Directed mutagenesis of the *Trypanosoma cruzi* trans-sialidase enzyme identifies two domains involved in its sialyltransferase activity

Lynne E. Smith and Daniel Eichinger

Departments of Pathology and 1Medical and Molecular Parasitology, New York University School of Medicine, 341 East 25th Street, New York, NY 10010, USA

1To whom correspondence should be addressed

Of the increasing number of sialidases found to be made by microorganisms, the trypanosome trans-sialidase is unique in its added ability to efficiently carry out a sialyltransferase reaction using preformed glycoconjugates. The enzyme is predicted to have a multidomain structure, with one domain containing sequence and expected structural features found in bacterial sialidases. The trans-sialidase is very similar in overall sequence to another trypanosome enzyme that has only sialidase activity. Hybrid expression constructs containing pieces of these trypanosome trans-sialidase and sialidase genes were used to determine which regions of trans-sialidase are required for sialyltransferase activity. Two domains were found to influence the enzymatic activity: the N-terminal catalytic domain, and a downstream domain that resembles an Fn3-like module.

Key words: mutagenesis/enzyme/sialyltransferase/trypanosome

Introduction

Several protozoan parasites of vertebrates (including *Trypanosoma cruzi*, *T. brucei*, and *Endotrypanum sp.*) have been shown to express a unique type of sialyltransferase called trans-sialidase (TS; Schenkman et al., 1991; Engstler et al., 1992; Medina-Acosta et al., 1995). This enzyme is capable of removing terminal α2,3-linked sialic acid from sialyl-β-galactose donor molecules and reattaching it to other β-galactose-terminated glycoconjugates (Vandekerckhove et al., 1992; Scudder et al., 1993). In this reaction the enzyme thus functions both as a sialidase, similar to viral neuraminidase and bacterial sialidases, and as a glycosyltransferase. It differs, however, from the sialyltransferases found in the Golgi apparatus which use only nucleotide-monophosphate-sugar donor substrates (Kornfeld and Kornfeld, 1985), although both enzyme types can be considered to use similar acceptor substrates. Neither the reaction mechanism nor the structural features of the TS protein that support its efficient sugar transfer activity are known. The trypanosome parasite, unable to synthesize sialic acid (Previato et al., 1985), uses the enzyme to scavenge the sugar from host glycoconjugates and to sialylate mucin-like acceptor molecules found on its plasma membrane. These molecules, and/or the enzyme itself, are involved in the obligate entry of the trypanomastigote form of the parasite into host cells where it differentiates and multiplies (Schenkman et al., 1991; Hall et al., 1992; Burleigh and Andrews, 1995).

The sequences of several genes encoding *T. cruzi* TS and TS-like proteins have been reported (Pereira et al., 1991; Pollevick et al., 1991; Uemura et al., 1992), and the predicted amino acid sequences of the subclass of enzymatically active gene products indicate that the parasite proteins have structural features previously found in influenza neuraminidase and several bacterial sialidases (Vimr, 1994). In particular, the trypanosome proteins contain all the amino acid residues (or classes) which have been described to interact with sialic acid in the substrate binding pockets of *Salmonella typhimurium*, *Vibrio cholerae* and *Micromonospora viridifaciens* sialidases (Crennell et al., 1993, 1994; Gaskell et al., 1995). The parasite enzymes also contain multiple copies of a conserved consensus sequence (aspartic acid box (ASP box)) found in viral, bacterial, and mammalian sialidases (Roggentin et al., 1989; Miyagi et al., 1993). Crystal structure analyses of the three bacterial sialidases indicate that their protein chains are folded into six-sided β-sheet barrels similar to that of influenza neuraminidase. The length of the open reading frames (ORFs) encoding the catalytic domain of the trypanosome enzymes, as well as the positions of the conserved sialic acid binding site residues and ASP boxes in primary structure alignments and hydrophaticity plots, predicts that the tertiary structure of the catalytic domain of trypanosome TS will be found to be similar to those of the viral and bacterial enzymes. The N-terminal catalytic domain in TS is followed, in order, by a domain of unknown function, a fibronectin type III domain (Fn3 domain), a large segment composed of 12 amino acid tandem repeats, and finally by a GPI anchor addition sequence (Pereira et al., 1991). The last two regions are not required for enzymatic activity (Schenkman et al., 1994a).

