Isomaltulose Synthase (PalI) of Klebsiella sp. LX3

CRYSTAL STRUCTURE AND IMPLICATION OF MECHANISM*

Received for publication, March 14, 2003, and in revised form, June 20, 2003 Published, JBC Papers in Press, June 20, 2003, DOI 10.1074/jbc.M302616200

Daohai Zhang‡‡, Nan Li§§, Shee-Mei Lok¶¶, Lian-Hui Zhang¶, and Kunchithapadam Swaminathan***†††‡‡‡ From the **Department of Pathology, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074, the ¶¶Laboratory of Macromolecular Crystallography, Laboratory of Biosignals and Bioengineering, Institute of Molecular and Cell Biology, 30 Medical Drive, Singapore 117609, and the **Department of Biological Sciences, National University of Singapore, Singapore 117543

Isomaltulose synthase from Klebsiella sp. LX3 (PalI, EC 5.4.99.11) catalyzes the isomerization of sucrose to produce isomaltulose (α-D-glucosylpyranosyl-1,6-D-fructofuranose) and trehalulose (α-D-glucosylpyranosyl-1,1-D-fructofuranose). The PalI structure, solved at 2.2-Å resolution with an R-factor of 19.4% and Rfree of 24.2%, consists of three domains: an N-terminal catalytic (βα)8 domain, a subdomain between Nβ3 and Nε3, and a C-terminal domain having seven β-strands. The active site architecture of PalI is identical to that of other glycoside hydrolase family 13 members, suggesting a similar mechanism in substrate binding and hydrolysis. However, a unique RLDRD motif in the proximity of the active site has been identified and shown biochemically to be responsible for sucrose isomerization. A two-step reaction mechanism for hydrolysis and isomerization, which occurs in the same pocket is proposed based on both the structural and biochemical data. Selected C-terminal truncations have been shown to reduce and even abolish the enzyme activity, consistent with the predicted role of the C-terminal residues in the maintenance of enzyme conformation and active site topology.

Isomaltulose synthase (PalI), also known as sucrose isomerase (EC 5.4.99.11), catalyzes the isomerization of sucrose to produce isomaltulose (α-D-glucosylpyranosyl-1,6-D-fructofuranose) and trehalulose (α-D-glucosylpyranosyl-1,1-D-fructofuranose) as the main products with residual amounts of glucosylfructose (1, 2), as shown in Scheme 1. Isomaltulose and trehalulose, two functional isomers of sucrose, have been suggested as non-carogenic alternatives to sucrose and are widely used in health products and the food industry (3). The isomaltulose synthase activity has been reported in a range of bacterial species (1, 4–6). The ratio of the enzyme products varies, from mainly isomaltulose (75–85%) to predominantly trehalulose (5–10%), depending on the bacterial strain (1, 4–7).

The molecular mechanism of isomaltulose synthase that controls sucrose isomerization has not been fully characterized, except the recent prediction by Veronese and Perlot (2, 8), in which sucrose binding, hydrolysis, and isomerization depend on the charges provided by residues in a closed shell.

To understand the mechanism of sucrose isomerization at the molecular level and identify the key amino acids involved in the enzyme reaction, we recently cloned the palI gene encoding isomaltulose synthase (PalI) from the bacterial isolate Klebsiella sp. LX3 (6). Sequence alignment and secondary structure prediction revealed that PalI is a novel member of glycoside hydrolase family 13. Family 13 contains enzymes that act on starch such as α-amylase and cyclodextrin glycosyltransferase (CGTase) as well as enzymes specific for the cleavage of other glycosidic linkage such as α-1,6- and α-1,1-bonds (9). The basic structural characteristics of this family of enzymes is that the catalytic core domain contains a (βα)8-fold. The potential catalytic triad (Asp241, Glu295, and Asp369) and two histidine residues (His147 and His368) in PalI are highly conserved in α-amylase and glycosyltransferase (6). These residues, as found in oligo-1,6-glucosidase from Bacillus cereus (Asp199, Glu255, Asp292, His303, and His329) (10) and in amylosucrase from Neisseria polysacchareais (Asp286, Glu328, Asp337, His340, and His362) (11), form a catalytic pocket that binds the substrate and hydrolyzes the glycosidic bond. The similarity of the active site architecture strongly suggests that PalI adopts the same molecular mechanism for hydrolysis of the glycosidic bond and formation of the glycosyl-enzyme complex. The reaction mechanism occurs via a general acid catalysis, as do those of all glycoside hydrolases (12). In addition, PalI adopts the same mechanistic scheme in the formation of enzyme-substrate intermediate. As shown in Scheme 2, the glycosidic bond is protonated by a proton donor and the anomic carbon of the glucose moiety is attacked by the nucleophilic acid, simultaneously, leading to the formation of the covalently linked enzyme-substrate intermediate. The glucosyl moiety can be transferred to a water molecule for hydrolysis and to the fructose moiety for sucrose isomers synthesis. In the isomerization step, the structure of fructose determines the balance in the formation of two sucrose isomers, isomaltulose and trehalulose (8).

