Propionyl-coenzyme A synthetases of *Ralstonia solanacearum* and *Salmonella choleraesuis* display atypical kinetics

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Abstract Propionyl-coenzyme A synthetases (PrpE) of *Salmonella choleraesuis* and *Ralstonia solanacearum* sharing 62% identity in amino acid sequence to each other were cloned, expressed in *Escherichia coli* and purified. Both enzymes catalyzed acetyl-, propionyl-, butyryl- and acrylyl-coenzyme A formation with the highest k_{cat}/K_m values for propionate. They displayed sigmoidal homotrophic autoactivation kinetics for propionate but not for the other acyl substrates tested. Besides, substrate inhibition kinetics was observed for co-substrates, i.e. ATP and CoA. Based on the kinetic data reported herein, the reaction mechanisms of the enzyme are discussed.

Key words: Propionyl-CoA synthetase; Kinetics; Autoactivation; Substrate inhibition; *Ralstonia*; *Salmonella*

1. Introduction

Acyl-coenzyme A (CoA) synthetase belongs to the acyl-adenylate/thioester-forming family of enzymes and catalyzes the primary route for fatty acid activation in organisms [1,2]. The most intensively characterized member of this family is acetyl-CoA synthetase (ACS), which catalyzes two-step reactions for the formation of acetyl-CoA from acetate, CoA and ATP via acetyl-AMP [3]. ACS has been identified in a variety of organisms including animals, plants, bacteria and archaea [1,4–7], and their enzymatic and structural characteristics have been studied in detail [8,9]. Other members of the acyl-CoA synthetase group include butyryl-CoA synthetase [10] and long-chain acyl-CoA synthetases [2,11]. In addition, the presence of propionyl-CoA synthetase (PCS) activity (preferential activation of propionate) has been detected in guinea pig liver mitochondria [12] and *Rhodopseudomonas sphaeroides* [13], and a gene (*prpE*) encoding PCS has been cloned from *Salmonella typhimurium* [14].

Propionate is one of the important intermediate metabolites and/or the end product of fermentation processes, and is also known to become toxic to cellular metabolism in patients after it is transformed to propionyl-CoA [15–17]. Another importance of propionate metabolism is associated with the usage of propionate derivatives as antimicrobial agents in medical, agricultural and industrial processes [18,19]. Many organisms can metabolize propionate by ACS (albeit slowly), while some organisms are known to possess PCS, which imparts a selective advantage for their survival in propionate-containing environments. So far, only one PCS, PrpE of *S. typhimurium*, has been cloned and studied in detail [14] and its preferential transformation of propionate has been demonstrated experimentally [14,20]. In addition to this enzyme, open reading frames (ORFs) in genome-sequenced bacteria of *Vibrio cholerae*, *Brucella suis*, *Escherichia coli*, *Ralstonia solanacearum*, *Salmonella enterica* and *Bordetella parapertussis* have been annotated to be genes for PCS (*prpE*) according to the similarities in primary structures. Phylogenetic comparisons of these genes have indicated that PCSs exhibit substantial similarities to ACSs and they form a separate cluster [14]. However, due to the limitation in enzymatic characterization of PCSs, the enzymatic and evolutionary relationships between PCSs and ACSs are still unclear.

In the present study, we examined the enzymatic characteristics of two PCSs cloned from *Salmonella choleraesuis* (PrpE-SC) and *R. solanacearum* (PrpE-RS). The homologies in amino acids of PrpE-SC and PrpE-RS with PrpE cloned from *S. typhimurium* (PrpE-ST) [20] were 99% and 62%, respectively. The primary purpose of this study was to gain experimental evidence that PCS is present in bacteria other than *S. typhimurium*. PrpE-RS was selected in our analysis, because PCS has been suggested to be an important enzyme for biopolymer production [21] and is considered to be involved in antimicrobial resistance in *Ralstonia* spp., including *R. solanacearum*, an important plant pathogen [22]. PrpE-SC, which is highly homologous to PrpE-ST, was also analyzed for comparing the kinetic properties. During the course of this study, we found that both PCSs did not display typical hyperbolic (Michaelis–Menten-type) kinetics to propionate, and therefore, another purpose of the present study was to describe the kinetics of these PCSs in detail, which may help in understanding the reaction mechanisms of the enzyme.

