Studies on the Biosynthesis of Bialaphos (SF-1293). 18.

2-Phosphinomethylmalic Acid Synthase: A Descendant of (*R*)-Citrate Synthase?†

Sir:

Bialaphos is a tripeptide produced by *Streptomyces hygroscopicus* SF-1293 and consists of two L-alanine residues and the unusual glutamic acid analogue, phosphinothricin possessing a unique C–P–C bond[2]. This metabolite acts as a potent inhibitor of glutamine synthetase and is commercially used as a herbicide.

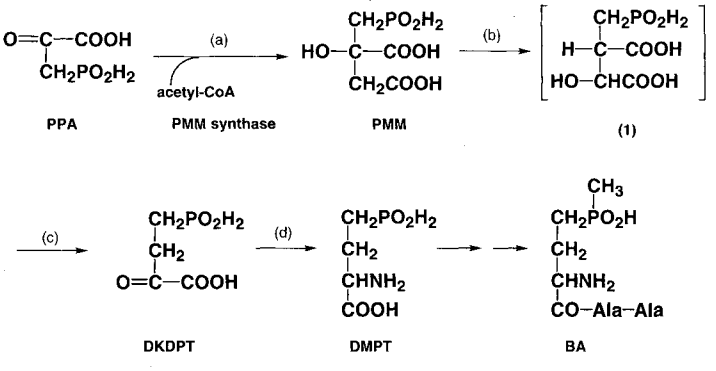
During the biosynthetic studies of bialaphos (BA), we proved that the biosynthetic pathway of BA consisted of at least 14 and most likely more than 20 steps. The mechanism leading from 2-phosphinomethylmalic acid (PMM) to deamino-α-ketodemethylphosphinothricin (DKDPT), however, remained to be clarified (Fig. 1)[3]. Accumulation of PMM by *S. hygroscopicus* upon addition of monofluoroacetic acid[4], a strong inhibitor of aconitase, suggested the operation of the TCA cycle or its related metabolic pathway for transformation of phosphinopyruvic acid (PPA) to DKDPT. Structural similarities of bialaphos biosynthetic intermediates to the members of the TCA cycle, *i.e.*, PPA, PMM, DKDPT and demethylphosphinothricin (DMPT) to oxalacetic acid, citric acid, α-ketoglutaric acid and glutamic acid, respectively, corroborated our above assumption. By analogy to the TCA cycle, a phosphinate analog of isocitric acid (Fig. 1 (1)) yet to be identified was assumed to be a substrate for aconitase or its related enzyme of the bialaphos biosynthetic pathway.

Formation of PMM by the condensation of PPA and acetyl-CoA proceeds in a manner similar to the production of citric acid, and the catalyzing enzyme, PMM synthase, was isolated and characterized[5]. The stereochemical course of this enzyme, however, turned out to be opposite to that of citrate synthase of porcine heart origin belonging to the ubiquitous (*S*)-type citrate synthase. Interestingly, PMM synthase showed similarities to (*R*)-citrate synthase isolated from *Clostridium acidi-urici* such as metal ion requirement and sensitivity to enzyme inhibitors suggesting that PMM synthase belongs to the group of (*R*)-citrate synthase[5]. On the other hand, citrate synthase from *S. hygroscopicus* SF-1293, which was assumed to be of (*S*)-type according to its properties, exhibited quite different characteristics from of PMM synthase[6].

To analyze the relationship between the bialaphos biosynthetic pathway and the TCA cycle in detail, transformation of PMM was attempted by using bialaphos non-producing *Brevibacterium lactofermentum*. Primary structure of PMM synthase was also determined by nucleotide sequencing of the corresponding gene. These results are reported in this paper.

Since PMM was expected to be converted to DKDPT by TCA cycle enzymes probably *via* the phosphinic acid analog of isocitric acid (Fig. 1, (1)), biotransformation of PMM was tested by utilizing bialaphos non-producing

Fig. 1. Conversion of phosphinopyruvic acid to demethylphosphinothricin.