To understand the specific structural features required for isomerization in PalI, the PalI protein was overexpressed, purified, and crystallized (13). Here we describe, at 2.2-Å resolution, the crystal structure of PalI and the potential function of an RLDRD motif, at the structural level, in determining the mechanism of sucrose hydrolysis and isomerization. The function of the C-terminal domain in the enzymatic activity is also reported. Based on these data, we present a mechanism to...
explain the sucrose isomerization process. We believe this paper is the first structure-function report on PalI, a sucrose isomerase that converts sucrose to isomaltoolose and trehalulose simultaneously.

**EXPERIMENTAL PROCEDURES**

**Strains, Plasmids, and Site-directed Mutagenesis—**Escherichia coli DH5α were used as host cells for plasmid propagation and protein overexpression. Site-directed mutation of palI was performed by using the QuikChange™ site-directed mutagenesis kit (Stratagene). The double-stranded DNA vector pGEX (6) was used as the template, and two synthetic oligonucleotides containing the desired mutation were used as primers. Five mutants, PalI:D241A, PalI:E295A, PalI:D368A, PalI:H145A, and PalI:H368A, were created in which the indicated residues were replaced by Ala. For the C-terminal deletion mutants PalI:Delta57, PalI:Delta72 and PalI:Delta45, a stop codon was separately introduced after the indicated amino acid positions. All point mutations (Table I) were carried out according to standard procedures.

**Enzyme Purification and Assays—**Overexpression and purification of enzymes (PalI and its mutant versions) were carried out as described previously (6). Further purification was performed by gel filtration chromatography with a HiPrep Sephacryl S-200, 16/60 column (Amerhar) that are highly conserved in members of family 13. The active site is shown in Fig. 2A as an example of the quality of the 2Fo – Fc electron density. This active site cleft is surrounded by a loop (residues 321–340), forming a pocket with the dimensions of 20 × 20 × 25 Å, large enough to accommodate a sucrose molecule. The overall surface around the active site pocket of PalI is highly negatively charged (data not shown). This highly negative character of the (βα)7 barrel domain is important for sugar-protein interactions. The subdomain, which is inserted between Nβ3 and Naα3, consists of two α-helices and three anti-parallel β-strands (Fig. 1A) and has no known function either in PalI or in other family 13 members. Only one strong salt bridge (Lys248Na− . . .Asp311β2) with a distance of 2.6 Å connects the subdomain and the N-terminal domain. The C-terminal domain is made of two antiparallel β-sheets. Five β-strands (Cβ1, Cβ2, Cβ3, Cβ5, and Cβ7) form the larger β-sheet, and two strands (Cβ4 and Cβ6) form the smaller one. The six loop segments that are present between the pairs of adjacent β-strands in the C-terminal domain are named as Clp1–Clp6, respectively. A network of salt bridges and hydrogen bonds between the two C-terminal β-sheets as well as the N- and C-terminal domains ensure the conformational stability of the structure in general and the active pocket in particular.