2. Materials and methods

2.1. Materials

Propionyl-CoA and CoA were purchased from Sigma Chemicals (St. Louis, MO, USA). Butyryl-CoA was obtained from Calbiochem-Novabiochem (La Jolla, CA, USA), and all other chemicals unless stated otherwise were obtained from Wako Pure Chemicals (Osaka, Japan). Genomic DNA of *R. solanacearum* GMI1000 was kindly provided by Dr. Christian Boucher (CNRS-INRA, France).

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Abbreviations: PCS, propionyl-coenzyme A synthetase; ACS, acetyl-coenzyme A synthetase; PCR, polymerase chain reaction; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

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The type strain, *S. choleraesuis* subsp. *choleraesuis* JCM6980, was obtained from the Japan Collection of Microorganisms.

2.2. Polymerase chain reaction (PCR) cloning and construction of expression vectors

ORF Rso3786 encoding the putative PCS was amplified by PCR from the genomic DNA of *R. solanacearum* GMI1000 using a forward primer (5′-CCATATGCCCATGTCCGAC-3′) and a reverse primer (5′-CGAGCTCACGCCTGCATCG-3′), which incorporated the *Nde*I and *Sac*I restriction sites (underlined) at the 5′ and 3′ ends of the gene, respectively. After the PCR product (approximately 1.9 kb) was purified by gel electrophoresis, it was cloned into the pGEM-T Easy plasmid (Promega, Madison, WI, USA), and *E. coli* JM109 (Takara, Kyoto, Japan) was transformed with the resultant plasmid (pGEM-prpE-RS). The nucleotide sequence of the cloned fragment was confirmed using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Plasmid pGEM-prpE-RS was digested with *Nde*I and *Sac*I, and a 1.9-kb fragment containing *prpE-RS* was ligated into pET28a(+) (Novagen, Madison, WI, USA) using Ligation-High T4 DNA ligase (Toyobo, Osaka, Japan). The pET28a(+) harboring *prpE-RS* with His_6-coding nucleotide bases at the N-terminal end (pET-prpE-PS) was transferred into *E. coli* Rosetta(DE3) (Novagen).

Similarly, the *prpE* of *S. choleraesuis* JCM6980 was PCR-amplified using a forward primer (5′-CCATATGTCTTTTAGCGAATTTTAT-3′) and a reverse primer (5′-CAAGCTTATTCCTCGATCGCC-3′), which incorporated the *Nde*I and *Hin*dIII restriction sites (underlined) at the N- and C-terminal ends of the gene, respectively. The PCR product was first cloned into pGEM-T Easy, sequenced and subsequently ligated into pET28a(+) as described above. The resultant plasmid (pET-prpE-SC) was transferred into *E. coli* Rosetta(DE3).

2.3. Expression and purification of the recombinant enzymes

The *E. coli* Rosetta(DE3) cells harboring pET-prpE-RS or pET-prpE-SC were grown overnight at 37°C in 2 ml of SOC medium [23] supplemented with 50 μg/ml kanamycin. This 2-ml seed culture was inoculated to 200 ml of SOC medium supplemented with 50 μg/ml kanamycin and incubated at 20°C. When the optical density at 600 nm of the culture had reached approximately 0.6, expression of the target gene was induced by adding isopropyl-β-D-thiogalactose at 0.5 mM, and the cultivation was continued for approximately 12 h. Cells were then harvested by centrifugation at 4000×*g* for 20 min at 4°C, washed twice with 20 mM sodium phosphate buffer (pH 8.0) and then suspended in buffer A (30 ml of 50 mM sodium phosphate buffer [pH 8.0] containing 400 mM NaCl and 10 mM imidazole) supplemented with lysozyme at 1 mg/ml. After incubation for 30 min on ice, the cells were further lysed by sonication. Cell debris was removed by centrifugation at 10 000×*g* for 30 min, and the supernatant containing crude enzyme was applied to 1 ml of a fresh Ni-NTA resin column (Qiagen, Valencia, CA, USA). The bound enzyme was eluted with a linear gradient of 10–100 mM imidazole in buffer A. The active fraction was pooled and dialyzed overnight at 4°C against 20 mM sodium phosphate buffer (pH 7) (for PrpE-SC) or 20 mM Tris–HCl buffer (pH 8) (for PrpE-RS) containing 20% glycerol.

