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cDNA encoding the glycosyl-phosphatidylinositol-specific phospholipase C of *Trypanosoma brucei*

(glycolipid anchor/variant surface glycoprotein)

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ABSTRACT **VSG lipase of *Trypanosoma brucei* specifically cleaves the glycosyl-phosphatidylinositol membrane anchor of the trypanosome variant surface glycoprotein (VSG), releasing this protein from the plasma membrane. It also cleaves similar membrane anchors on some mammalian proteins. VSG lipase may play a role in processes such as parasite differentiation or antigenic variation. We describe here the cloning and sequencing of a cDNA encoding VSG lipase from *T. brucei*.**

The variant surface glycoprotein (VSG) of *Trypanosoma brucei* is anchored to the plasma membrane by a covalently attached glycosyl-phosphatidylinositol. Ferguson *et al.* have recently reported the complete structure of this glycolipid anchor (1). It contains dimyristoyl phosphatidylinositol glycosidically linked to nonacetylated glucosamine. Attached to the glucosamine is an oligosaccharide composed of mannose and galactose, and a phosphodiester links ethanolamine to one of the mannosyl residues. Finally, an amide bond joins ethanolamine and the α-carboxyl of the protein's C-terminal amino acid residue.

Glycosyl-phosphatidylinositol anchors of similar structure are also found on cell-surface proteins in other protozoa and in higher eukaryotes including humans (for reviews, see refs. 2–4). These proteins include Thy-1, alkaline phosphatase, acetylcholinesterase, and decay accelerating factor of mammals and several surface antigens and membrane-bound enzymes in protozoa. A glycosyl-phosphatidylinositol related in structure to the membrane anchors has been postulated to mediate some of the actions of insulin (5).

The function of glycosyl-phosphatidylinositol membrane anchors is not yet clear, but in some cases they may facilitate specific and regulated release of proteins from cell surfaces by phospholipases or other enzymes that cleave the glycolipid. This possibility is especially appealing in the case of the trypanosome VSG; this protein is known to be lost from the cell when the parasite enters its tsetse fly vector (6, 7) and possibly also following endocytosis (4, 8) or during antigenic variation. VSGs of different trypanosome variants differ dramatically in amino acid sequence; therefore, if they were anchored by a hydrophobic peptide sequence it might be difficult to achieve specific release by proteolysis. However, the presence of a common glycolipid anchor on all VSGs would provide a uniform cleavage site for specific release by a phospholipase.

In 1983, Cardoso de Almeida and Turner (9) discovered a membrane-bound enzyme activity in *T. brucei* that cleaves the VSG glycolipid. When purified to homogeneity, this enzyme consists of a single polypeptide of about 37–40 kDa (10–12). It is a Ca^{2+}-independent phospholipase C which efficiently cleaves VSG membrane anchors, but it has little or no activity against free phosphatidylinositol. This enzyme was the first example of a class of enzymes called glycosyl-phosphatidylinositol-specific phospholipases. More recently, there have been reports of a similar enzyme in rat hepatocytes that may be involved in insulin action (13) and a glycosyl-phosphatidylinositol-specific phospholipase D, of unknown function, from mammalian serum (14, 15).

The trypanosome enzyme, which we designate VSG lipase, is apparently quiescent on living bloodstream parasites, yet upon cell lysis under nondenaturing conditions it cleaves all of the VSG from the membranes within several minutes. Its dormancy in living cells could be due to regulation of the enzyme activity; the enzyme could be specifically activated for release of VSG during appropriate stages of the trypanosome life cycle. As part of a study of the biological role and mode of regulation of VSG lipase, we have cloned and sequenced its cDNA.* It encodes a polypeptide of 40,760 Da, a value consistent with the protein's electrophoretic behavior. Despite the fact that VSG lipase appears to be a membrane protein, the deduced sequence does not include strongly hydrophobic domains or an N-terminal signal sequence. There are no significant similarities between the VSG lipase sequence and those of other known proteins including several recently reported mammalian phosphatidylinositol-specific phospholipase Cs.