**RESULTS AND DISCUSSION**

**Overall Structure—**As shown in Fig. 1A, the PalI molecule consists of three domains: the N-terminal catalytic (βα)7 β-strand domain (residues 43–146 and 216–521, colored blue), a subdomain (residues 147–215, magenta), and the C-terminal domain (residues 522–598, red), as reported for oligo-1,6-glucosidase (Fig. 1B) and amyllosucrase (Fig. 1C). The (βα)7 domain, as the main body of the structure, is sandwiched between the subdomain and C-terminal domain. It consists of the well-characterized (βα)7 barrel with eight alternating β-strands (Nβ1–Nβ8) and α-helices (Na1–Na8). This domain contains the residues involved in catalysis and substrate-binding. The cavity contains the acidic residues (Asp241, Glu285, and Asp306, PalI numbering) that are highly conserved in members of α-amylase family 13. The active site is shown in Fig. 2A as an example of the quality of the 2Fo – Fc electron density. This active site cleft is surrounded by a loop (residues 321–340), forming a pocket with the dimensions of 20 × 20 × 25 Å, large enough to accommodate a sucrose molecule. The overall surface around the active site pocket of PalI is highly negatively charged (data not shown). This highly negative character of the (βα)7 barrel domain is important for sugar-protein interactions. The subdomain, which is inserted between Nβ3 and Naα3, consists of two α-helices and three anti-parallel β-strands (Fig. 1A) and has no known function either in PalI or in other family 13 members. Only one strong salt bridge (Lys248Na− . . .Asp311β2) with a distance of 2.6 Å connects the subdomain and the N-terminal domain. The C-terminal domain is made of two antiparallel β-sheets. Five β-strands (Cβ1, Cβ2, Cβ3, Cβ5, and Cβ7) form the larger β-sheet, and two strands (Cβ4 and Cβ6) form the smaller one. The six loop segments that are present between the pairs of adjacent β-strands in the C-terminal domain are named as Clp1–Clp6, respectively. A network of salt bridges and hydrogen bonds between the two C-terminal β-sheets as well as the N- and C-terminal domains ensure the conformational stability of the structure in general and the active pocket in particular.

**Active Site Architecture—**Structural alignment results from the DALI server (15) show that the tertiary structures of PalI and oligo-1,6-glucosidase can be aligned in four parts (residues 43–257, 260–351, 383–566, and 576–598) with 46.4% sequence identity. A total of 544 Ca atoms could be superimposed with root mean square deviations in the range 0.46–1.69 Å. The
**Scheme 2. Possible mechanism of sucrose isomerization.** Sucrose (top left panel) forms intermediates with PGI. Pathway A shows the hydrolysis of sucrose and pathways B and C show the isomerization of sucrose to form isomaltulose and trehalulose, respectively.
TABLE I
Specific activity of PalI mutants

| Protein   | Description              | Specific activity (units/mg) |
|-----------|--------------------------|-------------------------------|
| PalII     | Native protein           | 330.5 ± 2.56                 |
| PalII:H145A | His^{141} replaced by Ala | 2.15 ± 0.28                  |
| PalII:H368A | Asp^{211} replaced by Ala | 8.85 ± 0.65                  |
| PalII:D241A | Asp^{241} replaced by Ala | 0.66 ± 0.44                  |
| PalII:E295A | Glu^{295} replaced by Ala | 0                           |
| PalII:D369A | Asp^{369} replaced by Ala | 4.1 ± 0.21                   |

C-terminal deletion

| Protein   | Description              | Specific activity (units/mg) |
|-----------|--------------------------|-------------------------------|
| PalII:J587 | C termini after Leu^{587} deleted | 305.05 ± 3.45                |
| PalII:J572 | C termini after Asp^{572} deleted | 32.95 ± 1.71                 |
| PalII:J545 | C termini after Tyr^{545} deleted | 0                           |

Table II
Data collection and refinement statistics

| Data collection | Unit-cell parameters (Å) | Space group |
|-----------------|--------------------------|-------------|
| Wavelength (Å)  | a = 59.24, b = 94.15, c = 111.29 | P2_12_2_ |
| Resolution range (Å) | 20–2.2 | |
| Total no. of reflections | 199,659 | |
| Total no. of unique reflections | 29,756 | |
| Redundancy | 6.1 | |
| Completeness (%) | 99.2 (96.6) | |
| R_{sym} (%) | 5.3 (26.0) | |
| Refinement | |
| R-factor (%) | 19.4 | |
| R_{free} (%) | 24.2 | |
| Root mean square deviation from | |
| ideal values | |
| Bond length (Å) | 0.006 | |
| Bond angle (°) | 1.30 | |
| Average temperature factor (Å^2) | 37.5 | |
| No. of protein atoms | 4648 | |
| No. of water molecules | 303 | |

Note: Values in parentheses are for highest resolution shell (2.28–2.20 Å) a R_{sym} = \sum |I_j| - \sum |I_j| / \sum |I_j|.

