The Class I hyaluronan synthase (HAS) is a unique glycosyltransferase synthesizing hyaluronan (HA), a polysaccharide composed of GlcUA and GlcNAc, by using one catalytic domain that elongates two different monosaccharides. As for the synthetic mechanism, there are two alternative manners for the sugar elongation process. Some bacterial HASs add new sugars to the non-reducing end of the acceptor to grow polymers. On the other hand, some vertebrate enzymes seem to transfer sugars to the reducing end. Expression of vertebrate HASs as active and soluble proteins will accelerate further precise insight into mechanisms of sugar elongation reactions by natural HASs. Since large scale production of HA polymers and oligomers would become powerful tools both for basic studies and new biotechnology to create functional carbohydrates in medicinal purposes, advent of an efficient method for the expression of HASs in *Escherichia coli* is strongly expected. Here we communicate the first success of the production of recombinant human HAS2 proteins composed of only the catalytic region in *E. coli* as the active form. It was demonstrated that an engineered HAS2 expressed in *E. coli* exhibited significant activity to synthesize a mixture of HAS oligomers from 8-mer (HAS8) to 16-mer (HAS16). Engineered HAS2 prepared herein elongated sugars from exogenous tetrasaccharide to form polymers with a direction to the non-reducing end. According to the present results, large scale production of engineered recombinant HASs is to be performed using *E. coli* that will provide practical and economic advantages in manufacturing enzymes for use in the synthesis of various oligomeric HA molecules and their industrial applications.

Hyaluronan (HA)3 is a linear unbranched polymer composed of a repeating disaccharide unit of GlcUA (β-1,3) and GlcNAc (β-1,4). HA is a major constituent of glycosaminoglycan in the extracellular matrix of most tissues and organs, and this polysaccharide plays important roles in cell migration, proliferation, and the development of tissue architecture. The enzymes responsible for the synthesis of HA, HAS genes have been cloned from bacteria to mammalian cells (1–5). HASs are the first glycosyltransferases that can catalyze transfer reactions of two distinct sugars in contrast to the vast majority of other known glycosyltransferases, which transfer only one saccharide moiety (6–7). HASs are classified into two groups, Class I and Class II, based on the homology of amino acid sequences and topology. PmHAS from *Pasteurella multocida* is the only known member of the Class II HAS. On the other hand, Class I HASs include streptococcal, viral, and vertebrate enzymes. In human HASs, for example, there have been three related enzymes, HAS1 (8), HAS2 (5), and HAS3 (9). Particularly it was reported that HAS2 shows much higher enzyme activity and produces the highest molecular weight of HA among common HASs (10, 11). Human HAS2 was cloned in 1996 by Yamaguchi et al. (5), and it was suggested that this enzyme seems to have the most potent enzymatic activity among the known human HASs from a viewpoint of the possibility for the production of engineered glycosyltransferases used in enzyme-assisted glycosynthetic strategy (12–14).

The peptide chains of the Class I HASs in the membrane region also have similar predicted topologies (15). As for the HAS activity, it seems that the proteins should have five to seven membrane-associated regions and a cytoplasmic region (16). This large central cytoplasmic domain of about 260–320 amino acid residues is flanked by two membrane-associated regions at the amino and the carboxyl terminus. The efficient synthetic process of HA molecules at the plasma membrane clearly suggests that the required enzymatic machinery of the Class I HASs would be located in a specific cytoplasmic region and/or at the cell membrane domains. However, it should be noted that the exact catalytic regions of Class I HASs have not been previously identified.

In regard to the synthetic mechanism of HA, it has been reported that PmHAS (Class II HAS) added sugars to the non-reducing terminus of the growing chain at the saturated GlcUA of a tetramer of HA as an acceptor (17). On the other hand, the exact mechanism of the synthesis and polymerization of HA molecules by Class I HASs has not yet been systematically elucidated. *Streptococcus pyogenes* HAS has been reported to add sugars to the non-reducing terminus of the growing chain as determined by selective labeling and degradation studies (18). Using a similar approach, however, two laborato-
ries working with the enzyme preparations from mammalian cells concluded that the new sugars were added to the reducing end of the nascent chain (19-21). Our interest has been directed toward these different polymerization manners by Class I HASs. Despite their similarity in the homology of the HAS polypeptides between vertebrates and streptococcus, why do they actually utilize different reaction pathways?