2.4. Estimation of protein concentration and molecular weight

Protein concentrations were measured using Coomassie plus protein assay reagent kit (Pierce, Rockford, IL, USA) with bovine serum albumin as the standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of the enzyme samples was done as described by Laemmli [24], and protein bands were visualized by staining the gel with Coomassie brilliant blue R250. A wide range (3.5–205 kDa) protein marker (Technical Frontier, Tokyo, Japan) was used to estimate molecular weights of the PrpE proteins. For gel filtration analysis, the purified PrpE-RS or PrpE-SC were dialyzed overnight against 50 mM Tris–HCl buffer pH 8 containing 0.1 M NaCl and applied to Superose 6 HR10/30 (Amersham Pharmacia Biotech, Tokyo, Japan) equilibrated with the same buffer. The proteins were eluted at a flow rate of 0.1 ml/min. A standard protein mixture (Oriental Yeast, Osaka, Japan) consisted of glutamate dehydrogenase (290 kDa), lactate dehydrogenase (142 kDa), enolase (67 kDa), adenylate kinase (32 kDa) and cytochrome *c* (12.4 kDa).

2.5. Enzyme assay

The PCS activity of PrpE-RS was determined at 37°C by the method of Jones and Lipmann [25] with modifications. The reaction mixture (200 μl) contained 2 mM of sodium propionate, 5 mM ATP, 3 mM $MgCl_2$, 0.5 mM dithiothreitol, 300 mM hydroxylamine, 50 mM Tris–HCl buffer pH 8 and a suitable amount of enzyme. The assay was initiated by adding CoA (2.5 mM), after the reaction mixture was kept at 37°C for 10 min. After incubation for 20 or 30 min, the reaction was terminated by adding 200 μl of 2.5% (w/v) $FeCl_3$ in 2 M HCl containing 10% perchloric acid. The denatured protein was removed by centrifugation at 9000 rpm for 5 min, and the absorbance at 520 nm was measured. The standard curves for acetyl-CoA, propionyl-CoA and butyryl-CoA were prepared for estimating the enzyme activity. The above procedure was adapted for the determination of PrpE-SC activity in 50 mM sodium phosphate buffer pH 7 without dithiothreitol. One unit of activity was defined to be 1 μmol of acyl-CoA produced per minute.

2.6. Effects of pH and temperature on enzyme activity and stability

The optimum pH was determined by measuring the activity (2 mM propionate) at 37°C for 30 min in the following buffers (50 mM): sodium citrate (pH 2.7–5.1), 2-[*N*-morpholino]ethane sulfonic acid (pH 5.3–6.6), sodium phosphate (pH 6.2–8.2), Tris–HCl (pH 7.3–8.7), 2-[*N*-cyclohexylamino]ethane sulfonic acid (pH 8.3–10.1), 3-[cyclohexylamino]-1-propane sulfonic acid (pH 9.6–11.2). To determine the optimum temperature, the enzyme activity (2 mM propionate) was measured at 16–65°C.

For examining the pH stability, the enzyme (8.4–10 μg protein/ml) was incubated in the above buffer for 30 min at 37°C, before the residual activity was measured under the standard conditions described in above. To determine the thermal stability, the enzyme (8.4–10 μg/ml) was incubated for 30 min in 50 mM Tris–HCl pH 8 (PrpE-RS) or Na-phosphate buffer pH 7 (PrpE-SC) at different temperatures (16–65°C), before the residual enzyme activity was measured.

2.7. Kinetic analyses

The enzyme activity was measured at different concentrations of fatty acyl substrates, CoA and ATP, and the results were analyzed using the Grafit software program, version 4 (Erithacus software, Middlesex, UK). Kinetics were analyzed according to the Michaelis–Menten equation and the Hill equation [26]. The Hill coefficient (n) was determined by linear regression of the plot for $\log[v/V_{max}-v]$ vs. $\log[S]$.

3. Results and discussion

3.1. Production of PrpE in E. coli

Using Ni-NTA affinity chromatography, PrpE-RS and PrpE-SC proteins were purified to almost homogeneity (Fig. 1) with specific activities (for propionate) of 1.1 and 1.0 units/mg protein, respectively. These values correspond to the specific activity reported for the *Salmonella* PrpE [14]. In the SDS–PAGE analysis, the purified enzymes migrated as single bands with a molecular mass of approximately 70 kDa (Fig. 1), which agreed with the mass calculated on the basis of their primary structures (70.9 kDa for PrpE-RS and 71.3 kDa for PrpE-SC). In the gel filtration analysis, intrapolation from the calibration curve determined the molecular masses of the PrpE proteins to be approximately 70 kDa. These data indicate that PrpE-RS and PrpE-SC are monomeric enzymes.