MATERIALS AND METHODS

Purification of VSG Lipase. VSG lipase was purified from *T. brucei* (ILTat 1.3) by two different methods. To generate rabbit antiserum and the mouse monoclonal antibody, it was purified by phenyl-Sepharose and carboxymethyl-Sephadex chromatography as described (method 1; see ref. 11).

For amino acid sequencing, VSG lipase was affinity purified by a procedure similar to that of Bulow and Overath (method 2; see ref. 10). A VSG lipase-specific murine monoclonal antibody, 2A6-6, which binds the native active enzyme, was generated by standard methods and a screening assay like that used by Bulow and Overath (10). Antibody was adsorbed from hybridoma culture supernatants onto protein A-Sepharose (Pharmacia) and covalently coupled to the resin by the method of Schneider *et al.* (16). An *n*-octyl glucoside extract of 5.4×10^{10} trypanosomes (40 ml; ref. 11) was passed through a 0.1-ml column of the resin overnight at 4°C. The column was then washed at 4°C at 0.5 ml/min with 2.5 ml of each of the following solutions in succession: (*i*) TEN (50 mM Tris·HCl/5 mM EDTA/150 mM NaCl, pH 7.5) containing 1% Nonidet P-40 (NP-40) and 0.5 M NaCl; (*ii*) TEN containing 1% NP-40; (*iii*) TEN containing 0.1% NP-40. Finally, the enzyme was eluted from the column with 2.5 ml of 50 mM NaP_i/0.1% NP-40, pH 12.0, at the same flow rate.

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Abbreviations: NP-40, Nonidet P-40; VSG, variant surface glycoprotein.

*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04124).

Fractions (0.25 ml) were immediately neutralized with 0.1 ml of 1 M Tris·HCl (pH 7.5); those containing VSG lipase activity (assayed as described in ref. 11) were pooled. Greater than 80% of the activity loaded onto the column was recovered in ≈1.5 ml of the eluting buffer, and $NaDodSO_4$/PAGE analysis of this material revealed a homogeneous 36-kDa protein (see Fig. 3*A*). Its specific activity, measured as described in ref. 11, was $\approx 10^7$ units/mg. This preparation is designated affinity-purified VSG lipase.

Production of Antiserum. Rabbit antiserum was obtained by immunization with VSG lipase (15 μg, prepared by method 1) excised from a $NaDodSO_4$/polyacrylamide gel. Gel pieces were equilibrated in 0.15 M NaCl, sonicated, and injected as an emulsion with Freund's complete adjuvant by standard procedures. VSG lipase-specific antiserum was obtained after the rabbit was given two comparable booster injections with Freund's incomplete adjuvant.

Screening of cDNA Library. About 10^5 clones of an amplified λgt11 cDNA library prepared from RNA from *T. brucei rhodesiense* (WRATat 1.1) bloodstream trypanosomes (generously provided by J. Donelson, University of Iowa) were screened with VSG lipase-specific antiserum (diluted 1:2000) (17). Alkaline phosphatase conjugated to goat anti-rabbit immunoglobulin was used to detect bound antibodies. Several clones, each containing a 0.95-kilobase (kb) insert, were obtained. One, designated DH1, was used as a hybridization probe (18) to screen a second *T. brucei rhodesiense* (WRATat 1.1) λgt11 cDNA library (also provided by J. Donelson) by standard methods (19). Of 10^5 recombinants screened, 6 cross-hybridizing clones were detected. A 1.4-kb cDNA, designated DH6, was used for DNA sequencing.

cDNA Sequencing. cDNA inserts were subcloned into the plasmid vector Bluescript (Stratagene, San Diego, CA) by established methods (19). Plasmid constructs were amplified in *Escherichia coli* strain DH5α (Bethesda Research Laboratories) and sequenced with Sequenase (United States Biochemicals, Cleveland, OH) following the manufacturer's protocol. To completely sequence both DNA strands, deletions were created from each end of the 1.4-kb cDNA using exonuclease III and mung bean nuclease as directed by the supplier (Stratagene).