Recently it was demonstrated that some of eukaryotic glycosyltransferases such as β-1,4 galactosyltransferase, α-2,3 sialyltransferase, α-2,6 sialyltransferase, and α-1,6 fucosyltransferase could be expressed as active and soluble forms in Escherichia coli (22-24). However, the number of recombinant glycosyltransferases produced in E. coli is still limited. It is likely that the recombinant human HASs (rhHASs) expressed in E. coli might lose a chance to bind a nascent HA chain because E. coli host cells do not produce UDP-GlcUA and UDP-GlcNAc required for the synthesis of HA polysaccharide. However, it is anticipated that the exogenous HA-derived oligosaccharides as acceptor substrates can access the active site of rhHAS and be utilized for sugar elongation reactions in the presence of exogenous glycosyl donor substrates. It has also been reported that PmHAS added new sugar residues to the saturated tetramer prepared by means of ovine testis hyaluronidase at the non-reducing end (17). Moreover the recent work of DeAngelis et al. (25) clearly demonstrated that the engineered PmHAS could be converted into two single action glycosyltransferases, and reactor enzymes immobilized onto solid supports were utilized in an alternating fashion to make pure HA molecules of a single length in a controlled and stepwise manner. In vertebrates HASs, it is known that HAS of Xenopus Dg42 expressed in yeast exhibited a significant activity as HA synthase (26).

In this report, we communicate the production in E. coli and characterization of some modified recombinant human HAS2s (rhHAS2s) containing the sequences corresponding to a common catalytic region found in human HAS2. It is demonstrated that rhHAS2s produced in this study show potent activity to synthesize HA polymers in vitro by using exogenous tetrasaccharide as an acceptor substrate, and sugar elongation was performed at the non-reducing end of the saturated GlcUA residue.

EXPERIMENTAL PROCEDURES

Materials and Strains—The starting vector for the expression of PCR-mediated fusion proteins, pMALp2x, was purchased from New England Biolabs, Inc. Reagents for the molecular biology were obtained from Toyobo Co., Ltd. (Osaka, Japan) and Takara Co., Ltd. (Shiga, Japan) unless noted. Custom oligonucleotides were purchased from Hokkaido System Science Co. (Hokkaido, Japan). UDP-GlcUA and UDP-GlcNAc were purchased from Sigma. Radiolabeled sugar nucleotides UDP-[14C]GlcUA and UDP-[3H]GlcNAc were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO) and PerkinElmer Life Sciences, respectively. Even-numbered HA oligosaccharide standards ([GlcUA-GlcNAc]n, n = 2 or 3) were gifts from Denki Kagaku Kogyo K K. (Tokyo, Japan). All other reagents were the highest grade available from either Sigma or Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise noted.

Plasmid Construction—Human HAS2 gene was obtained from the human small intestine cDNA plasmid pHpAN05. The coding open reading frame of 552 residues was amplified by 25 cycles of the polymerase chain reaction with Tag DNA polymerase and oligonucleotide primers designed to add flanking BamHI and HindIII restriction sites. The cassette was subcloned into pMALp2x vector expression in E. coli and constructed on phHAS2ORF. Subsequently, as shown in Fig. 1, to create the PCR-mediated rhHAS2 fused with maltose-binding protein, three kinds of encoding genes composed of only cytoplasmic region were generated by PCR as template on phHAS2ORF.

These plasmids corresponded to amino acids 122–214, 122–355, and 122–347, respectively. The following primers were used: 122–414: forward primer, 5’-CCGGATCCGAGGAATCCAGTGAC-3’ (this primer contains BamHI site), and reverse primer, 5’-CCCAAGCTTCTTGGACATT3A-3’ (this primer contains HindIII site); 122–355: reverse primer, 5’-CCCAAGCTTCTTGGACATT3A-3’ (this primer contains HindIII site); and 122–347: reverse primer, 5’-CCCAAGCTTCTTGGACATT3A-3’ (this primer contains HindIII site).

The PCR products prepared as template on phHAS2ORF were purified by using the MinElute™ gel extraction kit (Qiagen, Germany) after digestion with BamHI and HindIII. The products were ligated overnight with T4 DNA ligase (Invitrogen). E. coli strain JM109 was transformed with the newly constructed plasmid, and colonies were maintained on LB containing 100 μg/ml ampicillin at 37 °C. Sequences of the resultant constructs were determined using an Applied Biosystems 310 automated DNA sequencer.

