The Toxoplasma Adhesive Protein MIC2 Is Proteolytically Processed at Multiple Sites by Two Parasite-derived Proteases

Vern B. Carruthers§§, Gale D. Sherman§, and L. David Sibley‡

From the §Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110 and the ¶Department of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

MIC2 is an adhesive protein that participates in host cell invasion by the obligate intracellular parasite Toxoplasma gondii. Earlier studies established that MIC2 is secreted into the culture medium by extracellular parasites and that release is coincident with proteolytic modification. Since little is known about proteolytic processing of proteins secreted by T. gondii, we undertook this study to investigate the proteolytic events that accompany secretion of MIC2. We demonstrate that the C-terminal domain of MIC2 is removed by a protease, termed MPP1, when MIC2 is released into the culture supernatant. Additionally, prior to release, a second protease, termed MPP2, trims the N terminus of MIC2, resulting in the release of heterogeneous species of MIC2. Although MPP1 activity was unaffected by any of the protease inhibitors tested, MPP2 activity was blocked by a subset of serine and cysteine protease inhibitors. These results establish that MIC2 is proteolytically modified at multiple sites by two distinct enzymes that probably operate on the parasite surface.

Cell invasion is a critical step during infection of susceptible hosts by intracellular protozoan parasites. Although the mechanisms of parasite invasion vary considerably, a common theme includes the participation of parasite-derived proteases that have been described in Leishmania (1), Trypanosoma cruzi (2), and Plasmodium (3–5). In contrast, apart from a recent description of the effects of serine protease inhibitors (6), little is known regarding the involvement of proteases in host cell invasion by the related apicomplexan parasite Toxoplasma gondii.

T. gondii is a widely distributed intracellular protozoan that parasitizes virtually all warm-blooded vertebrates, including humans. T. gondii tachyzoites (a rapidly dividing stage responsible for acute infection) actively invade host cells in several discrete steps. First, the parasite uses gliding motility to emerge from an infected cell, to move across the substratum, and to reach adjacent host cells (7). Second, the parasite adheres via its apical (anterior) pole to the host cell (8, 9). Third, using contractile proteins, including actin and myosin, the parasite penetrates the host cell (10, 11) to form a parasitophorous vacuole, in which it rapidly proliferates to produce additional infective tachyzoites. Notably, T. gondii is capable of invading a wide variety of host cells in vitro, suggesting that it expresses ligands that recognize receptors commonly expressed on the surface of vertebrate cells. Consistent with this idea, recent studies suggest that T. gondii uses ubiquitously expressed glycosaminoglycans as one of its receptors for invasion of host cells (12).

Although ligands used by T. gondii to attach to host cells have yet to be characterized in detail, recent studies suggest that some adhesins may be released from apical secretory organelles called micronemes. For example, MIC1 possesses two degenerate adhesive sequences related to a domain of thrombospondin, and it is capable of binding host cells in vitro (13). Similarly, the 115-kDa antigen MIC2 harbors several thrombospondin-like repeat sequences (M-domain) and an integrin-like adhesive domain (I-domain) (14). One or both of these adhesive domains may be responsible for the host cell binding activity recently demonstrated for MIC2 (15). MIC2 is mobilized from the micronemes to occupy the parasite apical surface at the time of attachment to host cells (16). Thereafter, MIC2 relocates to the posterior end of the tachyzoite as the parasite penetrates into the host cell (15). Collectively, these findings suggest that while on the cell surface, MIC2 participates in adhesion. Consistent with this model, a homologue of MIC2 expressed by Plasmodium sporozoites is essential for invasion of host cells (17).

Although we have previously shown that MIC2 is shed into the culture medium as several similarly sized species (14, 18), the molecular details of this presumed proteolytic processing remain obscure. Here we show that two distinct parasite-derived proteases, denoted MPP1 and MPP2, process MIC2 at its C and N termini, respectively. We show here that MPP1 cleaves MIC2 proximal to its transmembrane anchor, releasing the adhesive domains of MIC2 into the culture medium, whereas MPP2 functions by trimming the N terminus of MIC2 down to the beginning of the integrin-like adhesive domain.

EXPERIMENTAL PROCEDURES

Cell Culture—T. gondii strain 2F was propagated in human foreskin fibroblast (HFF)§ cells as described (10). Tachyzoites were harvested from lysed out HFF cells after a 2-day passage by twice passing them through a 20-gauge needle followed by filtration through a 3-μm pore size membrane to remove host cell debris. Tachyzoites were washed twice by centrifugation (1000 × g, 10 min, room temperature) in invasion medium (Dulbecco’s modified Eagle’s medium, 3% fetal bovine serum, and 20 mM HEPES, pH 7.5) prior to use in experiments.