RESULTS

Isolation and Characterization of cDNAs. We first isolated a phage clone from a λgt11 cDNA library by immunoscreening with a VSG lipase-specific antiserum. This clone contained a 0.95-kb cDNA insert designated DH1. The probing of western blots of infected cell extracts, using the same antiserum, indicates that this clone encodes a 145-kDa immunoreactive isopropyl thiogalactoside-inducible protein (data not shown).

We then obtained a 1444-base-pair (bp) cDNA, designated DH6, by screening a second library with radiolabeled DH1 sequence as a hybridization probe. Based on sequencing, the 3′ end of DH6 overlaps ≈800 bp of the DH1 sequence and contributes an additional 600 bp of sequence at the 5′ end. Both strands of DH6 were completely sequenced (Fig. 1). The sequence of the "sense" strand is presented in Fig. 2.

DH6 contains an open reading frame encoding a polypeptide of 358 amino acid residues, beginning with a methionine codon at nucleotide 240. This polypeptide sequence is also shown in Fig. 2. To prove that this sequence is that of VSG lipase, we determined amino acid sequences of tryptic peptides derived from affinity-purified enzyme (prepared by method 2).

Amino Acid Sequencing of VSG Lipase Peptides. Initial attempts to obtain the VSG lipase N-terminal amino acid sequence were unsuccessful, possibly because the protein has a blocked N terminus. Therefore, we digested affinity-purified enzyme with trypsin and separated the resulting

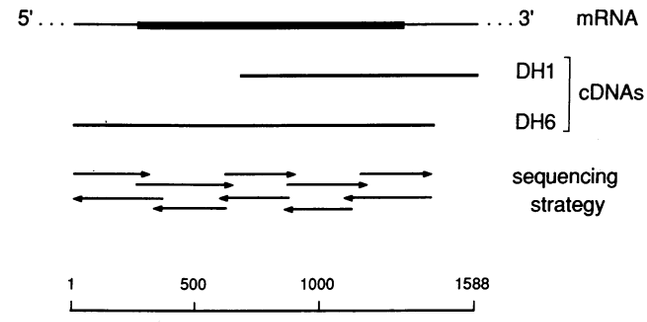

FIG. 1. Maps of VSG lipase mRNAs and cDNAs, and sequencing strategy. Solid bar on mRNA is region encoding VSG lipase. Scale is in base pairs.

tryptic peptides by reverse-phase HPLC (Fig. 3*B*). The amino acid sequences of three peptides, designated T1, T2, and T3, are shown in Table 1. All three of these peptides are contained in the amino acid sequence predicted by the cDNA (the peptide sequences are underlined in Fig. 2). Therefore, the DH6 cDNA must encode VSG lipase.

Properties of the cDNA Sequence. Translation almost certainly begins at the methionine codon at nucleotide 240 because it is the only initiation codon present between in-frame termination codons (at nucleotides 60 and 66) and the sequence that encodes peptide T3 (beginning at nucleotide 258). Thus, DH6 is comprised of a 239-bp 5′ untranslated sequence, 1077 bp encoding VSG lipase, and a 128-bp 3′ untranslated sequence. An additional ≈150 bp of 3′ untranslated sequence was obtained from cDNA DH1 (data not shown).

DH6 represents most of the 5′ end of the VSG lipase mRNA because the sequence at its extreme 5′ end closely resembles part of the miniexon sequence, a 39-mer which is present at the 5′ end of all known mRNAs of trypanosomes and related organisms (for review, see ref. 22). The 13-nucleotide miniexon-like sequence of DH6 is identical to the 3′ end of the *T. brucei brucei* miniexon sequence in all but one position (20). The substitution of a thymine for an adenine at nucleotide 5 (Fig. 2) could imply that the *T. brucei rhodesiense* miniexon differs from that of *T. brucei brucei*; however, all trypanosomatid miniexons that have been sequenced have adenine at this position (23). Alternatively, it could reflect a reverse transcriptase error during cDNA synthesis or the use of a miniexon gene that differs from the majority of the roughly 200 miniexon genes present in the parasite genome (22).