Abbreviations: PPA, phosphinopyruvic acid; PMM, 2-phosphinomethylmalic acid; DKDPT, deamino-α-keto-demethylphosphinothricin; DMPT, demethylphosphinothricin; BA, bialaphos. Compound (**1**) is a putative intermediate.

† For part 17[1].

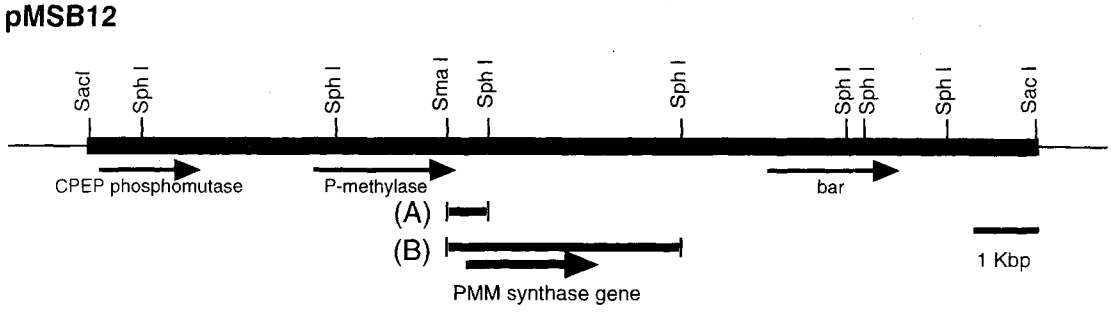
B. lactofermentum ATCC13869 known to be a potent glutamic acid producer with high aconitase and isocitrate dehydrogenase activities[7]. The organism was cultivated at 30°C for 24 hours in the medium containing glucose 50 g, KH_2PO_4 1 g, $MgSO_4$ 0.4 g, $MnSO_4$ 2 mg, $FeSO_4$ 2 mg, soybean hydrolysate 200 g, thiamine-HCl 200 μg, biotin 2 μg and urea 10 g in one liter water (pH 6.6). Cells were harvested by centrifugation, washed twice with 50 mM Tris-HCl (pH 7.5) and suspended in the same buffer at a ratio of 0.1 g (wet weight) per 1ml of buffer. The cells were then disrupted by sonication and unbroken cells and cell debris were removed by centrifugation. The reaction mixture (0.5 ml) containing 50 mM Tris-HCl (pH 7.5), 150 mM an amino donor (L-aspargic acid or NH_4Cl), 10 mM $MnSO_4$, 0.4 ml of the cell extract and 100 mM a plausible substrate was incubated at 30°C for 16 hours. The amount of glutamic acid or DMPT formed in the reaction mixture was estimated using an automatic amino acid analyzer. As shown in Table, PMM and citric acid were converted into DMPT and glutamic acid, respecively, by the cell extracts of *B. lactofermentum* with citric acid being approximately 10 times more efficient. In addition, cell extracts of bialaphos non-producing *S. lividans* as well as commercial glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase of porcine muscle origin converted DKDPT to DMPT effectively (data not shown) suggesting that the transamination of DKDPT was also catalyzed by ubiquitous transaminases. These results strongly imply that the conversion of PMM to DKDPT proceeded mechanistically in a manner analogous to or identical with the TCA cycle pathway, but do not necessarily mean that the TCA cylcle is the only pathway for the formation of DKDPT. Less efficient conversion of PMM to DMPT by *B. lactofermentum* as just explained may mean the presence of the hitherto unidentified enzyme system specific for the biosynthesis of bialaphos. In this regard, it may be important to note that WOHLLEBEN *et al.* recently identified *acnP* encoding an aconitase-like gene from another bialaphos producing organism, *Stretptomyces viridochromogenes*[8]. The gene product AcnP catalyzed the isomerization of PMM and was highly similar to the *Escherichia coli* aconitase AcnA and to other members of the aconitase family. This finding may indicate that the BA producing organisms possess a specific enzyme system required for the conversion of PPA to DKDPT.

Table. Transformation *Brevibacterium lactofermentum*.