The low overall level of amino acid similarity (35%) between the bacterial and trypanosome enzymes renders uninformative any cross-genera sequence comparisons designed to reveal unique amino acids or subdomains involved in the sialic acid transfer function of TS. Another trypanosome species, *T. rangeli*, was previously shown to express a sialidase type enzyme that hydrolyzes the same sialylated donor substrates as TS (Pereira and Moss, 1985), but lacks sialic acid transfer activity (Pontes de Carvalho et al., 1993). The relatedness of the *T. cruzi* and *rangeli* parasites suggested that sequence comparisons between the two parasite enzymes might reveal structural differences relevant to the sialic acid transfer function of TS. We recently described the cloning and expression of a gene fragment encoding *T. rangeli* sialidase (Smith et al., 1996). The *T. rangeli* protein contains the same predicted structural features as TS, including ASP boxes and a C-terminal Fn3 domain. Most importantly, the same amino acids within the presumed sialic acid binding pocket of TS are found in the *T. rangeli* sialidase, suggesting that amino acids outside of this pocket are involved in the TS sialic acid transfer reaction.

Here we describe the construction, expression, and functional activity analysis of *T. cruzi* trans-sialidase/*T. rangeli* si-
L.J. Smith and D. E. Kichinger

and D. E. Kichinger

alidase hybrid proteins which served to roughly define a region in the TS catalytic domain required for transfer function, and the site specific mutagenesis of this region to further delimit the involved segment. The amino acids in this segment are predicted to lie in a loop just outside of the sialic acid binding pocket, and in this position are likely to interact with the galactose portion of donor/acceptor molecules. By exchanging a single amino acid within this segment, we added sialyltransferase activity to a hybrid protein that originally expressed only sialidase activity, thereby creating a novel trans-sialidase enzyme. The C-terminal Fn3-like domain of TS, although well separated from the catalytic domain in the primary structure, was also found to influence the relative levels of the sialidase and sialyltransferase activities of TS.

Results

The approach taken to dissect TS for the region required for sialyltransferase activity was based on the similarity in the positions of the sialic acid binding pocket residues and Asp boxes in primary structure alignments of the catalytic domains of T. cruzi TS and T. rangeli sialidase with the S. typhimurium sialidase. Figure 1 shows a schematic of the β-sheet and strand structure of the bacterial sialidase with the approximate positions of the sialic acid binding pocket residues indicated. The amino acids that accommodate the sialic acid N-acetyl group on C5 (M99, W121, W128, L175) and the glycerol chain on C6 (W128) are all within β-sheets 2 and 3 of the enzyme. On the other hand, the amino acids (E231, R246, R309, Y342, E361, with the exception of R37 in β-sheet 1) that interact with C1 and C2 at the opposite "end" of the sialic acid molecule are located in β-sheets 4–6. Since hydrolysis and subsequent transfer of sialic acid to galactose occurs here, the gene segments of TS and T. rangeli sialidase corresponding to β-sheets 4–6 were exchanged with one another, using the pQE-based expression constructs pΔ4 (expressing TS) and pG5EX (expressing sialidase) previously described (Schenkman et al., 1994a; Smith et al., 1996).

The region of T. rangeli sialidase containing the three presumed β-sheets 4–6, plus the flanking region between the sixth β-sheet and the Fn3 domain, was inserted behind β-sheets 1–3 of TS. The resulting construct, pS7-l, encoded a product with sialidase, but no trans-sialidase activity (Figure 2). This glycolytic activity was greatest at the lowest pH tested, (4.0), but dropped off abruptly with any increase in pH (Figure 3). The fact that this hybrid expressed any activity at all was signifi-

---

Fig. 1. Alignment of the β-sheet/strand structure of the S. typhimurium sialidase with the ORFs of trypanosome enzymes. The six β-sheets (B1-6) of the bacterial sialidase are each made up of four strands (1–4) that are connected by loops (not shown to scale). The approximate positions of the amino acids that comprise the sialic acid binding pocket are indicated as bold letters (R, arginine; M, methionine; W, tryptophan; L, leucine; E, glutamic acid; Y, tyrosine). N and C indicate the termini of the bacterial sialidase ORF. Schematics of the ORFs of T. cruzi trans-sialidase and T. rangeli sialidase catalytic domains are aligned below to indicate the conserved positions of the Asp boxes in primary sequence alignments. Vertical dotted lines indicate the positions within the presumed secondary structure (based on that of S. typhimurium sialidase) of the junction points of the hybrid proteins shown in Figure 2 (E, EcoNI).
The expression constructs assayed are indicated to the right, and correspond to those in Figure 2.