alignment between PalI and amylosucrase is very poor, resulting in several shorter fragments. However, superimposition of the five conserved amino acids (His^{141}, Asp^{241}, Glu^{295}, Asp^{369}, and His^{368} in PalI) in the substrate-binding pocket of PalI, oligo-1,6-glucosidase, and amylosucrase (Fig. 2B; oligo-1,6-glucosidase data not shown) shows a high degree of structural similarity of the active site architecture with a root mean square deviation of 0.41 Å between PalI and oligo-1,6-glucosidase and 0.89 Å between PalI and amylosucrase. To verify the importance of these conserved residues, the five residues (His^{141}, Asp^{241}, Glu^{295}, Asp^{369}, and His^{368}) were replaced individually by Ala, and the created mutant PalI proteins were purified (Table I). With the specific activity of the wild type enzyme (335.3 units mg^{-1}) defined as 100%, the remaining activity of the mutants PalI:D241A, PalI:E295A, PalI:D369A, PalI:H145A, and PalI:H368A is only 0.2, 0.123, 0.65, and 2.68% of the native PalI, respectively, strongly suggesting that these conserved residues are essential for PalI activity.

The substrate recognition scheme and binding sites have been identified in CGTase (23), TAKA-amylase with substrate analogs (24), amylosucrase with β-glucose, and mutated amylosucrase with sucrose (25, 26). Structural comparison of PalI with amylosucrase is of particular interest as these two enzymes use sucrose as their sole substrate. The superimposition of the structure of PalI with the complex of amylosucrase and isomaltulose as standard per min under the conditions specified.
bridge to O6 of Asp\(^{241}\), which is essential for the correct positioning of the nucleophile. Similarly, His\(^{145}\) forms a hydrogen bond to O6 and His\(^{368}\) to O2, as is the case for the equivalent residues His\(^{187}\) and His\(^{392}\) in amylosucrase (11, 25). In addition, a salt bridge between Asp\(^{102}\) and Arg\(^{456}\) is formed in PalI. The equivalent salt bridges between Asp\(^{144}\) and Arg\(^{509}\) in amylosucrase (25) and Asp\(^{60}\) and Arg\(^{415}\) in oligo-1,6-glucosidase (10) have been reported. One notable feature in amylosucrase, when compared with TAKA-amylase, is that the subsite is modified from Lys to Ala\(^{289}\) (amylosucrase numbering), providing the specificity of amylosucrase for the furanosyl ring of sucrose (11). The residues in PalI at the equivalent positions follow those of amylosucrase, mainly with Ala\(^{244}\), implying similar modifications of PalI at the +1 subsite to accommodate sucrose as the major substrate. Evidently, PalI adopts a mechanism similar to that in amylosucrase for sucrose binding, hydrolysis, and formation of covalent intermediate.

**A Motif Influencing Sucrose Isomerization**—The hydrolysis of sucrose by PalI constitutes only a minor part of the reactions mediating the synthesis of sucrose isoforms (6). Amylosucrase catalyzes the transfer of a d-glucopyranosyl moiety in the active site cleft to an acceptor molecule in a ravine formed by its domain B\(^{'}\) that plays the pivotal role in transferase reaction (11, 26). In PalI, however, breakage of \(\alpha\)-1,2-linkage in sucrose and formation of \(\alpha\)-1,6- and \(\alpha\)-1,1-linkages occur in the same pocket. To elucidate the mechanism of isomerization of PalI, the crucial structural features that interact with fructofuranose at the active site cleft and determine the change of fructofuranose to fructopyranose must be determined. This is because the conversion of fructofuranose to fructopyranose is the key step for trehalulose formation (8). Two residues in amylosucrase, Asp\(^{394}\) and Arg\(^{446}\), directly interact with the fructosyl ring of sucrose through hydrogen bonds (25). The equivalent residues in PalI are Asn\(^{570}\) and Arg\(^{533}\), respectively. Amylosucrase and oligo-1,6-glucosidase significantly differ from PalI in that PalI contains a flexible loop region from