Substrate	Amino donor	Product	Amount of product (%)
none	NH_4Cl	—	0
	L-Asp	—	0
	NH_4Cl+L-Asp	—	0
Citric acid	NH_4Cl	Glu	39
	L-Asp	Glu	32
	NH_4Cl+L-Asp	Glu	52
PMM	NH_4Cl	DMPT	6.2
	L-Asp	DMPT	3.3
	NH_4Cl+L-Asp	DMPT	3.8
PPA	L-Asp	PA	40

As mentioned above, PMM synthase giving rise to a product with the (*R*)-configuration is apparently different from the ubiquitous (*S*)-citrate synthase. Thus, we then determined the nucleotide sequence of the PMM synthase gene to reveal the primary structure of this unique enzyme. We had previously reported the sequence of the amino

Fig. 2. Restriction map of pMSB12.



PMM synthase gene is indicated by a thick arrow. Other bialaphos biosynthetic genes, CPEP phosphonomutase gene[11], P-methylase gene[12] and bialaphos resistance gene (bar)[13] located on pMSB12 are indicated by thin allows.

Fig. 3 (A).

```
CCCGGGGGAGAGCCCCCAGACCGTGCGGAACACCATCGACTTCATCAACTCCGCGGGACCGGACACCTTCGCGGTCAACC  80
Sma I
ACTGGTACTACATGCAGTCCACGCCCATCCACGTCCGGGCGCCCCAGTTCGACCTCACCGGCAACGGCCACACCTGGTCG 160
CACCGCACCATGAACTCTCATCAGGCGCTCGAAGCGGCCGACGAGATATTCGACTCGGTGGTCAACGCCGCCTGGATGCC 240
CGTGAACGGACTCGACTTCTGGGGCGTCCCCTACCTCCTGGGCAAGGGCATGAGCCACGCCGAGATCGTCCGGTTCCTCG 320
ACCTCGCCAAACCGCTCACCGTGGCCAACGTCTCCCGCGGCAGCGCGGAGGAGGCCGCCACCGCGGAGCGCCGGTTCCGC 400
GACTTCGTCACCGGCCTGGACCTTGCACCCGCCCGCTACCGCACCGCAGGGAGTGAGTTTCGATGACCGTCCAGAATCCT 480
                                                    M  T  V  Q  N  P
CAGGAACCCGAGTACTTCCCGGAGGTCTTCCCCCAAGACGCCTTCCCCCAGTACGCCTGGGACGAGGGCATGCGGCCGAT 560
Q  E  P  E  Y  F  P  E  V  F  P  Q  D  A  F  P  Q  Y  A  W  D  E  G  M  R  P  I
CACTCTGCCGCACGAGGTGTGGCTGTCGGAGACCACCCACCGGGACGGCCAGCAGGGGGGACTGCCCCTGTCGCTGGACA 640
 T  L  P  H  E  V  W  L  S  E  T  T  H  R  D  G  Q  Q  G  G  L  P  L  S  L  D  T
CCAGCCGCCGTATCTACGACATCCTCTGCGAGATCACGGACGACTCCACGGCGATCCGGCACGCCGAGTTCTTCCCCTAC 720
  S  R  R  I  Y  D  I  L  C  E  I  T  D  D  S  T  A  I  R  H  A  E  F  F  P  Y
CGCGACTCCGACCGCAACGCCCTGATCTACGCCCTGGAAAGGCACCGGGACGGCGCTCCGATCGAGCCCACCACCTGGAT 800
R  D  S  D  R  N  A  L  I  Y  A  L  E  R  H  R  D  G  A  P  I  E  P  T  T  W  I
CCGGGCCCGCCGGGAGGACGTCGAGCTGATCAAGCGGATCGGTGTCATCGAGACCGGCCTGCTCAGCTCCTCCTCCGACT 880
 R  A  R  R  E  D  V  E  L  I  K  R  I  G  V  I  E  T  G  L  L  S  S  S  S  D  Y