Preparation of Membrane Fraction—Membranes from E. coli were obtained by modifications of a proteolipid method as reported previously (27, 28). The fusion plasmids for the rhHAS2 fusion constructs were grown in JM109 cells. Cells containing the HAS-encoding MBP fusion plasmids were grown at 37 °C overnight in Luria broth containing 0.2% glucose. The overnight culture was diluted 1:100 to start a new culture in LB containing 100 μg/ml ampicillin, 0.2% glucose; cells were grown with vigorous shaking to midlog phase (0.4–0.6 A600). The cells were then induced with 100 μM isopropyl-β-D-thiogalactoside and grown on an additional 4 h at 37 °C. The cells were harvested by centrifugation at 4 °C for 10 min at 10,000 × g. The cell pellet was thawed and resuspended in 2.7% of the original culture volume with 20% sucrose, 30 mM Tris-HCl, pH 7.4 including 0.5 μg/ml leupeptin, 10 mM MgCl2, 1 mM dithiothreitol, and 0.3 mg/ml lysesyme in 0.1 M EDTA, pH 8. The suspension was incubated for 30 min on ice and centrifuged to fractionate the periplasm (10,000 × g for 30 min at 4 °C). The cells were resuspended by pipetting in a similar buffer containing 46 μg/ml phenylmethylsulfonyl fluoride and 50 mM MgCl2 followed by sonication (Bioruptor sonifier from Cosmo Bio Co., Ltd.). The cellular debris was removed by centrifugation (10,000 × g for 30 min at 4 °C). The supernatant fraction was diluted 20-fold in phosphate-buffered saline containing 10% glycerol, 1 mM dithiothreitol, and the membrane fraction was harvested by ultracentrifugation (100,000 × g for 1 h). The prepared membrane fraction containing active enzyme was suspended in 50 mM Tris, pH 7.4 at a concentration of 1–3 mg/ml protein. Although the maltose binding vector usually targets the recombinant protein to the periplasm, the present rhHAS2 fusions were obtained as a membrane fraction. These active fractions were found to dissolve in supernatant after centrifugation for 20,000 × g for 5 min (3, 28).

Quantification of protein in the crude membrane preparations was carried out using the BCA™ protein assay kit (Pierce) using bovine serum albumin as a standard.

Determination of Expressed Fusion Proteins—Proteins separated by SDS-PAGE (4–20% gel) were transferred to nitrocellulose membrane at 100 V for 2 h in a Bio-Rad Trans-Blot Cell blot apparatus (Bio-Rad) and blocked with Block Ace (Dainipponseiyaku Co., Ltd., Osaka, Japan) for 30 min. Membranes were then exposed to an anti-MBP monoclonal antibody (from New England Biolabs, Inc.) in buffer containing 0.1% Block Ace in Tris-buffereed saline/Tween 20 for 1 h followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad). Immune complexes were detected using a Konica immunostaining kit (Konica Corp., Tokyo, Japan).

Acceptor Oligosaccharides—Even-numbered HA oligosaccharides ([GlcNAc-GlcUA]n) were generated by degradation of macromolecular HA (from pig skin) with either ovine testicular hyaluronidase or Streptococcus dysgalactiae hyaluronidase (n = 2 or 3) in 20 mM sodium acetate, 0.15 mM sodium chloride, pH 5.7, at 30 °C overnight. The preparation of hyaluronate of HA by treating with ovine testicular hyaluronidase involved a saturated GlcUA residue at the non-reducing end of HA (HAR). Ovine hyaluronidase produces oligosaccharides with an elimination mechanism to cleave the polysaccharide chain, resulting in an unsaturated GlcUA residue at the non-reducing terminus of each fragment (17). For further purification and desalting, some crude preparations were subjected to gel filtration with Bio-Gel P-4 (Bio-Rad) eluted with 0.2 mM ammonium formate, 30 mM sodium acetate, 1 M NaCl, 1 mM dithiothreitol, 1 mM EDTA, 15% glycerol, 0.5 mM UDP-GlcNAc, and 0.5 mM UDP-GlcUA. General conditions for the HA synthesis were as follows.