It is possible that neither DH1 nor DH6 cDNAs represent the complete 3′ end of VSG lipase mRNA. Since neither sequence has more than 8 adenines at its 3′ end, it is uncertain whether these are encoded in the gene or are added post-transcriptionally. When available, the sequence of the VSG lipase gene may clarify this point.

Analysis of the Deduced Amino Acid Sequence. The predicted amino sequence of VSG lipase (358 residues) given in Fig. 2 has a calculated molecular mass of 40,760 Da, in agreement with reports of 37–40 kDa based on $NaDodSO_4$/PAGE (10–12). Since VSG lipase has been assumed to be a membrane-associated protein (see *Discussion*), we performed a hydropathy analysis of the amino acid sequence (24) to identify domains that might interact with the lipid bilayer. The results of this analysis (Fig. 4) are discussed below.

DISCUSSION

Clone DH6 encodes the entire VSG lipase sequence. The original clone corresponding to this sequence (DH1) was isolated by immunoscreening with a rabbit antiserum. Although this clone could have encoded a protein that contaminated or cross-reacted with VSG lipase, protein sequencing

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                    **** ********
1                   CTGTTCTATATTGTAGCGGCCAAGCCCCGTGGTGTTGGAATTTTCTTCATAAACGTGATTAGGTG

66 TAACTAAAGCACCTTCTGACTGTCGTCGTAGTTGTTGTTATTACTTCTTCTTCCTTTTTTTCTTTTTCTTTTGTTTGTTTGTCGTTT

153 GTGTGTGGGTGCGTGTGGGTGTGTGAGAGAGAAAGAAAGAAAGAAAAAGAGGGGAAGGGGACTTTGAGACGGTTAAGAATCATTGTA

240 ATG TTT GGT GGT GTA AAG TGG TCA CCG CAG TCA TGG ATG AGT GAC ACG CGG TCT TCC ATT GAG AAG
    Met Phe Gly Gly Val Lys Trp Ser Pro Gln Ser Trp Met Ser Asp Thr Arg Ser Ser Ile Glu Lys22

306 AAA TGT ATT GGT CAA GTA TAT ATG GTA GGA GCA CAT AAT GCA GGA ACA CAC GGC ATA CAA ATG TTT
    Lys Cys Ile Gly Gln Val Tyr Met Val Gly Ala His Asn Ala Gly Thr His Gly Ile Gln Met Phe44

372 TCC CCG TTT GGA TTA GAT GCC CCT GAA AAG TTA CGG AGC CTC CCT CCA TAT GTG ACC TTT CTT TTA
    Ser Pro Phe Gly Leu Asp Ala Pro Glu Lys Leu Arg Ser Leu Pro Pro Tyr Val Thr Phe Leu Leu66

438 AGA TTT CTT ACT GTT GGT GTG AGC AGC AGA TGG GGA CGT TGT CAA AAT CTT TCT ATT CGA CAG CTT
    Arg Phe Leu Thr Val Gly Val Ser Ser Arg Trp Gly Arg Cys Gln Asn Leu Ser Ile Arg Gln Leu88

504 TTG GAT CAT GGG GTG CGT TAT CTC GAC TTA CGC ATG AAC GTA AGT CCG GAT CAG GAA AAT AAA ATT
    Leu Asp His Gly Val Arg Tyr Leu Asp Leu Arg Met Asn Val Ser Pro Asp Gln Glu Asn Lys Ile110

570 TAC ACA ACT CAT TTC CAT ATT TCT GTT CCA CTA CAA GAG GTT CTG AAG GAT GTC AAG GAT TTC TTG
    Tyr Thr Thr His Phe His Ile Ser Val Pro Leu Gln Glu Val Leu Lys Asp Val Lys Asp Phe Leu132

636 ACC ACA CCT GCA AGC GCC AAC GAA TTT GTC ATT CTC GAT TTC TTG CAT TTC TAC GGA TTT AAT GAG
    Thr Thr Pro Ala Ser Ala Asn Glu Phe Val Ile Leu Asp Phe Leu His Phe Tyr Gly Phe Asn Glu154