ACCACACCTTCCACAAGTTCGGCTCGGGCGGCCGGACCCAGGCCGCGTCGATGTACCTGGACGCGGTGACCATGGCGCTC 960
  H  T  F  H  K  F  G  S  G  G  R  T  Q  A  A  S  M  Y  L  D  A  V  T  M  A  L
GACCACGGCATCCGGCCCCCGCGTCCACCTGGAGGACACGACCCGGTCCTCTCCCGACTTCGTCCGCGCCCTGGTCGAAGA 1040
D  H  G  I  R  P  R  V  H  L  E  D  T  T  R  S  S  P  D  F  V  R  A  L  V  E  E
GGTACTGAAGACCGCCGAACGCTACCCGGCCGAACTCCAGCCCCGCTTCCGGGTCTGCGACACCCTCGGCATCGGCCTGC 1120
 V  L  K  T  A  E  R  Y  P  A  E  L  Q  P  R  F  R  V  C  D  T  L  G  I  G  L  P
CCTACGACGACGTGAGCCTGCCCCGCAGCATCCCCCGCTGGATCCGGCTGCTGCGCGGCTTCGGTCTCTCCCCGTCCCAG 1200
  Y  D  D  V  S  L  P  R  S  I  P  R  W  I  R  L  L  R  G  F  G  L  S  P  S  Q
ATCGAGCTGCACCCGCACAACGACACATGGCTGGTCGTCGCGAACTGCCTGGCAGCCATCCGCGAGGGCTGTGGCGTGAT 1280
I  E  L  H  P  H  N  D  T  W  L  V  V  A  N  C  L  A  A  I  R  E  G  C  G  V  I
CAGCGGGACGACGCTGGGCACGGGTGAACGCACCGGCAATGCACCGCTGGAGGCCGTCATGGTGCACCTGCTCGGGATGG 1360
 S  G  T  T  L  G  T  G  E  R  T  G  N  A  P  L  E  A  V  M  V  H  L  L  G  M  G
GCTACTGGTCCGGGGCCCGGGTCAACCTGCCCGCGGTCAACAAGCTCGTCGAGTTGTACGAGGGCATCGGAGCCGGCCCG 1440
  Y  W  S  G  A  R  V  N  L  P  A  V  N  K  L  V  E  L  Y  E  G  I  G  A  G  P
TCGCAGAAGTACCCCTTCTTCGGCCGCGACGCCTACGTCACCCGGGCCGGTATCCACGCCGACGGCCTGAACAAGTTCTG 1520
S  Q  K  Y  P  F  F  G  R  D  A  Y  V  T  R  A  G  I  H  A  D  G  L  N  K  F  W
GTGGATGTACGCACCGTTCAACGCCCCGCTGCTCACCGGCCGGGAGCTGGACGTCGCCCTCACCAAGGACTCCGGCCAGG 1600
 W  M  Y  A  P  F  N  A  P  L  L  T  G  R  E  L  D  V  A  L  T  K  D  S  G  Q  A
CGGGGCTGCTGTTCGTCCTGAACAAGCGGCTCGGGCTGCAGTTGGAGAAGGGCGACCCGCGGGTCGCCGAGGTGCTGGCC 1680
  G  L  L  F  V  L  N  K  R  L  G  L  Q  L  E  K  G  D  P  R  V  A  E  V  L  A
TGGATGGACCGGCAGTGGGACGCCGGCCGGGTCTCGGCCGTCGAGTGGAGCGAGTTGGAGCCGGTGGTCGAGAAGGCGTT 1760
W  M  D  R  Q  W  D  A  G  R  V  S  A  V  E  W  S  E  L  E  P  V  V  E  K  A  F
CGCCACCGAGGAAGGGGTGGGCTGACATGTCCGGACCGGCGCGAGGAGGCAACCCCTCATGACCGTCGCCACATCGGGTG 1840
 A  T  E  E  G  V  G
AGGCGTCGGGCAGCACCGGCCGTCCGCCGTCCTGCCCCGTCGTCGCGGAGTTCGAGCACCAGGTGCGAACCGGCCCGGAC 1920
CGGCCCGCCGTCGTCCTGCCCCGGCGGGACGGTCGGCTACCGGGAGCTGGGCGCGCGCGCCGACGCCATCGCCCGCGCGCT 2000
CACCGACCGGCGGGGCGAAGGGGACCGAAGCCGGTCCCCCTGATGGTGCTGGACCCGGCCTGGATGCTCGCTTGCATGC  2079
                                                                                Sph I
```