To initiate the enzymatic reaction, 500 μl of enzyme solution was added to 1 ml of 50 mM Tris-HCl, pH 7.4 including 0.5 μg/ml leupeptin, 5 mM MgCl2, 20 mM D-glucuronic acid, and 50 mM UDP-GlcUA required for the synthesis of HA polysaccharide. It has also been reported that the exogenous HA-derived oligosaccharides can access the active site of rhHAS and be utilized for sugar elongation reactions in the presence of exogenous glycosyl donor substrates. It has also been reported that PmHAS added new sugar residues to the saturated tetramer prepared by means of ovine testis hyaluronidase at the non-reducing end (17). Moreover the recent work of DeAngelis et al. (25) clearly demonstrated that the engineered PmHAS could be converted into two single action glycosyltransferases, and reactor enzymes immobilized onto solid supports were utilized in an alternating fashion to make pure HA molecules of a single length in a controlled and stepwise manner. In vertebrates HASs, it is known that HAS of Xenopus Dg42 expressed in yeast exhibited a significant activity as HA synthase (26).

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series of 100 μg of d-GlcUA or oligosaccharide acceptors were added, and the mixture was incubated for 1 h at 37 °C. The reactions were terminated by boiling the mixtures for 10 min. HA products were fractionated by gel filtration chromatography using Superdex peptide HR 10/30 (Amersham Biosciences) eluted with 0.2 M ammonium acetate. Sugar-containing fractions were lyophilized, and the molecular masses were measured with MALDI-TOF MS.

MALDI-TOF MS Analysis—Determination of the molecular weight of the HA products was performed by positive ion mode MALDI-TOF MS analysis on a REFLEX™ III and BIFLEX™ (Bruker, Bremen, Germany). The samples were dissolved in distilled water and subsequently mixed well at a ratio of 1:1 with a matrix solution of N-2,5-dihydroxybenzoic acid solution in 80% methanol (w/v). Linear mass scans were recorded over 400 Da by using a pulse delay time of 20,000 ns.

RESULTS

Production of rhHAS2 Fusion Proteins in E. coli—It has been reported that the major catalytic regions of eukaryotic HAS seem to exist in the cytoplasmic region by topology research and Kyte-Doolittle hydropathy analysis (5, 15, 30), although the essential structure of the catalytic domain is still putative and has not been identified previously. In this study, we designed three constructions: (i) a peptide composed of the two common catalytic domains of HAS in the cytoplasmic region and the sequence including a part of the transmembrane region (rhHAS2-(122–414)), (ii) a peptide containing only two catalytic sites (rhHAS2-(122–355)), and (iii) a small peptide lacking one of the catalytic sites (rhHAS2-(122–347)) as illustrated in Fig. 1.

These constructs were expressed as induction proteins with isopropyl-β-D-thiogalactoside in E. coli. As described under "Experimental Procedures," each DNA fragment was amplified from a human small intestine cDNA library by PCR. Then PCR products were placed into pMALp2x vectors encoding MBP (42.9 kDa) and transformed into JM109 cells. The sequences of the fusion junctions were confirmed by DNA sequencing. Constructs were checked for expression of fusion protein by Western blot analysis, and the molecular weights of the subunits for
FIG. 3. Reaction of rhHAS2-(122–414) peptide with GlcUA as an acceptor. HAS activity was monitored using UDP-[14C]GlcUA (300 mCi/mmol) and UDP-[3H]GlcNAc (50 μCi/mmol). Samples were assayed for HAS activity in 200 μl of 50 mM Tris-HCl solution, pH 7.4, containing 20 mM MgCl₂, 1 mM dithiothreitol, 1 mM EGTA, 15% glycerol, 0.5 mM UDP-GlcNAc, and 0.5 mM UDP-GlcUA. To initiate the enzymatic reaction, 500 μg of rhHAS2-(122–414), 100 μg of GlcUA as substrate, 0.1 μCi of UDP-[14C]GlcUA, and 2.4 μCi of UDP-[3H]GlcNAc were added, and the mixture was incubated for 1 h at 37°C. After the incubation, samples were boiled to stop the reaction, and mixtures were
all constructs were found to be consistent with MBP fusion proteins (Fig. 2). On the other hand, the open reading frame region of the rhHAS2 protein was not expressed in E. coli (data not shown) probably because of the complicated topology and high hydropobicity of vertebrate HASs.