702 AGA CAT ACG ATG AAG CGC TTT GTT GAG GAG CTG CAA GCA CTT GAG GAG TTT TAC ATT CCC ACA ACC
    Arg His Thr Met Lys Arg Phe Val Glu Glu Leu Gln Ala Leu Glu Glu Phe Tyr Ile Pro Thr Thr176

768 GTC TCT TTA ACC ACA CCA CTT TGT AAC CTC TGG CAG TCA AAC AGA CGT ATT TTT CTT GTT GTG AGA
    Val Ser Leu Thr Thr Pro Leu Cys Asn Leu Trp Gln Ser Asn Arg Arg Ile Phe Leu Val Val Arg198

834 CCT TAT GTA GAA TAC CCT TAT GCA CGA CTC CGC AGT GTT GCG CTT AAA TCC ATT TGG GTT AAT CAA
    Pro Tyr Val Glu Tyr Pro Tyr Ala Arg Leu Arg Ser Val Ala Leu Lys Ser Ile Trp Val Asn Gln220

900 ATG GAG TTG AAT GAT CTT CTC GAC CGG TTG GAG GAA CTC ATG ACT CGT GAT TTG GAA GAT GTC AGT
    Met Glu Leu Asn Asp Leu Leu Asp Arg Leu Glu Glu Leu Met Thr Arg Asp Leu Glu Asp Val Ser242

966 ATT GGC GGG GTT CCA TCT AAA ATG TAC GTC ACG CAA GCT ATC GGT ACG CCG CGA AAT AAC GAC TTT
    Ile Gly Gly Val Pro Ser Lys Met Tyr Val Thr Gln Ala Ile Gly Thr Pro Arg Asn Asn Asp Phe264

1032 GCG GTG GCA GCG TGT TGT AGC GCG TGT CCC GGT TCA CAT CCC GAT TTG TAT TCC GCT GCA AAG CAT
     Ala Val Ala Ala Cys Cys Ser Ala Cys Pro Gly Ser His Pro Asp Leu Tyr Ser Ala Ala Lys His286

1098 AAA AAT CCA CAT CTT TTG CAG TGG TTT TAT GAT TTA AAT GTT AAT GGT GTT ATG CGA GGG GAG CGT
     Lys Asn Pro His Leu Leu Gln Trp Phe Tyr Asp Leu Asn Val Asn Gly Val Met Arg Gly Glu Arg308

1164 GTG ACT ATA AGA CGG GGA AAC AAT ACA CAT GGA AAT ATA CTT TTG CTT GAT TTC GTG CAA GAA GGC
     Val Thr Ile Arg Arg Gly Asn Asn Thr His Gly Asn Ile Leu Leu Leu Asp Phe Val Gln Glu Gly330

1230 ACT TGT ACC GTT AAG GGA GTC GAC AAA CCG ATG AAT GCC GTT GCA TTA TGC GTT CAT TTA AAC ACC
     Thr Cys Thr Val Lys Gly Val Asp Lys Pro Met Asn Ala Val Ala Leu Cys Val His Leu Asn Thr352

1296 AAC CAA ACC GCA AGG TCA TAA AAATGTGGGAAAAAAAACAAATCTCTGCTGCGTTTGTGACTGAAACAAGGGTTTAAAAG
     Asn Gln Thr Ala Arg Ser  o

1376 TAAAAAAAAAAGTTAAGTGGACGACGAAATAACAACAAAGGGAAGGAAGGAAAATTAAAATAAAAAAAA 1444
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FIG. 2. cDNA and amino acid sequences of VSG lipase. The sequence of the sense strand of DH6 and the deduced amino acid sequence are shown. Asterisks overlie nucleotides that match the miniexon sequence determined by Dorfman and Donelson (20). Open circle indicates stop codon. Residues confirmed by amino acid sequencing are underlined. Nucleotide numbering is at left and amino acid residue numbering is at right. All sequences in the coding region were confirmed on both strands except for the nucleotide at position 462, which was ambiguous on one of the strands.

experiments ruled out these possibilities. For sequencing, the enzyme was affinity purified with a monoclonal antibody that binds the native active enzyme (method 2), whereas the protein used for preparing the rabbit antiserum was prepared by a different procedure (method 1); thus, the possibility that the cDNA represents a contaminating protein is highly unlikely. The amino acid sequences of three tryptic peptides confirmed ≈10% of the sequence deduced from the cDNA, making the possibility of fortuitous cross-reaction very remote.