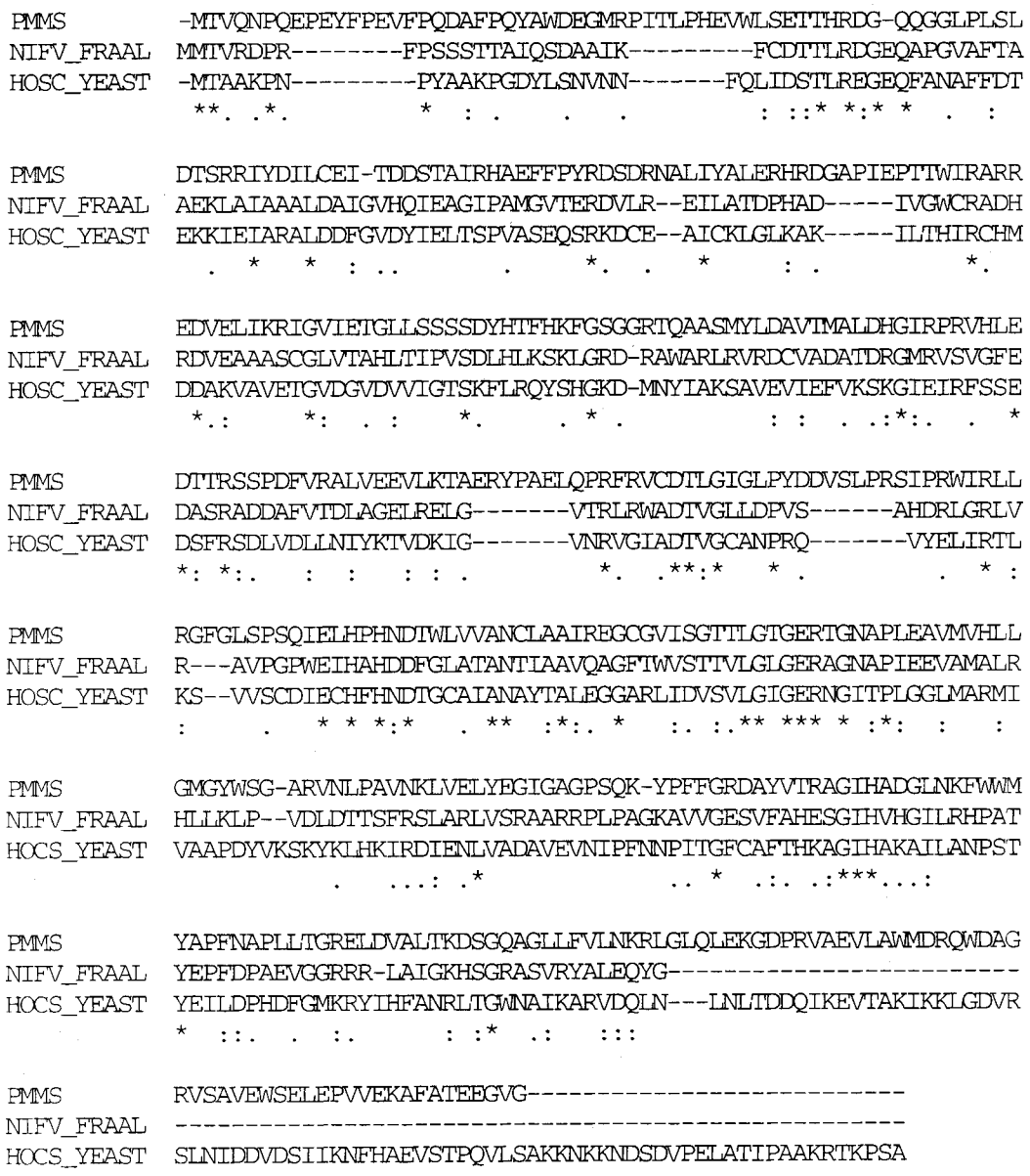
Nucleotide sequence of the PMM synthase gene. The deduced amino acid sequence of the enzyme is shown under the nucleotide sequence. The previously determined amino acid sequence of the *N*-terminus portion is underlined[6]. These nucleotide sequence data have been deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases under accession No. AB029822.

