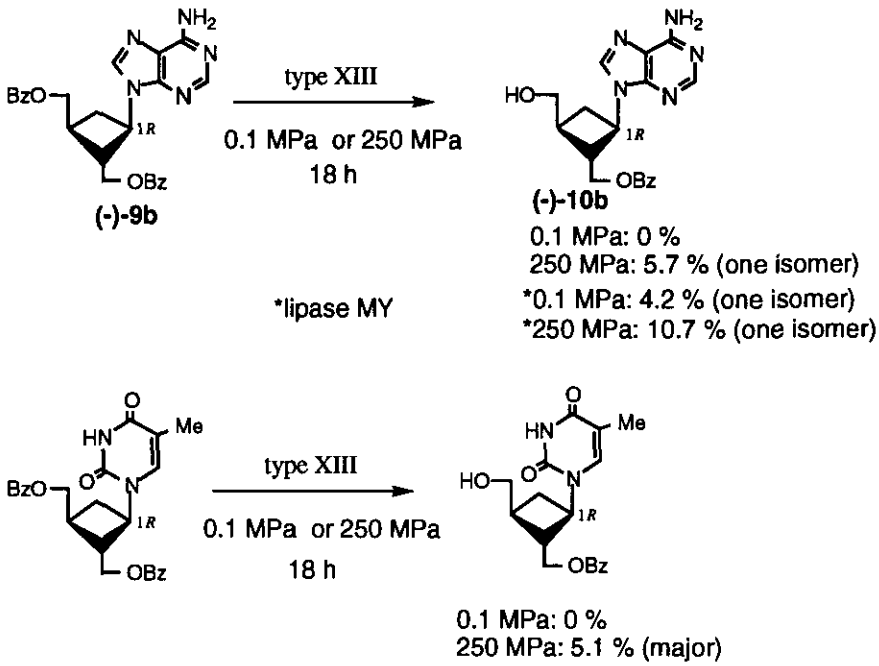


(-)-**9a,b**
a : P=Ac
b : P=Bz

(-)-**10a,b** (+)-**11a,b** (-)-**12**

Entry	Lipase	P	Unit / mmol	Time	(-)-**9**	(-)-**10**	(+)-**11**	(-)-**12**
1	lipase MY	Ac	8.7×10^4	5 d	58	40	1	1
2	lipase MY	Ac	8.7×10^4	7 d	38	51	trace	-
3	lipase MY	Ac	8.7×10^4	21d	24	70	1	5
4	lipase Type I	Ac	3.1×10^3	17d	96	3	0	2
5	lipase Type VII	Ac	3.2×10^5	17d	89	10	0	2
6	lipase Type XIII	Ac	1.1×10^4	17d	88	8	0	4
7	lipase MY	Bz	8.7×10^4	6d	0	87	0	2

* The yields in Entries 1, 4, 5, and 6 were determined by HPLC analyses (μBondapak C18; MeOH-H_2O=1:2). Entries 2, 3, and 7 were isolated yields.



It was found that lipase catalyzed hydrolysis was also facilitated by high pressure. However, the effect was small, compared with ADase mediated deamination. To obtain more effective activation of the enzyme by high pressure, more detailed investigation concerning other kinds of lipases will be needed.

4. CONCLUDING REMARKS

It has become apparent that hydrolysis by ADase or lipases is facilitated by high pressure. Mechanism for this acceleration can be considered as follows: 1) enzyme catalyzed hydrolysis proceeds *via* polar transition state, which has a negative activation volume. 2) The three-dimensional structure of enzyzme is changed by high pressure and hence the active site of the enzyme becomes close to that of the substrate. Today, an enzyme has been extensively used for the preparation of chiral chemicals in the field of chemical industry. Although temperature is the most important factor in the enzymatic reaction, the present study has revealed that the pressure also plays a significant role. This high pressure technique would be applicable widely to the enzyme catalyzed reaction, which is rather inert either under atmospheric pressure or at higher temperature, and may also be useful for elucidation of mechanism for the enzymatic reaction.[52-54]

ACKNOWLEDGEMENT

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