Received for publication, September 1, 1999, and in revised form, February 21, 2000

This paper is available online at http://www.jbc.org

¶ To whom correspondence should be addressed: Dept. of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health, 615 N. Wolfe St., Baltimore, MD 21205. Tel.: 410-614-5592; Fax: 410-955-0105; E-mail: vcarruth@jhsph.edu.

† This work was supported by the Johns Hopkins University School of Hygiene and Public Health (to V. B. C.) and by National Institutes of Health Grant AI36034 (to L. D. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Molecular Microbiology and Immunology, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205. Tel.: 410-614-5592; Fax: 410-955-0105; E-mail: vcarruth@jhsph.edu.

§ These abbreviations used are: HFF, human foreskin fibroblast; PAGE, polyacrylamide gel electrophoresis; bis-Tris propane, 1,3-bis(tris(hydroxymethyl)methylamino)propane; CyD, cyclohexalasin D; TLCK, 1-1-chloro-3-[4-tosylamido]-7-amino-2-heptanone HCl; ALLN, N-acetyl-Leu-Leu-norleucinal; ALLM, N-acetyl-Leu-Leu-methioninal; mAb, monoclonal antibody; ESA, excretory secretory antigen.

1 V. B. Carruthers and L. D. Sibley, unpublished data.

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**Preparation of Recombinant Proteins and Antisera**—Preparation of recombinant MIC2 (ectodomain) has been described previously (14). DNA encoding the C-terminal domain of MIC2 (residues 727–769) was polymerase chain reaction-amplified (94 °C for 5 min and then 20 cycles of 94 °C for 40 s, 65 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 7 min) from a tachyzoite cDNA library constructed using KlenTaq polymerase (Invitrogen) and primers MICW.721.F (5'-AGTT ACCACTACTATTGAGCT-3') and MIC2.726.R (5'-GATCCTCGAG CTACTCCATCCACATCCT-3'). The resulting polymerase chain reaction product was gel-purified and digested with SacI (which cleaved a natural restriction site present at nucleotide 2182 relative to the first nucleotide of the initiator methionine) and XhoI (which cleaved at a site engineered into MICW.2306.R). The restricted polymerase chain reaction product was then gel-purified again and ligated into SacI/XhoI-digested and gel-purified pScreen1b (Novagen). This resulted in a translational fusion of the T7 phage gene 10 product (encoded by pScreen1b) to the C-terminal domain of MIC2. The ligature mixture was electrotransformed into Escherichia coli strain XL1-Blue, and the resulting transformants were screened for inserts. A randomly chosen clone harbouring the C-terminal domain insertion insert was transferred to Escherichia coli strain BL21 for expression. Gene 10/C-terminal domain fusion protein was purified by nickel chelation chromatography using a His-Bind column (Novagen) according to the manufacturer's instructions. Gene 10 protein (expressed by pScreen1b) was also purified in the same manner.

**Surface Biostimulation**—Freshly harvested tachyzoites (~5 × 10^5) were resuspended in invasion medium containing 1% cytD and used to infect monolayers of HFFs by incubation for 30 min at 37 °C with 7% CO_2~/. Following infection, monolayers were washed with phosphate-buffered saline containing 1 mM CaCl_2 and 1 mM MgCl_2 at room temperature and treated with Me_2SO (negative control) or 50 μg/ml sulfo-succinimidobiotin (Pierce) in Me_2SO for 30 min at room temperature. Monolayers were washed and solubilized (1 h, 0 °C) in radioimmune precipitation assay buffer (50 mM Tris–HCl, pH 7.4, 1% Triton X-100, 0.5% sodium deoxycholate, 0.2% SDS, 100 mM NaCl, and 5 mM EDTA (sodium salt)) supplemented with 10 μg/ml RNase A, 20 μg/ml DNase I, and protease inhibitors (see above). Insoluble material was removed by centrifugation (13,000 × g, 15 min, 4 °C). After adjustment to pH 8.0 with 1 M NaOH, streptavidin–agarose (Sigma) was added (50% slurry in radioimmune precipitation assay buffer) and incubated for 1 h at room temperature with agitation. Streptavidin–agarose beads were pelleted by centrifugation (1000 × g, 1 min, room temperature), and proteins in the supernatant (SA-S) were acetone-precipitated and solubilized in SDS-PAGE sample buffer. Streptavidin–agarose beads were washed with radioimmune precipitation assay buffer, and proteins in the pellet (SA-P) were solubilized in SDS-PAGE sample buffer. Samples were aliquoted and stored at −20 °C.