Fig. 3 (B).

```
PMMS    MTVQNPQEPEYFPEVFPQDAFPQYAWDEGMRPITLPHEVWLSETTHRDGQQGGLPLSLDT
CLSA    LNLKDVEEPNLYRDIFPYHEVPKIKFSTDEIKVDIPDEIWITDTTFRDGQQSMTPFTVEQ
        :.::: :**: : ::** . .*:  :. .   : :*.*:*:::**.*****.  *::::

PMMS    SRRIYDILCEITDDSTAIRHAEFFPYRDSDRNALIYALERHRDGAPIEPTIWIRARREDV
CLSA    IVTIFDYLNKLDNNTGVIRQTEFFLYTNRDKEALMECMNRGYKFP--QITIWIRANKDDF
           *:* * :: ::: .**::*** * : *::**: .::*  . .  : ******.::*.

PMMS    ELIKRIGVIETGLLSSSSDYHTFHKFGSGGRTQAASMYLDAVTMALDHGIRPRVHLEDIT
CLSA    KLVKDIGIKETGILMSCSDYHIFKKLKMT-RTETYNKYVEIVEEALSNGIVPRCHLEDIT
        :*:* **: ***:* *.**** *:*:    **:: . *:: *  **.:** ** **** *

PMMS    RSSPD-FVRALVEEVLKTAERYPAELQPRFRVCDILGIGLPYDDVSLPRSIPRWIRLLRG
CLSA    RADFFGFVVPLVNKLMELSNKYG--IQVKIRACDTLGLGVAFPGVELPRSVPAIISGLRK
        *:.   ** .**:::: :::*   :* ::*.*****:*:.: .*.****:*  *  **

PMMS    FGLSPS-QIELHPHNDIWLVVANCLAAIREGCGVISGTTLGIGERTGNAPLEAVMVHLLG
CLSA    YCGVPSTALEWHGHNDFYYVVPNATAAWLHGCSAVNTTLLGIGERTGNCPLEGMVFQYCQ
        :   **  :* * *** : **.*. **  .**..:. * ** ******.***.:::.:

PMMS    MGYWSGARVNLPAVNKLVELYEGIGAGPSQ-KYPFFGRDAYVTRAGIHADGLNKFWWMYA
CLSA    LKGNPG--MNLHAITEMSKYFENSMKYEIPPRTPFVGIDFNVTRAGIHADGILKDQEIYN
        :   .*  :** *:.:: : :*.        : **.* *  **********: *   :*

PMMS    PFNAPLLTGRELDVALTKDSGQAGLLFVLNKRLGLQLE----KGDPRVAEVLAWMDRQWD
CLSA    IFDIEKILDRPVLVAVNEYSGLAGIAAWINTYFKLNKENEVDKKDSRVAEIKKWVDNLYE
         *::  : .* : **:.: ** **:   :*. : *: *    * *.****:  *:*. ::

PMMS    AGRVSAVEWSELEPVVEKAFATEEGVG
CLSA    NGRTTPITNKELE--------------
         **.:.:  .***
```

Homology between the amino acid sequences of PMM synthase (PMMS) and the predicted protein of *Clostridium acetobutylicum* (CLSA).

Fig. 3 (C).