To determine if any of the TS-derived sequence at the C-terminal end of the catalytic domain of pS3—2 was required to reveal the sialyltransferase activity resulting from addition of the PGS triplet, the same Mon-2 oligo was used to mutagenize plasmid S7—1, which had the entire C-terminal half of the catalytic domain derived from G5. This mutant (S7—1mon-2) also acquired a similar level of sialyltransferase activity as the mon2 mutant of S3—2 (Figure 2).

Each of these three amino acids from TS was next introduced individually into the pS3—2 and pS7—1 sialidase expression constructs. Inserting proline for glutamine added sialyltransferase activity to both expression constructs, whereas the other two amino acid substitutions (D to G, and C to S) resulted only in retention of sialidase activity (Figure 4). The level of sialyltransferase activity of the P to Q mutant was approximately 25% that of wild-type TS (Figure 2).

We had previously described the inability to express enzyme activity from constructs expressing only the catalytic domains (i.e., without any attached C-terminal domains) of either the TS or T. rangeli sialidase genes (Smith et al., 1996). To express a protein product with any enzyme activity, these genes required the full length ORFs encoding the N-terminal catalytic domain and the C-terminal Fn3-like domain (which was noted by others to contain a conserved sequence VTVxNVxLYNR; Cross and Takle, 1993), plus the intervening domain of unknown function. A recent description of the crystal structure of sialidase from M. viridifaciens (Gaskell et al., 1995) revealed the presence of two additional domains attached to its β-barrel.

<table>
<thead>
<tr>
<th>Activity</th>
<th>S</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>5122</td>
<td>5166</td>
<td></td>
</tr>
<tr>
<td>9786</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Hybrid expression constructs and enzymatic activities. The top two schematics represent the ORFs of the full-length T. cruzi trans-sialidase (TS154Δ4A) and T. rangeli sialidase (G5SEX) expression constructs (Smith et al., 1996). These and all other expression constructs contain the intervening and Fn3-like domains downstream of the catalytic domain from TS154Δ4 (to the right of the Bs restriction site), plus a small number of C-terminal tandem repeats (not shown). Hybrid constructs made with these two genes are indicated below, and shaded and unshaded segments represent T. cruzi TS- and T. rangeli sialidase-derived sequences, respectively. When a mutagenic oligonucleotide was used, the template and amino acids exchanged by the oligo are indicated (PGS, P). Enzyme activity encoded by each construct is indicated to the right, in fluorescence units for sialidase (S), which was assayed at pH 5.0, and in c.p.m. for trans-sialidase (TS), which was assayed at pH 7.2 (E. E. ConN; Bs, BstWI).

The next hybrid construct, pS3—2, inserted all of β-sheets 4 and 5 and the first strand of sheet 6 from T. rangeli sialidase in place of the TS sequence. The product of this hybrid expressed only sialidase activity (Figure 2). Again, the reciprocal hybrid construct protein product was inactive (data not shown).

The third hybrid, pS8—1, replaced the fourth strand of the fourth β-sheet (B4S4), the entire fifth β-sheet and the first strand of the sixth β-sheet (B6S1) of the pS3—2 gene product with the same region from TS. Now the encoded product expressed both sialidase and sialyltransferase activities. The pS8—1-encoded protein had reduced sialidase activity and the broad pH optimum range similar to the wild type TS enzyme. The sequence and encoded activity differences between the pS3—2 and pS8—1 constructs served to define a segment of 88 amino acids that contained residues required for TS sialic acid transfer activity.

Next, using pS3—2 as template, oligo-directed mutagenesis was used to systematically exchange small portions of the T. rangeli sialidase-derived 88 amino acid-encoding segment with those found in TS. Of the eight oligos designed to span this region, the changes introduced by only one were informative. Oligonucleotide Mon-2 was designed to replace the 3 contiguous amino acids, Q, D, and C (284—286) of sialidase with the P, G, and S, respectively (315—317), found in the corresponding positions of TS. Exchange of these three amino acids resulted in a hybrid gene (s3—2mon2) that encoded a protein with sialidase and sialyltransferase activities. The sialidase activity of this mutant exhibited the same pH sensitivity as the pS3—2 encoded product (not shown). Its level of sialyltransferase activity ranged from 12 to 25% that of the wild type T. cruzi TS enzyme.