**RESULTS AND DISCUSSION**

MIC2 Is Secreted without Its C-terminal Domain—We have previously shown that MIC2 is released into the culture medium from extracellular tachyzoites as a secreted/excreted antigen (ESA) (14, 18). The released forms of MIC2 (MIC295–100) were 15–20 kDa smaller than cellular MIC2 (MIC2115) when analyzed by SDS-PAGE, suggesting that the released forms are proteolytically processed. To examine this further, we used a mAb (6D10) specific to the M-domain of MIC2 (Fig. 1A) and a mouse anti-C-terminal domain antibody (Mac-dom) to determine if they could recognize the released forms of MIC2 (MIC295–100). The results (Fig. 1B) indicate that although mAb 6D10 recognized both the cellular and released forms (MIC2115 and MIC295–100, respectively), Mac-dom recognized only the cellular form (MIC2115). These results imply that the released
forms (MIC2\textsuperscript{95–100}) were produced by proteolytic removal of the C-terminal domain from the cellular form (MIC2\textsuperscript{115}), and that this event was coincident with release of MIC2\textsuperscript{95–100} into the supernatant (ESA). These experiments were carried out in the absence of host cells, suggesting that a parasite-derived protease was responsible for generating the released forms (MIC2\textsuperscript{95–100}). We named this protease MPP1 (microneme protein protease 1). The 15–20-kDa size reduction we observed for MIC2 after release is also consistent with cleavage occurring just N-terminal to the transmembrane sequence. Although the released forms (MIC2\textsuperscript{95–100}) were easily detectable in the ESA fraction, we failed to detect the C-terminal peptide either in the parasites or in the ESA fraction, even after long exposures. We also failed to detect the C-terminal peptide after briefly treating parasites with highly effective stimulators of microneme secretion, including A23187 (18) and ethanol (21) (data not shown). These results suggest that the C-terminal peptide is rapidly degraded after removal from the cellular form (MIC2\textsuperscript{115}).

The C-terminal Domain Is Associated with MIC2 during Parasite Invasion—In a previous study, we showed that MIC2 is secreted onto the apical surface of the parasite during attachment, and then it redistributes to the posterior end as the parasite penetrates into the host cell (15). Having demonstrated that the released forms of MIC2 (MIC2\textsuperscript{95–100}) do not possess the C-terminal domain, we wanted to determine whether surface MIC2 retains the C-terminal domain. To do this, we used immunofluorescence microscopy and antibodies specific to the extracellular adhesive domains (RorM2) and the C-terminal domain (M\textalpha C-dom). In the presence of saponin, both antibodies exclusively stained the apical region of extracellular parasites (Fig. 2, first row), indicating that they spe-
ingly, full-length MIC2 (MIC2115) readily bound to host cells, 
bind to host cell receptors (15). Interes-
the junction is thought to be the site of parasite ligand and host 
membranes formed at the time of apical attachment. The moving 
a zone of tight apposition of the parasite and host cell mem-
brane (14), these results suggest that MIC2 is a trans-
secretion is significantly up-regulated (16), and an increased 
secreting proteins into the supernatant (SA-S). Although we expected to 
detect the full-length cellular form of MIC2 (MIC2115) on the 
parasite surface, surprisingly little of this form 
was detected. Instead, a species migrating at 110 kDa 
(MIC2110) was apparent (Fig. 3B). This Δ5-kDa reduction in 
apparent molecular mass was not due to biotin labeling be-
cause other labeled parasite proteins (e.g. SAG1) did not ex-
hit a change in mobility. The sharp appearance and slower 
mobility of the MIC2110 band clearly distinguished it from the 
released forms (MIC295–100) detected in an ESA preparation. 
Also, unlike the released forms (MIC295–100), surface MIC2 
(MIC2110) possessed at least some portion of the C-terminal 
domain, based on reactivity with MoC-dom (Fig. 3B). It is 
possible that the surface form (MIC2110) was generated by 
limited proteolysis of the C-terminal domain, leaving at least 
one epitope recognized by MoC-dom. However, since removal of 
~5 kDa from the C terminus of the cellular form (MIC2115) 
would likely eliminate the entire C-terminal domain, these
results suggest that MIC2 is instead processed at its N terminus while on the parasite surface. As controls for labeling and partitioning, we probed Western blots with antibodies to SAG1, a well characterized surface antigen, and actin, a cytosolic marker. As expected, SAG1 was mainly in SA-P, whereas parasite actin was exclusively in SA-S (Fig. 3B). Similarly, unlabelled cellular MIC2 (MIC2115) from micronemal stores was detected in SA-S (Fig. 3B).