Homology between the amino acid sequences of PMM synthase and homocitrate synthase of *Frankia alni* (NIFV FRAAL, DDBJ Q47884) and *Saccharomyces cerevisiae* (HOSC YEAST, DDBJ P48570). The conserved amino acids are marked by asterisks. These amino acid sequences were aligned by CLUSTAL W (1.7) multiple sequence alignments program.

terminal 27 amino acids of PMM synthase and revealed that this sequence was encoded in a 600 bp *Sma*I-*Sph*I fragment of the chromosomal DNA portion of pMSB12[6] (Fig. 2, fragment (A)). In view of the molecular weight of PMM synthase, 48000 daltons, determined by SDS-PAGE[5], we assumed the PMM synthase gene to be located in a 2.1 kb *Sma*I-*Sph*I fragment of pMSB12 (Fig. 2, fragment (B)). The nucleotide sequence of the fragment was determined on an automated sequencer (ABI PRISM 310, PE Appleid Biosystems). As shown in Fig. 3 (A), an open reading frame of 441 codons, starting at nucleotide 463 and terminating at nucleotide 1785, was identified.

The deduced amino acid sequence of PMM synthase showed significant identity (38.0%) to that of the predicted protein encoded by the *Clostridium acetobutylicum* genome found in TIGR Microbial Database (accession number AE001437, nucleotide 774682 to 775959) (Fig. 3 (B)) indicating that PMM synthase and the *C. acetobutylicum* protein had the same function. In view of the close taxonomical relationship between *C. acetobutylicum* and *Clostridium acidi-urici* possessing (*R*)-citrate synthase, the predicted protein of *C. acetobutylicum* may be concluded to be (*R*)-citrate synthase. In addition, PMM synthase showed similarity to several homocitrate synthases[9] catalyzing the condensation of acetyl-CoA and α-keto-glutaric acid (Fig. 3 (B)). This synthase catalyzes the first step of the α-aminoadipic acid pathway that leads to the biosynthesis of lysine in some yeast and fungi[9]. This enzyme was also detected in some nitrogen-fixing bacteria as the *nifV* gene product. In these organisms, homocitrate is a component of the iron-molybdenum cofactor of nitrogenase[10]. The absolute configuration of homocitric acid synthesized by homocitrate synthase was reported to be (*R*)[9,10] in both cases. Thus, the similarity between PMM synthase and homocitrate synthase means that the reaction mechanisms of these two enzymes are similar; both of them catalyze the condensation between acetyl-CoA and α-keto acid to form (*R*)-type citric acid analogs. It is thus suggested the evolutional origin of all of three enzymes, PMM synthase, (*R*)-citrate synthase and homocitrate synthase are identical.

The analysis of the primary structure of PMM synthase has revealed that PMM synthase belongs to the group of (*R*)-citrate synthase reported only to be present in few obligate anaerobic bacteria. It is interesting to note that although (*R*)-citrate synthase of *C. acidi-urici* is inactivated by oxygen[10], PMM synthase is stable under aerobic conditions. It may be argued that (*R*)-citrate synthase of *C. acetobutylicum* or other obligate anaerobes was transferred to the bialaphos producing *S. hygroscopicus* and that the enzyme was converted to a protein resistant to oxygen to be utilized for the biosynthesis of bialaphos under the aerobic conditions. Comparison of the structure of PMM synthase to that of (*R*)-citrate synthase would reveal the inactivation mechanism of (*R*)-citrate synthase of obligate anaerobic bacteria by oxygen.

Acknowledgments

We thank Ajinomoto Co. for providing us with *B. lactofermentum*. This work was supported in part by JSPS-RFTF96I00301 (T. H., T. M. and H. S.).

TOMOMI HIDAKA*
KUMIKO W. SHIMOTOHNO[a]
TAKEHIKO MORISHITA[b]
HARUO SETO[b]

Center for Gene Research, Ehime University,
3-5-7 Tarumi, Matsuyama 790-8566, Japan
[a] Kyoritsu College of Pharmacy,
1-5-30 Shiba-koen, Minato-ku, Tokyo 105-8512, Japan
[b] Institute of Molecular and Cellular Biosciences,
University of Tokyo,
Bunkyo-ku, Tokyo 113-0032, Japan

(Received July 14, 1999)