**HA Synthesis by rhHAS2 Produced in E. coli**—To investigate whether vertebrate HAS genes on plasmids inserted into eukaryotic hosts have the ability to produce HA polymer in vitro, rhHAS2 fusion protein (rhHAS2-(122–414)) produced in E. coli was preliminarily subjected to the reaction with two radiolabeled sugar nucleotides, UDP-[14C]GlcUA and UDP-[3H]GlcNAc, as donors in the presence of the GlcUA as a monosaccharide acceptor substrate. As shown in the chromatographic analyses of the products (Fig. 3, A and B), rhHAS2-(122–414) apparently incorporated both [14C]GlcUA and [3H]GlcNAc to produce some new compounds showing higher molecular weight than [14C]GlcUA or [3H]GlcNAc. It was also suggested that the HA product could be digested by treating with S. dysgalactiae hyaluronidase to give a mixture of low molecular weight fragments. In addition, Fig. 3D shows a MALDI-TOF mass spectrum of the HA fractions produced using normal (non-radiolabeled) sugar nucleotides as donors instead of radiolabeled substrates with GlcUA acceptor. Although the significant peak due to 5-mer (HA5) at 1016.28 was detected, it seems that the polypeptide did not exhibit satisfactory HAS activity to produce longer HA polymers from the monosaccharide acceptor. It was also suggested that GlcNAc cannot be used as a glycosyl acceptor for this enzyme (data not shown).

Since it has been known that a tetrasaccharide derivative (HA4) could become exogenous and a potential acceptor substrate for PmHAS (17), our attention was next directed toward the reaction of rhHAS2 with this tetrasaccharide substrate as an acceptor. Interestingly it was shown that rhHAS2-(122–414) seemed to produce much longer HA oligosaccharides than HA5, and the existence of the HA8, HA10, and HA14 could be detected by MALDI-TOF mass spectrum of the crude product as indicated in Fig. 4A. Further purification of the mixture by using Superdex peptide HR 10/30 and MS analyses of each fraction revealed that the mixture contained HA8, HA10, HA12, HA14, and HA16. Since we have preliminarily found the reproducible accumulation of HA14 in the above experiments using rhHAS2-(122–414), HA synthetic activity of the three polypeptides rhHAS2-(122–414), rhHAS2-(122–355), and rhHAS2-(122–347) were discussed by comparing the production of HA14 as indicated in Fig. 5B. It was suggested that rhHAS2-(122–414) and rhHAS2-(122–355) peptides showed potent HAS activity and the advent of a major peak at 2850.17. On the other hand, rhHAS2-(122–347) exhibited activity that was lower than that of the two other peptides (Fig. 5C), suggesting that a part of the deletion of the catalytic region of HAS2 drastically reduced its HAS activity. In addition, HA14 synthesized by rhHAS2-(122–414) was sensitive to the treatment by S. dysgalactiae hyaluronidase, the intensity of the MALDI-TOF MS peak corresponding to the HA14 apparently disappeared, and a new peak due to disaccharide product was observed (Fig. 5D).

These results provided direct evidence that rhHAS2-(122–414) and rhHAS2-(122–355) synthesize HA by using UDP-GlcUA and UDP-GlcNAc as donors and the tetrascarhide as a potent acceptor in vitro. Furthermore it was also suggested that rhHAS2-(122–414) may be utilized for the synthesis of various lengths of HA polymers from HA8 to HA16 as indicated in Fig. 4. rhHAS2-(122–414) could not synthesize longer (high molecular weight) HA chains even by using longer incubations (over 24 h) or reactions with higher UDP-sugar concentrations (data not shown). Incubation of the tetrascarhide acceptor with a single donor, UDP-GlcNAc, did not yield HA5, suggesting that the recombinant truncated HAS2 polypeptides cannot be utilized for the stepwise synthesis of HA performed by using the engineered PmHAS method reported by DeAngelis et al. (25). For the purpose of the regulation of the molecular size of the HA, some improvement or optimization of the reaction conditions will be required.