3.2. Optimum conditions and enzyme stability

The optimum conditions for PCS activity of PrpE-RS and PrpE-SC were found to be around pH 8.0 at 37°C and around pH 7.0 at 45°C, respectively. Fig. 2 presents results of stability analyses, and it was clear that these enzymes have similar thermal and pH stabilities. It is noteworthy that they were very unstable at moderately low pH; the PCS activity was almost negligible after incubation at pH 5 for 30 min, while no significant loss of activity was observed after incubation at

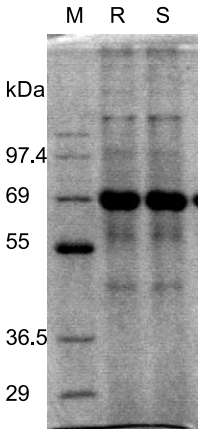

Fig. 1. SDS–PAGE of purified PrpE-RS and PrpE-SC. Lanes: M, molecular mass marker; R, PrpE-RS; S, PrpE-SC.

pH 9 and higher. This asymmetric pH dependence is interesting and considered not to be due to electrostatic effects on overall protein folding; rather, it is likely ascribable to some specific reaction mechanisms of PCS. Recently, workers have suggested that the pH dependence of the thermodynamic stability of a protein arises as a consequence of different pK_a values between folded and unfolded states, in which pK_a values of some specific residues (Asp and Glu, in particular) are important [27]. In the case of acyl-CoA synthetases, crystal structure analysis of ACS has suggested that the enzyme adopts two different orientations to catalyze two half reactions by rotating its C-terminal domain [9]. Based on these findings, we hypothesize that the moderately low pH may have resulted in irreversible denaturation of the flexible catabolic sites.

3.3. Substrate specificity

Acetate, propionate and butyrate and their analogues, including acrylate, isobutyrate, phenylacetate, ethylacetate, phenylpropionate, sodium lactate, β-alanine, formic acid, pyruvate, 2-phenylbutyric acid, phenylpropionate, 2-mercaptopropionic acid, 3-mercaptopropionic acid, 3-phenylpropionic acid, 2-hydroxyphenylpropionic acid and *p*-nitrophenyl propionate (a total of 19 substrates), were tested to determine if they served as substrates for PrpE-RS and PrpE-SC. Among them, PrpE-RS and PrpE-SC transformed only acetate, propionate, butyrate and acrylate (Table 1). All other substrates with substitutions at the C1, C2, C3 and C4 carbons with thiol, phenyl-carboxylic, methyl, or amino groups did not serve as substrates for the enzymes. Acrylate, having a deletion of hydrogen atoms at C2 and C3, was found to be a good substrate, but its K_m values were significantly higher than that

Table 1
Substrate specificity of PrpE-RS and PrpE-SC[a]

Substrate	PrpE-RS	PrpE-SC
Acetate	1.67 (150)[b]	1.32 (128)
Propionate	1.11 (100)	1.03 (100)
Butyrate	0.67 (60)	0.14 (14)
Acrylate	1.21 (109)	0.21 (20)

[a]Activity (μmol/min/mg) was measured at 37°C at a substrate concentration of 2 mM.
[b]Values in parentheses indicate percentages relative to the activity for propionate.

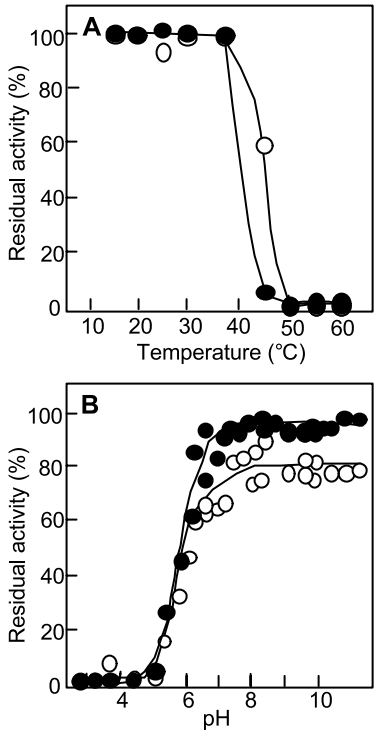

Fig. 2. Stability of PrpE-RS (●) and PrpE-SC (○). Residual activities after incubation (for 30 min) under different temperatures (A) or pHs (B) are shown. The details of the experiments are described in Section 2.