To determine if any of the TS-derived sequence at the C-terminal end of the catalytic domain of pS3—2 was required to reveal the sialyltransferase activity resulting from addition of the PGS triplet, the same Mon-2 oligo was used to mutagenize plasmid S7—1, which had the entire C-terminal half of the catalytic domain derived from G5. This mutant (S7—1mon-2) also acquired a similar level of sialyltransferase activity as the mon2 mutant of S3—2 (Figure 2).

Each of these three amino acids from TS was next introduced individually into the pS3—2 and pS7—1 sialidase expression constructs. Inserting proline for glutamine added sialyltransferase activity to both expression constructs, whereas the other two amino acid substitutions (D to G, and C to S) resulted only in retention of sialidase activity (Figure 4). The level of sialyltransferase activity of the P to Q mutant was approximately 25% that of wild-type TS (Figure 2).

We had previously described the inability to express enzyme activity from constructs expressing only the catalytic domains (i.e., without any attached C-terminal domains) of either the TS or T. rangeli sialidase genes (Smith et al., 1996). To express a protein product with any enzyme activity, these genes required the full length ORFs encoding the N-terminal catalytic domain and the C-terminal Fn3-like domain (which was noted by others to contain a conserved sequence VTVxNVxLYNR; Cross and Takle, 1993), plus the intervening domain of unknown function. A recent description of the crystal structure of sialidase from M. viridifaciens (Gaskell et al., 1995) revealed the presence of two additional domains attached to its β-barrel.
catalytic domain. In this case a C-terminal jelly roll domain containing a galactose binding pocket was proposed to position, via an intervening Ig-like domain, a bound galactose molecule to within 30 Å of the sialic acid binding pocket of the catalytic domain. The gross similarity between the domain structure of this bacterial sialidase and those of the parasite enzymes, and the apparent requirement of the C-terminal domain of the parasite enzymes for activity, prompted us to align the amino acid sequences of the bacterial and parasite protein C-terminal domains (Figure 5). The R572 and E578 residues of *M. viridifaciens* sialidase that interact with O3 and O4 of the bound galactose were in fact conserved in the *T. cruzi* and *T. rangeli* proteins. The aligned arginine in the parasite proteins was the same one found at the end of the conserved VTVxN-VxLYNR sequence that defines the trypanosome TS/sialidase superfamily of cell surface proteins (Campetella et al., 1992; Cross and Takle, 1993; Schenkman et al., 1994b). Oligo-directed mutagenesis of the corresponding R (to A) in each parasite enzyme greatly reduced their sialidase activities when using MU-NANA as substrate, and significantly increased the sialyltransferase activity of TS (Figure 5). Changing the conserved E (to V) in each parasite enzyme had the same effects on the two enzymatic activities.

### Discussion

Sequence alignments of *T. cruzi* TS, *T. rangeli* sialidase, and *S. typhimurium* and *M. viridifaciens* sialidases predict that the three amino acids exchanged by the Mon-2 oligo fall within a loop connecting the last strand of β-sheet 4 and the first strand of sheet 5 in the parasite enzymes. This loop in the bacterial enzymes has amino acid side chains that extend into the space just outside of the sialic acid binding pocket (Crennell et al., 1993; Gaskell et al., 1995). Therefore, these residues in the parasite proteins might be capable of interacting with the penultimate (galactose) residue of a donor substrate, or with the terminal galactose of an acceptor substrate. This predicted loop is 4–5 amino acids longer in both trypanosome proteins than in any of the bacterial enzymes, and varies in sequence between the *T. cruzi* and *T. rangeli* enzymes in places other than the three amino acids exchanged by oligo Mon-2. These other differences (when exchanged independently of the Mon-2 residues) were not found to be significant with respect to sialyltransferase function (data not shown).