![Fig. 2. Catalytic pocket, isomerization region, and N-C terminus interactions in PalI.](image)
glucosidic bond formation. As such, the unique location of the cose and fructose binding to the enzyme and the isomaltulose synthase motif influence the stability of gltrehalulose formation. Evidently, the charge distributions in the native (28). The isomaltulose content is decreased and the trehalulose glucose, fructose, isomaltulose, and trehalulose synthesized Pal
the active site cleft (Fig. 2, A and B). Not surprisingly, the RLDRD motif is not present in oligo-1,6-glucosidase and amylosucrase, as these two enzymes are functionally different from PalI. Notably, all known isomaltulose synthases contain the RLDRD sequence at equivalent regions (5, 27). This specific region has also been identified by sequence alignment (28). The crystal structural analysis further indicates its unique location and possible interactions with the substrate. The role of the charged residues in this motif was investigated by creating the mutant versions of PalI and analyzing the relative amount of glucose, fructose, isomaltulose, and trehalulose synthesized (28). The isomaltulose content is decreased and the trehalulose content is increased in all of the PalI mutants, compared with the native PalI. However, mutation of Asp327, Arg328, and Asp329 did not significantly affect the ratio of sucrose hydrolysis to sucrose isomerization activity (≈0.06), but resulted in a 13–25-fold increase in trehalulose production. Only the mutation of residue Arg325 enhanced both sucrose hydrolysis and trehalulose formation. Evidently, the charge distributions in the isomaltulose synthase motif influence the stability of glucose and fructose binding to the enzyme and α-1,1- and α-1,6-glucosidic bond formation. As such, the unique location of the RLDRD motif highlights its importance in the isomerization process and therefore in the control of PalI product specificity.

**Mechanistic Implication of Sucrose Isomerization**—Comparison of PalI with the amylosucrase-sucrose complex structure should reveal the mechanism of interactions between the side chains of active site residues and the glucosyl or the fructosyl moiety. In PalI, as shown in Scheme 2, A, Glu295 interacts with a bound sucrose molecule by protonating the glycosidic bond. It, therefore, serves to activate Asp244 to nucleophilically attack C1 to form the β-glucosyl-enzyme intermediate, as described for other members of glycosyl hydrolase family 13 (9, 10, 11). The residues that most probably interact with glucosyl moiety at −1 subsite include the conserved active residues, Asp369, Arg379, His414, and His468 and the salt bridge residues, Asp102 and Arg456. These remote glucose-binding subsites may prevent the release of glucose and thus confer less hydrolytic activity on PalI.

The mechanism of sucrose isomerization is speculative, although the biochemical functions of the motif RLDRD provide molecular evidence that the isomerization of sucrose is controlled by the charged residues in the proximity of the active site cleft. Based on our data, we propose that enzyme-bound sucrose interacts with the conserved residues of the active site and the isomaltulose synthase motif. The fructofuranose formation of the fructose moiety is tightly preserved by the charged residues of the RLDRD motif so that isomaltulose is the main isomer synthesized (Scheme 2, B). Disruption of the charge distribution balance by mutations of the RLDRD motif or the pH changes enhances the tautomerization of fructofuranose to fructopyranose (6), thereby forming the sucrose isomer, trehalulose (Scheme 2, C). Molecular dynamics analysis suggests that the fructose moiety bound to the mutant enzyme displays variable conformation (29). This electrostatic shift in the active site pocket also appears to cause the movement of 6′-OH group toward C2′ to form fructopyranose as well as the rotation of the C1′-OH toward C1 of glucosyl ring. Although direct evidence for the proposed mechanism is not yet available, further structural studies of PalI-substrate complex would allow us to present a complete isomerization scheme.

**C-terminal Domain**—The C-terminal domain of PalI inter-

acts with the N-terminal domain by forming salt bridges and hydrogen bonds (Fig. 2C). All hydrogen bonds between the two domains are clustered in two regions. In the first region, residues of N60 and the loops formed by residues 398–401 and 515–521 in the N-terminal domain make hydrogen bonds with residues in Cβ1-Cp1-Cp2 segment. The second region involves residues 379–384 and 497–501 in the N-terminal domain and Clp3 and Clp5 in the C-terminal domain. An interdomain salt bridge (Arg381-Ny2 . . . Glu380-Oε1), which is not present in oligo-1,6-glucosidase (10), connects Clp3 in the C-terminal domain and the loop after the active site in the N-terminal domain (Fig. 2C).

To understand the influence of the C-terminal domain on PalI functions, we have constructed three C-terminal deletion mutants, PalI-Δ587, PalI-Δ572, and PalI-Δ545, which are truncated after the indicated residue. The truncated PalI mutants were overexpressed, purified, and the remaining activity has been assayed. As shown in Table I, the mutant PalI-Δ587, created by the deletion of Clp6 and Cβ7, reduces the enzyme activity to 92.3% of the native PalI. Further deletion of Cβ5 and Clp5 in PalI-Δ572 leads to about 90% loss of activity, and deletion of Cβ3-Cβ7 in PalI-Δ545 completely abolishes the enzyme activity. C-terminal deletions interrupt the interdomain interactions and may cause significant changes in the structure of the N-terminal domain.