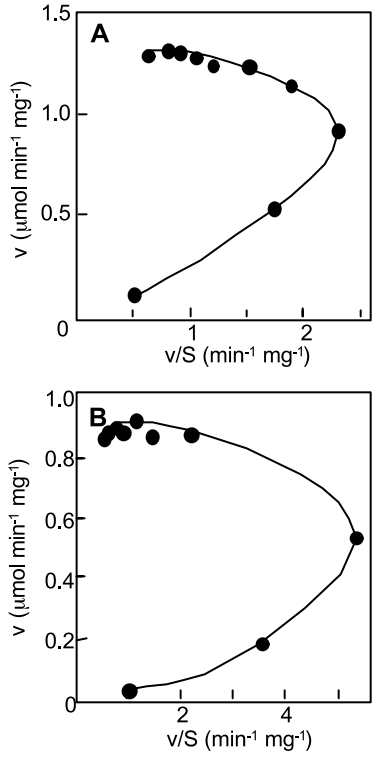

Fig. 3. Eadie–Hofstee plots of the activities of PrpE-RS (A) and PrpE-SC (B) towards propionate. The activities were measured using 10 different concentrations of propionate ranging from 25 to 2000 μM.

Table 2
Kinetic constants of PrpE-RS and PrpE-SC for different substrates and co-substrates

Substrate	PrpE-RS				PrpE-SC			
	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m (s^{-1} mM^{-1})	n	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m (s^{-1} mM^{-1})	n
Propionate	0.26 ± 0.02	1.8 ± 0.02	6.8	1.38	0.080 ± 0.002	1.2 ± 0.02	15	1.25
Acetate	1.9 ± 0.06	6.8 ± 0.06	3.6	0.96	1.9 ± 0.08	4.8 ± 0.07	2.5	0.98
Butyrate	13 ± 0.85	1.7 ± 0.05	0.13	0.84	82 ± 5.0	0.87 ± 0.03	0.01	0.96
Acrylate	1 ± 0.10	1.3 ± 0.06	1.3	1.47	1.3 ± 0.07	1.1 ± 0.01	0.8	0.81
CoA	0.69 ± 0.05	2.4 ± 0.18	3.5	NA[a]	0.9 ± 0.04	1.0 ± 0.02	1.1	NA
ATP	0.65 ± 0.04	1.5 ± 0.04	2.3	NA	0.33 ± 0.01	1.1 ± 0.03	3.3	NA

[a]Not analyzed.

of propionate (see below), suggesting that those hydrogens at C2 and C3 are involved in substrate binding. These results suggest that PrpE-RS and PrpE-SC are acetate- and propionate-preferring acyl-CoA synthetases.

3.4. Kinetics

In order to examine if PrpE-RS is PCS, detailed kinetic analyses for the enzymatic activities toward the available acyl substrates (acetate, propionate, butyrate and acrylate [acrylate is not an authentic acyl compound]) and co-substrates (ATP and CoA) were carried out. The activities were determined in wide ranges of substrate concentrations, showing that the activity curves did not follow typical hyperbolic saturation profiles in some cases (Fig. 3). In such cases, kinetic constants in the Michaelis–Menten equation were determined using values in ranges where the hyperbolic saturation relationships could reasonably be applied (Table 2). As presented in this table, both enzymes exhibited the lowest K_m values for propionate. Although the k_{cat} values of these enzymes were the highest for acetate, estimation of k_{cat}/K_m values indicates that the preferential substrate was propionate. When the kinetic trends of these enzymes were compared, PrpE-SC was shown to be more specific for propionate than PrpE-RS.

The kinetic properties of PrpE-SC were very similar to those of PrpE-ST [14,20], while the values were different to some extent (e.g. K_m values for propionate of PrpE-SC and PrpE-ST were 80 and 20 μM, respectively). PrpE-SC is 99% identical in amino acid sequence to PrpE-ST; the different residues may have affected the kinetic values, although they are considered to be peripheral ones. Other possibilities include differences in conditions for the enzyme assay and that PrpE-SC was produced as a recombinant protein having the 6×His tag at the N-terminal end. The third possibility is, however, not likely, since the crystallography of ACS, the homologous enzyme, has shown that its N-terminus has a relaxed structure (freely moving out of the enzyme) and is situated at the opposite end from the catalytic center [9].