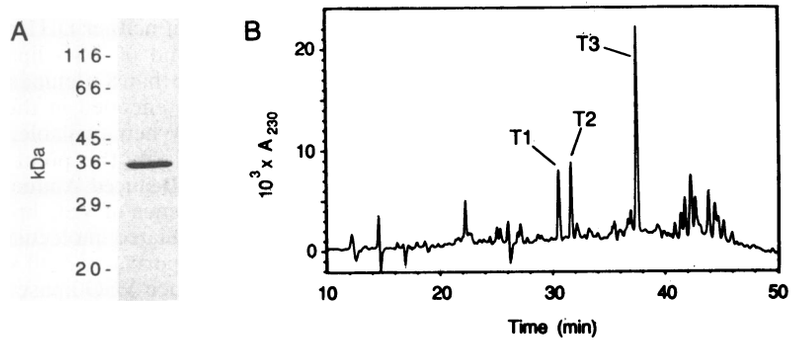

FIG. 3. Tryptic peptides of VSG lipase. Affinity-purified VSG lipase (60 μg in 1.45 ml) was concentrated to 1.0 ml and adjusted to pH 8.5 with NaOH. $NaDodSO_4$ was added to a final concentration of 0.1% and the mixture was incubated for 2 min at 100°C. The protein was treated sequentially with dithiothreitol (5 μmol in 5 μl, 75 min, 37°C), iodoacetic acid (10 μmol in 10 μl, 135 min, 25°C), and with dithiothreitol again (5 μmol in 5 μl, 30 min, 25°C). The protein was then precipitated with chloroform and methanol (21). (*A*) Coomassie-stained $NaDodSO_4$/polyacrylamide gel of the alkylated affinity-purified VSG lipase (2 μg). (*B*) HPLC of alkylated VSG lipase (≈58 μg) digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (2 μg) in 0.2 ml of 50 mM NH_4HCO_3 (pH 8) at 37°C for 18 hr. Trifluoroacetic acid (0.01 vol) was added and the reaction products were fractionated by reverse-phase HPLC using a large-pore C_{18} column (Alltech, no. 71079). After a 5-min wash with 0.1% trifluoroacetic acid, tryptic peptides were eluted with a linear gradient of acetonitrile (0–60%) in 0.1% trifluoroacetic acid over 60 min (flow rate, 1 ml/min). Background absorbances (230 nm), present in a mock digest from which VSG lipase was omitted, were automatically subtracted from the profile shown. Peptides T1, T2, and T3 were analyzed for amino acid sequence (see Table 1).

Table 1. Amino acid sequences of VSG lipase tryptic peptides

Peptide	Sequence determined	Yield, %	Corresponding nucleotides of DH6
T1	MYVTQAIGTPR	29	987–1019
T2	DLEDVXIGGVPXX	12	948–986
T3	WSPQSWMSDTR	ND	258–290

HPLC fractions (0.5 ml) containing peptides T1, T2, and T3 were lyophilized and analyzed with a gas-phase automated amino acid sequencer (Applied Biosystems, Foster City, CA). Amino acids are denoted by the single-letter code. Yield refers to the derivatized amino acid detected in the first cycle expressed as a percentage of the theoretical yield of 1.6 nmol (58 μg) of VSG lipase used for sequencing. Losses could be due to incomplete trypsinization or could have occurred during HPLC or concentration. ND, not determined.

The molecular mass of the homogeneous VSG lipase polypeptide is 37–40 kDa (10–12). This value is similar to that deduced from the cDNA sequence (40,760 Da), indicating no posttranslational processing events grossly alter the protein's apparent molecular mass. Although we cannot rule out the possibility that cleavage of the peptide was compensated by addition of some moiety, experiments with lectins (9) have failed to detect glycosylation at the four potential N-linked glycosylation sites (residues 82, 101, 315, and 353).