The finding that a single proline substitution into the sialidase-expressing construct added sialyltransferase function was surprising. The proline is not likely to be directly involved in
protein–sugar interactions, as amides are the type of amino acid side chain in lectins most often found hydrogen bonded to sugar OH groups (Weiss and Drickamer, 1996). The gain of function resulting from insertion of a second prololne into this segment of the T rangeli protein indicates that all the other amino acids essential for transfer function are already present, and that only a conformational alteration of the loop is necessary to add some level of transfer function. We do not know at this time how the prololne described here changes the local structure of the T rangeli sialidase, and whether the significant repositioned residues are in fact found within the same or adjacent loops. There are three prololnes in this presumed loop of the T cruzi TS protein, suggesting that further conformational adjustments may improve on the low (25% of wild type) level of sialyltransferase function of the Mon-10 mutant of hybrid s7–1.

A prololne in a different position of TS was previously shown to be required for high levels of TS sialyltransferase activity in proteins that already expressed some level of transferase activity (Cremona et al., 1995). Both the T cruzi clone 154 TS and the T rangeli G5 sialidase contain this particular prololne, which is predicted to be part of strand 1 of β-sheet 4. It, most likely, is critical for positioning the adjacent upstream glutamic acid, which is one of the conserved amino acids of the substrate binding pocket, and thereby directly effects the enzyme’s sialidase activity.

The implication that the amino acids which are essential for sialyltransferase activity are present in the T rangeli enzyme is consistent with the notion that the T rangeli enzyme expressed in the insect stage of the parasite originally had and then lost sialyltransferase activity (Briones et al., 1995), gained mutations that increased the sialidase activity at low pH levels, and drifted in regions of the protein not required for sialidase function. It is worth noting, therefore, that a reported sequence (Buschiazzo et al., 1993) of a trans-sialidase-like gene from an unspecified strain of T rangeli is predicted to encode a protein with the PGS sequence in the critical loop, rather than the QDC sequence of the three T rangeli sequences described previously (Smith et al., 1996). Although no evidence was presented for enzymatic activities expressed by this unspecified strain or the protein product of the described gene, it leaves open the possibility that a different stage of the T rangeli parasite does express TS activity, as postulated by those authors, and that the T rangeli genome contains two types of sialidase-related genes. The sequences of trans-sialidases from earlier diverging trypanosomatids, such as T brucei, will be interesting for comparison in this regard.

The role of the Fn3-like domain in the parasite enzymes remains unclear based on the limited analysis described here. It contains conserved amino acids that may be involved in galactose binding, by comparison to the M viridifaciens sialidase. M viridifaciens makes two forms of sialidase from the same gene, one which contains only the N-terminal catalytic domain, and another which has the attached Ig-like and jelly roll domains (Sakurada et al., 1992). Both of these forms express sialidase activity, and the growth medium of a casein-induced culture of M viridifaciens, which should express the full length gene product, contained readily detectable sialidase but no trans-sialidase activity when assayed under the same conditions as the parasite enzymes, (D.E., unpublished observations). Previous deletion constructs indicated that an intact Fn3-like domain is required for expression of enzymatically active products from both T cruzi TS (Schenkman et al., 1994) and T rangeli sialidase (Smith et al., 1996) genes. On the other hand, the Fn3-like domain of TS is apparently not sufficient to confer sialyltransferase activity to a protein with sialidase activity, since it is found in the wild type T rangeli sialidase protein, and since exchanging the Fn3-like domain from TS with that of the T rangeli sialidase did not add sialyltransferase function to the sialidase. Individual elimination of the conserved R and E residues of this domain of TS actually served to increase its apparent sialyltransferase activity. The fact that mutations in this region did have an effect on enzyme activity indicates that the parasite proteins are folded so as to bring the substrate-binding region of the catalytic domain and (at least) the R and E of the Fn3-like domain close together, as is proposed for the sialic acid and galactose binding sites of the M viridifaciens sialidase (Gaskell et al., 1995). Whether or not the Fn3-like domain of TS forms an essential component of a binding pocket for donor and/or acceptor substrates will best be resolved with additional mutagenesis and kinetic studies, in combination with protein crystal structure data obtained from enzymatically active forms of these proteins. Such detailed information about the roles of the catalytic and Fn3-like domains of TS should also help to establish the feasibility of eventually adding glycosyltransferase activity to other glycosidases.

Materials and methods

Materials

Oligonucleotides were purchased from Operon Technologies, Inc. (Alameda, CA). Methyl umbelliferyl-N-acetylneuraminic acid (MU-NANA), sialylactose (SL), IPTG, and buffers were purchased from Sigma.