The Eadie–Hofstee plots (Fig. 3) indicated that PrpE-RS and PrpE-SC did not exhibit typical Michaelis–Menten kinetics for propionate. Hence, the Hill equation was also applied to analyze the kinetics, and the Hill coefficients (n) were estimated (Table 2). The n values of 1.3 and 1.4 indicate that these enzymes displayed positive cooperativity for propionate activation (i.e. homotrophic autoactivation) [28,29]. On the other hand, for other acyl substrates except for the case of PrpE-RS for acrylate, the Eadie–Hofstee plots were almost straight (data not shown) and the n values were about 1 (Table 2). These results suggest that PrpE enzymes possess propionate-specific autoactivation mechanisms, although its specificity was not sufficiently high in PrpE-RS. To our knowledge, this is the first report to show homotrophic autoactivation mechanism for acyl-CoA synthetase. Similar homotrophic autoactivation kinetics have been reported for cytochrome P450 [29,30], acetyl-CoA carboxylase [31] and some other enzymes [32,33]. Studies on these enzymes have suggested that homotrophic autoactivation is observed when multiple substrate-binding sites are present (as observed for acetyl-CoA carboxylase [24]) or conformational change of the enzyme occurs in the presence of a substrate. In the case of PrpE, it would be interesting if there is a non-catalytic binding site for propionate.

As shown in Fig. 4, this study also found that the activities of PrpE-RS and PrpE-SC were reduced at higher concentrations of CoA and ATP, although concentrations where substrate inhibition was observed were much higher than those in bacterial cells. Similar inhibition by CoA has been observed for phosphopantetheinyl transferase [34]. Nevertheless, the in-

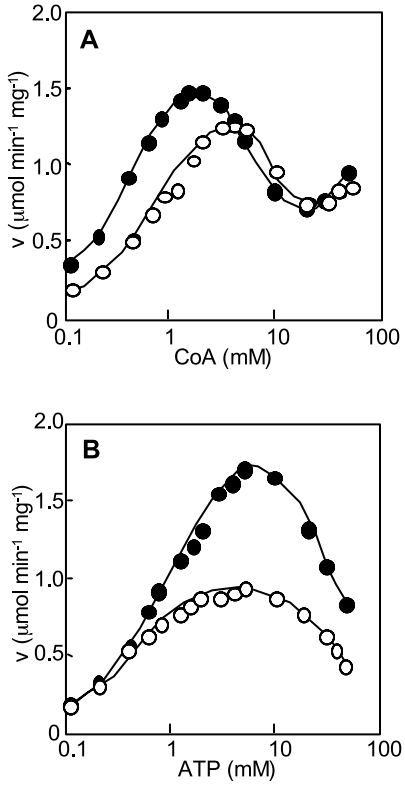

Fig. 4. Effects of CoA (A) and ATP (B) concentrations on the activities of PrpE-RS (●) and PrpE-SC (○). The activities were measured at a propionate concentration of 2 mM, and similar activity curves were also observed at propionate concentrations of 5 and 10 mM (data not shown).

formation is considered useful for deducing the reaction mechanism, and in this case, we deduce that high concentrations of co-substrates may have been inhibitory to the reversible conformational change for the two half reactions as observed for ACS [9].

3.5. Conclusions

This study gained the experimental evidence that *R. solanacearum*, an important plant pathogen, has PCS that may play roles in resistance to some antimicrobial chemicals. In addition, detailed kinetic analyses of two PCS enzymes showed that they employ atypical kinetics toward propionate, suggesting that their reaction mechanisms are more complex than those considered previously. Based on the kinetic data obtained in the present study, we described some hypotheses on the reaction mechanisms of PCS, which will be addressed in our ongoing protein structure analyses coupled to site-directed mutagenesis.