The preceding discussion is based on the comparison of the PalI structure with oligo-1,6-glucosidase and amylosucrase, together with biochemical analysis of a series of mutant PalI proteins. The unique RLDRD motif in the loop region participates in sucrose isomerization and thus influences product specificity. The protein-substrate complex structure should allow us to elucidate the real interaction of identified key residues with substrate and the true mechanism of sucrose isomerization.

**Acknowledgments**—We thank K. Miura (Spring8 in Japan) for data collection and M. James (University of Alberta) and D. Voet (University of Pennsylvania) for advice and revision of the manuscript.

**REFERENCES**

1. Huang, J. H., Hsu, L. H., and Su, Y. C. (1998) *J. Ind. Microbiol. Biotechnol.* 21, 22–25.
2. Veronese, T., and Perlot, P. (1999) *Enzyme Microb. Tech.* 24, 263–269.
3. Baer, A. (1989) *Wiss. Technol.* 4, 46–53.
4. Miyata, Y., Sugitani, T., Tsyuk, Y., Ebashi, T., and Nakajima, Y. (1992) *Biochem. Biotechnol. Biochem.* 54, 1689–1681.
5. Matthes, R., Klein, K., Schiewch, H., Kunz, M., and Munir, M. (1998) *U. S. Patent* 5, 786, 140.
6. Zhang, D. H., Li, X. Z., and Zhang, L. H. (2002) *Appl. Environ. Microbiol.* 68, 2676–2682.
7. Nagai-Miyata, J., Tsyuk, Y., Sugitani, T., Ebashi, T., and Nakajima, Y. (1995) *Biosci. Biotechnol. Biochem.* 57, 2049–2053.
8. Veronese, T., and Perlot, P. (1998) *FEBS Lett.* 441, 348–352.
9. Davies, G., and Henriassat, B. (1995) *Structure* 3, 853–859.
10. Watanabe, K., Hata, Y., Kuzaki, H., Katsube, Y., and Suzuki, Y. (1997) *J. Mol. Biol.* 269, 142–153.
11. Skov, L. K., Mirza, O., Henriksen, A., de Montalk, G. P., Renaud-Simeon, M., Sarcabal, P., Willemsen, R. M., Mooman, P., and Gajhede, M. (2001) *J. Biol. Chem.* 276, 29273–29278.
12. Koshland, D. E. (1953) *Biol. Rev. Camb. Philos. Soc.* 28, 416–436.
13. Li, Z., Zhang, D. H., Zhang, L. H., and Swaminathan, K. (2005) *Acta Crystallogr.* 59, 150–151.
14. Otwinowski, Z., and Minor, W. (1997) *Methods Enzymol.* 276, 307–326.
15. Holm, L., and Sander, C. (1995) *Trends Biochem. Sci.* 20, 478–480.
16. Collaborative Computing Project Number 4 (1994) *Acta Crystallogr.* 50, 760–763.
23. Uitdehaag, J. C. M., Mosi, R., Kalk, K. H., van der Veen, B. A., Dijkhuizen, L., Withers, S. G., and Dijkstra, B. W. (1999) *Nat. Struct. Biol.* 6, 432–436
24. Brzozowski, A. M., and Davies, G. J. (1997) *Biochemistry* 36, 10837–10845
25. Mirza, O., Skov, L. K., Remaud-Simeon, M., de Montalk, G. P., Albenne, C., Monsan, P., and Gajhede, M. (2001) *Biochemistry* 40, 9032–9039
26. Skov, L. K., Mirza, O., Sprogøe, D., Dar, I., Remaud-Simeon, M., Albenne, C., Monsan, P., and Gajhede, M. (2002) *J. Biol. Chem.* 277, 47741–47747
27. Bornke, F., Hajirezaei, M., and Sonnewald, U. (2001) *J. Bacteriol.* 183, 2425–2430
28. Zhang, D. H., Li N., Swaminathan, K., and Zhang, L. H. (2003) *FEBS Lett.* 534, 151–155
29. Immel, S., and Lichtenthaler, F. W. (1995) *Liebigs Ann.* 1925–1937