Bacterial strains

For plasmid preparations E.coli strain TG1 (F' trnD36 lacIq Δ(lacZ)M15 proA+ lacR75 Δ(hsdMrcB5) (qα m- m+) thi A(lac-proAB)) was obtained from ATCC (catalog number 15146) and grown as described previously (Sakurada et al., 1992).

Construction of hybrid genes

Overlap extension PCR was performed using a modification of the method of Von and Fried, 1989. Oligo DE17 (5'-CGG CAT GCC ACC CGG ATC GAG CTA) and the linking oligo DE37 (5'-CGA GGA CTG CCG GTT CAG AGC AGC) were used in the PCR with T.cruzi pTS154ΔAF (pD4) (Schenkman et al., 1994a) template to amplify the N-terminal half of the TS catalytic domain containing the presumed β-sheets 1–3, (see Figure 1). This reaction product, plus oligo RT154 (5’-CGG CAT GCC ACC CGG GTA CTT TCA CTG CTT CCG CTG) and T rangeli sialidase expression construct pG5EX (Smith et al., 1996) as template were combined and amplified for 10 cycles, and then oligo DE17 was added and amplification was continued for 20 more cycles. The resulting product was digested with NcoI and BsiWI and inserted in place of the same restriction fragment of the pD4 expression vector, yielding plasmid pS7–1 (see Figure 2). Two other constructs were made with additional sequence from the TS gene. The EcoNI–BsiWI fragment of pS7–1 was inserted in place of the same fragment of pD4, yielding pS3–2. An overlap extension PCR, containing oligo DE17, linking oligo DE47 (5’-AAT TCC CCA TGT CCG ATG CCT ACG ACA ACA AGC AAC AAT CAC TAC) to pTS154ΔAF (pD4) template in the second round, generated an NcoI/BsiWI insert which was inserted in place of the same fragment of pD4, yielding pS8–1.

Oligonucleotide-directed mutagenesis

Mutagenesis was carried out using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) following the manufacturer’s instructions. In order to distinguish mutated plasmids from wild type plasmids that carried through the
reaction, each oligo pair was designed to add or destroy a restriction site without changing the encoded amino acids except for those being changed by mutagenesis. The pair of oligos (Mon 2: 5’-TCA ACA TCG CAC GGA TCC CAG TGG CTG GTT CGA TGT TGG TGA and Mon 3: 5’-GAA CAG GAC TCT GGA CAG ACC TTC CTG GTG TTG CGG CTG CCG TTC GTG GCT GCT CTG CTG CTG CTG CTG CTG CTG GTG TGA) inserted the amino acids P, G, and S (residues 313–317, numbering based on T. cruzi trans-sialidase clone 154; Smith et al., 1996) in place of Q, D, and C (residues 284–286 of T. rangeli sialidase), respectively, into the hybrid constructs pS7-1 and pS7-2. Single amino acid changes to pS7-1 and pS7-2 were made using the following oligo pairs: Q284 to P (Mon 10: 5’-AAC TCA ACA ACT GCT CGG GGG GCA AGT TGG TGA GTT) in place of (Mon 11: 5’-AAC TCA ACA ACT GCA CAG GGC GAC TCT GCT GCT CTG CTG CTG CTG CTG GTG TGA) 

**E. coli** containing expression plasmids were grown overnight in LB broth plus 50 μg/ml ampicillin (LB/amp) at 37°C, diluted 1:10 in LB/amp containing 2 mM IPTG, and grown for 2.5 h. Bacterial lysates were prepared using the method described by Schupp et al., 1995. The total protein concentration in the lysates was determined by the method of Udenfriend et al., 1967. One unit of neuraminidase activity was defined as the amount of enzyme required to release 1 nmol of sialic acid from carboxymethylcellulose per minute at 37°C. The total protein concentration in the lysates was determined by the method of Udenfriend et al., 1967. One unit of neuraminidase activity was defined as the amount of enzyme required to release 1 nmol of sialic acid from carboxymethylcellulose per minute at 37°C.

## Abbreviations

TS, trans-sialidase; Fn3, fibronectin type III domain; IPTG, isopropyl-β-D-thiogalactopyranoside; MES, 2-(N-morpholino)ethanesulfonic acid; HEPES, (N-2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid).

## References


Received on October 11, 1996; revised on November 12, 1996; accepted on November 21, 1996

Trans-sialidase domains