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References

[1] Londesborough, J.C. and Webster, L.T. (1974) The Enzyme, 3rd edn., Vol. 10, pp. 469–488.
[2] Bar-Tana, J. and Shapiro, B. (1975) Methods Enzymol. 35, 117–122.
[3] Metzler, D.E. (2001) Biochemistry: The Chemical Reactions of Living Cells, 2nd edn., Vol. 1, Harcourt Academic, New York.
[4] Brown, T.D.K., Jones-Mortimer, M.C. and Konberg, H.L. (1977) J. Gen. Microbiol. 102, 327–336.
[5] De Virgilio, C., Burckert, N., Barth, G., Neuhaus, J.M., Boller, T. and Wiemken, A. (1992) Yeast 12, 1043–1051.
[6] Martinez-Blanco, H., Reglero, A., Fernandez-Valverde, M., Ferrero, M.A., Moreno, M.A., Penalva, M.A. and Luengo, J.M. (1992) J. Biol. Chem. 267, 5474–5481.
[7] Grundy, F.J., Waters, D.A., Takova, T.Y. and Henkin, T.M. (1993) Mol. Microbiol. 10, 259–271.
[8] Starai, V.J., Celic, I., Cole, R.N., Boeke, J.D. and Escalante-Semerena, J.C. (2002) Science 298, 2390–2392.
[9] Gulick, A.M., Starai, V.J., Horswill, A.R., Homick, K.M. and Escalante-Semerena, J.C. (2003) Biochemistry 42, 2866–2873.
[10] Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1981) Biochem. Biophys. Res. Commun. 103, 1231–1237.
[11] Tanaka, T., Hosaka, K., Hoshimaru, M. and Numa, S. (1979) Eur. J. Biochem. 98, 165–172.
[12] Groot, P.H. (1976) Biochim. Biophys. Acta 441, 260–267.
[13] Maruyama, K. (1982) J. Biochem. 91, 725–730.
[14] Horswill, A.R. and Escalante-Semerena, J.C. (1999) Microbiology 145, 1381–1388.
[15] Glasgow, A.M. and Chase, H.P. (1976) Pediatr. Res. 10, 683–686.
[16] Brass, E.P. and Beyerick, R.A. (1987) Metabolism 36, 781–787.
[17] Bain, M.D., Jones, M., Borriello, S.P., Reed, P.J., Tracey, B.M., Chalmers, R.A. and Stacey, T.E. (1988) Lancet i, 1078–1079.
[18] Cherrington, C.A., Hinton, M., Mead, G.C. and Chopra, I. (1991) Adv. Microb. Physiol. 32, 88–107.
[19] Sevoz, C., Benoit, E. and Buronfosse, T. (2000) Drug Metab. Dispos. 28, 398–402.
[20] Horswill, A.R. and Escalante-Semerena, J.C. (2002) Biochemistry 41, 2379–2387.
[21] Valentin, H.E., Mitsky, T.A., Mahadeo, D.A., Tran, M. and Gruys, K.J. (2000) Appl. Environ. Microbiol. 66, 5253–5258.
[22] Stapp, C. (1965) Zent.bl. Bakteriol. Parasitenkd. Infektionskr. Hyg. 119, 166–190.
[23] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring harbor Laboratory, Cold Spring Harbor, NY.
[24] Laemmli, U.K. (1970) Nature 227, 680–685.
[25] Jones, M.E. and Lipmann, F. (1955) Methods Enzymol. 1, 585–591.
[26] Segel, I.H. (1975) Enzyme Kinetics, Wiley-Liss, New York.
[27] Tollinger, M., Crowhurst, K.A., Kay, L.E. and Forman-Kay, J.D. (2003) Proc. Natl. Acad. Sci. USA 100, 4545–4550.
[28] Soars, M.G., Ring, B.J. and Wrighton, S.A. (2003) Drug Metab. Dispos. 31, 762–767.
[29] Hutzler, J.M. and Tracy, T.S. (2002) Drug Metab. Dispos. 30, 355–362.
[30] Harlow, G.R. and Halpert, J.R. (1998) Proc. Natl. Acad. Sci. USA 95, 6636–6641.
[31] Dehaye, L., Alan, C., Job, C., Douce, R. and Job, D. (1994) Eur. J. Biochem. 225, 1113–1123.
[32] Sakamoto, T., Sakata, S.F., Matsuda, K., Horikawa, Y. and Tamaki, N. (2001) J. Nutr. Sci. Vitaminol. (Tokyo) 47, 132–138.
[33] Honda, Y., Kitaoka, M., Tokuyasu, K., Sasaki, C., Fukamizo, T. and Hayashi, K. (2003) J. Biochem. 133, 253–258.
[34] Finking, R., Solsbacher, J., Konz, D., Schobert, M., Scafer, A., Jahn, D. and Marahiel, M.A. (2002) J. Biol. Chem. 277, 50293–50302.