VSG lipase contains no significant sequence similarities with other proteins presently listed in the protein sequence data base. In particular, it has no similarities with the sequences of four distinct mammalian phosphatidylinositol-specific phospholipase C isozymes (25–29).

Although the intracellular localization of VSG lipase is not yet known, several physical properties suggest that this enzyme is a membrane-associated protein. In cell extracts, the activity is present exclusively in the particulate fraction and has been solubilized only by detergents (8–12). The homogeneous isolated enzyme associates with liposomes (10), and the activity partitions into the hydrophobic phase during Triton X-114 phase separation (12).

Despite VSG lipase's apparent association with membranes, its deduced amino acid sequence does not resemble that of a typical membrane protein. In contrast to many eukaryotic membrane proteins, it does not have a cleavable N-terminal signal sequence. As shown in Fig. 2, only five amino acids separate the N-terminal methionine residue and the first residue of T3, a peptide isolated from the mature protein. Since T3 was generated by tryptic cleavage, the neighboring Lys-6 is probably also present in the protein. Therefore, at most only five residues could have been cleaved from the N terminus of the primary translation product to form the mature protein.

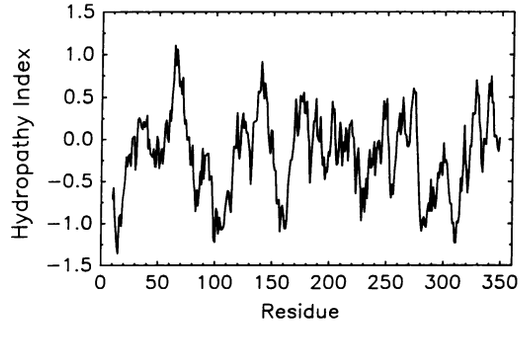

FIG. 4. Hydropathy analysis of the VSG lipase polypeptide. Hydropathy analysis of the deduced amino acid sequence was performed according to Kyte and Doolittle (24) using a span of 19 amino acid residues. Apolar residues have positive hydropathy values.

Not all membrane proteins have N-terminal signal sequences. For example, some mammalian plasma membrane proteins synthesized in the endoplasmic reticulum, such as asialoglycoprotein receptor, transferrin receptor, and HLA-DR invariant chain have uncleaved internal signal sequences (reviewed by Wickner and Lodish, ref. 30). These proteins are synthesized with their C-terminal domains on the luminal side of the endoplasmic reticulum membrane and N-terminal domains on the cytoplasmic side; these domains are joined by a transmembrane hydrophobic sequence. If VSG lipase were processed in this way, its C-terminal domain would be topologically on the same side of the membrane as the VSG substrate. However, the Kyte–Doolittle plot (Fig. 4) does not offer support for this model. There are no internal hydrophobic sequences that are strong candidates for transmembrane domains. One of the more prominent hydrophobic sequences is between residues 57 and 75. However, this sequence contains an arginine and several serines and threonines, and therefore its hydropathic index is less than that found for most transmembrane domains (24). An alternative possibility is that VSG lipase has multiple membrane-spanning amphipathic α-helices, but analysis of the sequence revealed no obvious candidates for such a structure.

It is possible that VSG lipase is held to the membrane by a covalently attached lipid. However it lacks the N-terminal signal sequence and C-terminal hydrophobic tail present in the precursors of proteins anchored by glycosyl-phosphatidylinositol (4). Alternatively, VSG lipase might be modified by a fatty acid. If it were anchored by a myristoyl group attached to its N terminus, like $p60^{src}$, or by a palmitoyl linked to an internal cysteine residue, like $p21^{ras}$, it is unlikely that it would localize on the same side of the membrane as its VSG substrate (31).

Further investigation will be necessary to determine the mode of interaction of the VSG lipase polypeptide with the membrane. These studies should contribute to an understanding of the biological function of this enzyme.

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