**Oligosaccharide Acceptors for rhHAS2 Peptides**—To investigate the suitability of exogenous oligosaccharide substrates and the mechanism of HA synthesis by rhHAS2-(122–414), sugar elongation reactions using some oligosaccharide materials as acceptor substrates were examined. As shown in Fig. 6, a tetrasaccharide carrying a saturated GlcUA residue at the non-reducing end was found to become a suitable and desirable acceptor substrate for rhHAS2-(122–414) among the materials used in this study. Interestingly a tetrasaccharide bearing an unsaturated GlcUA residue at the non-reducing end derived by the action of S. dysgalactiae hyaluronidase could not be used as a substrate for this reaction at all (Fig. 6D). This result clearly suggests that HA polymerization catalyzed by rhHAS2 was initiated at the non-reducing GlcUA residue of this tetrasaccharide. Neither hexamer prepared by treating HA with ovine testis hyaluronidase nor the structurally similar disaccharide chondrosine became an even tentative substrate (Fig. 6, B and C). Likewise the rhHAS2 did not use free GlcNAc as a glycosyl acceptor substrate (data not shown). GlcUA and GlcNAc monosaccharides are not acceptors for synthesis of HA14 even though GlcUA will serve as an acceptor for synthesis of HA5.

**DISCUSSION**

In this study, we found some new pieces of invaluable information on the expression of recombinant human HAS peptides and their ability to synthesize HA molecules. First it was found that the rhHAS2 peptides composed of only the partial catalytic domain located in the membrane-associated regions were successfully produced in E. coli, and the peptides showed a potent ability to synthesize HAS in vitro. The amino acid sequences in the catalytic region of a variety of vertebrate HASs have been known to show high homology with the region of 272–461 in human HAS2. Therefore, the recombinant human HAS2s prepared in this study, rhHAS2-(122–414) and rhHAS2-(122–355), were involved in the high homology region in the catalytic domain. At present, we can conclude that rhHAS2-(122–414) and rhHAS2-(122–355) containing partial structures of the cytoplasmic catalytic domain of human HAS2 synthesize relatively low molecular weight HA (from 8-mer to 16-mer) as elucidated mainly by MALDI-TOF MS. Moreover peptide domains found in the membrane-associated regions are not nec-
Fig. 4. Various sizes of hyaluronan synthesized by rhHAS2-(122–414) peptide. A, MALDI-TOF mass spectrum of the crude HA product. B, purified HA products from HA8 to HA16, respectively.
essential, at least, for this simple synthetic process observed in the action by rhHAS2-(122–414) and rhHAS2-(122–355). However, it seems that reproducible accumulation of the 14-mer (HA14) as a major product in this procedure means the lack of the capacity to synthesize further high molecular weight HA. Although the reason for the accumulation of HA14 is not clear, it would be affected by some intrinsic factors because this tendency could not be controlled by limiting time, substrate, or...
enzyme concerns. Therefore, it should be noted that membrane-associated peptide domains deleted in this study might have a crucial function to accelerate the highly efficient and high speed polymerization reaction of HA as well as transportation of high molecular weight HA from inside to outside of the cells.

As for the mechanism of HA synthesis by rhHAS2 peptides, it was clearly demonstrated that these rhHAS2s elongated sugar residues from the non-reducing end of the exogenous acceptor molecule, a saturated form of the tetrasaccharide derivative. However, it was previously demonstrated that the Class I HASs neither recombinant S. pyogenes HAS nor recombinant Xenopus DG42 used the saturated tetrasaccharide (HA4) as exogenous acceptor (17). Therefore, it could be speculated that the access of the tetrasaccharide to a catalytic site/cleft was blocked by the flanking membrane-associated regions. The tetrasaccharide derivative having unsaturated GlcUA at the non-reducing end did not become a good substrate for rhHAS2 at all. These results are not contradictory with the fact that S. pyogenes HAS, carrying a large cytoplasmic domain homologously similar to that of human HAS2 (30), also elongates sugars at the non-reducing end (18). It was also revealed that the hexasaccharide derivative could not be used for the synthesis of larger HA molecules. This may be caused by the limit of the spatial size of the carbohydrate-binding cavity or pocket of the rhHAS2 peptides. Although the essentials in the mechanism of HA synthesis by native human HASs expressed in mammalian cells have not been discussed thoroughly (19–21), rhHAS2 peptides expressed in E. coli in this study will become convenient tools for further investigation of the rela-
tionship between HAS functions and cardiolipin or ectoprotein kinase (27, 31–33) and their use as a novel class of engineered glycosyltransferases for medicinal purposes.

In conclusion, we have successfully expressed and produced modified rhHAS2 as the active form in E. coli. It was demonstrated that rhHAS2 polymerizes HA using an exogenous tetrasaccharide as acceptor substrate and elongates sugars from the non-reducing end of the saturated GlcUA residue in vitro.

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