References

1) NAKASHITA, H.; K. WATANABE, O. HARA, T. HIDAKA & H. SETO: Studies on the biosynthesis of bialaphos. Biochemical mechanism of C–P bond formation: Discovery of phosphonopyruvate decarboxylase which catalyzes the formation of phosphonoacetaldehyde from phosphonopyruvate. J. Antibiotics 50: 212~219, 1997
2) KONDO, Y.; T. SHOMURA, Y. OGAWA, T. TSURUOKA, H. WATANABE, K. TOTSUKAWA, T. SUZUKI, C. MORIYAMA, J. YOSHIDA, S. INOUE & T. NIIDA: Studies on a new antibiotic SF-1293. I. Isolation and physico-chemical and biological characterization of SF-1293 substance. Scientific Reports of Meiji Seika Kaisha 13: 34~41, 1973
3) SHIMOTOHNO, K. W.; H. SETO, N. OTAKE, S. IMAI & A. SATOH: Studies on the biosynthesis of bialaphos (SF-1293). 7. The absolute configuration of 2-phosphinomethylmalic acid, a biosynthetic intermediate of bialaphos. J. Antibiotics 39: 1356~1359, 1986
4) SETO, H.; S. IMAI, T. SASAKI, K. W. SHIMOTOHNO, T. TSURUOKA, H. OGAWA, A. SATOH, S. INOUE, T. NIIDA & N. OTAKE: Studies on the biosynthesis of bialaphos (SF-1293). 5. Production of 2-phosphinomethylmalic acid, an analogue of citric acid by *Streptomyces hygroscopicus* SF-1293 and its involvement in the biosynthesis of bialaphos. J. Antibiotics 37: 1509~1511, 1984
5) SHIMOTOHNO, K. W.; H. SETO & N. OTAKE: Studies on the biosynthesis of bialaphos (SF-1293). 8. Purification and characterization of 2-phosphinomethylmalic acid synthase from *Streptomyces hygroscopicus* SF-1293. J. Antibiotics 41: 1057~1065, 1988

6) SHIMOTOHNO, K. W.; S. IMAI, T. MURAKAMI & H. SETO: Purification and characterization of citrate synthase from *Streptomyces hygroscopicus* SF-1293 and comparison of its properties with those of 2-phosphinomethylmalic acid synthase. Agric. Biol. Chem. 54: 463~470, 1990
7) KAWABATA, Y.; K. TAKAHASHI-FUKE, E. SHIMIZU, T. NAKAMATSU & S. NAKAMORI: Relationship between the glutamate production and the activity of 2-oxoglutarate dehydrogenase in *Brevibacterium lactofermentum*. Biosci. Biotechnol. Biochem. 61: 1109~1112, 1997
8) SCHWARTZ, D.; J. RECKTENWALD, S. KASPER, G. KIENZLEN & W. WOHLLEBEN: "Symposium Papers, Japan-UK Joint Study on Molecular Genetics of Streptomyces, John Innes Research Centre, Norwich, UK, 1997" p. 21
9) THOMAS, U.; M. G. KALYANPUR & C. M. STEVENS: The absolute configuration of homocitric acid, an intermediate in lysine biosynthesis. Biochemistry 5: 2513~2516, 1966
10) GOTTSHALK, G.: Partial purification and some properties of the *R*-citrate synthase from *Clostridium acidi-urici*. Eur. J. Biochem. 7: 301~306, 1969
11) HIDAKA, T.; M. HIDAKA, T. UOZUMI & H. SETO: Nucleotide sequence of carboxyphosphonoenolpyruvate phosphonomutase gene isolated from a bialaphos producing organism, *Streptomyces hygroscopicus*, and its expression in *Streptomyces lividans*. Mol. Gen. Genet. 233: 476~478, 1992
12) KAMIGIRI, K.; T. HIDAKA, S. IMAI, T. MURAKAMI & H. SETO: Studies on the biosynthesis of bialaphos (SF1293) 12. C–P bond formation mechanism of bialaphos: discovery of a P-methylation enzyme. J. Antibiotics 45: 781~787, 1992
13) THOMPSON, C. J.; N. R. MOVVA, R. TIZARD, R. CRAMERI, J. E. DAVIES, M. LAUWEREYS & J. BOTTERMAN: Characterization of the herbicide-resistance gene bar from *Streptomyces hygroscopicus*. EMBO J. 6: 2519~2523, 1987