A Neutral or Alkaline Protease Sensitive to a Subset of Serine and Cysteine Protease Inhibitors Modifies MIC2—Since studying proteolytic processing of surface MIC2 is limited by the low amounts of material, we decided to further characterize the protease(s) that act upon MIC2 by monitoring the proteolytic products present in ESA (i.e. MIC295–100). To examine the sensitivity of the MIC2 protease(s) to protease inhibitors, we treated extracellular tachyzoites with inhibitors effective against each class of protease. As shown in Fig. 4A, proteolytic processing of MIC2 was affected by a subset of protease inhibitors, including chymostatin and two calpain inhibitors, ALLN and ALLM. These compounds inhibited the formation of the smallest species of MIC2 (MIC295). Although they partially inhibited microneme secretion (based on detection of a control unprocessed microneme protein, H4 (24)), chymostatin, ALLN, and ALLM did not substantially affect the release of MIC2 from the surface of the parasite, suggesting that they inhibit a protease that is distinct from MPP1. Thus, we will hereafter call the MIC295-forming protease MPP2. Although calpain-like proteases typically require calcium for high level activity, chelating calcium with EGTA had little or no effect on the formation of MIC295. Furthermore, calpain proteases are sensitive to leupeptin and E-64 (25), which had no effect on MPP2 activity. Thus, despite the effectiveness of calpain inhibitors, these observations suggest that MPP2 may not be a calpain-like enzyme. Rather, based on its inhibition by chymostatin, MPP2 may be a chymotrypsin-like serine protease.

Several other protease inhibitors affected the amount of MIC2 in ESA by inducing parasite lysis or by affecting microneme secretion directly. For example, EDTA and 1,10-phenanthroline produced partial lysis of parasites based on detection of a control cytoplasmic protein, β-galactosidase. Despite inducing some parasite lysis, the main effect of 1,10-phenanthroline was an inhibition of microneme secretion based on reduced abundance of MIC2 and H4 in ESA. Independently, 1,10-phenanthroline was observed to inhibit the secretion of MIC2 and MIC4 by extracellular parasites.3 The highest concentration of dichloroisocoumarin tested (50 mM) also inhibited microneme secretion, whereas lower concentrations had an inducing effect. Notably, high concentrations of dichloroisocoumarin were recently found to inhibit tachyzoite invasion of host cells (6). Since microneme secretion is likely required for invasion of host cells (15), our current results suggest that the inhibitory effects of dichloroisocoumarin on parasite invasion may be related to an inhibition of microneme secretion rather

3 J. L. Lovett and L. D. Sibley, unpublished data.

Fig. 3. MIC2 is proteolytically modified on the surface of tachyzoites apically attached to host cells. A, phase-contrast and immunofluorescence images of a tachyzoite apically attached to an HFF host cell. Tachyzoites were pretreated with CytD to prevent penetration into host cells. Infected monolayers were fixed, and MIC2 was detected by staining with RorM2. Arrowsheads indicate the point of apical attachment to the host cell. Bar = 2 μm. B, Western blots of tachyzoite lysate (RH; 2 × 10^6 cell equivalents), streptavidin-agarose (SA) supernatant (S) and pellet (P), and culture supernatant (ESA). Surface antigens from CytD-arrested, apically attached tachyzoites were labeled with sulfoconjugated biotin as described under “Experimental Procedures.” Lysates of labeled infected monolayers were incubated with streptavidin-agarose, separated into supernatant and pellet fractions, and Western-blotted with the antibodies shown to the left. The integrity of the tachyzoite plasma membrane was tested by detection of the major surface antigen SAG1 in SA-P and of parasite actin in SA-P. Host cell actin, which migrated just below parasite actin, was detected in both SA-S and SA-P, indicating some permeabilization of host cells. (A similar cross-reaction of rabbit anti-Tg actin antibody has been observed previously (10)).
than an effect of proteolysis. On the other hand, we found that 4-(2-aminoethyl)benzenesulfonyl)/fluoride hydrochloride actually stimulated microneme secretion, yet it markedly inhibited tachyzoite invasion (6), suggesting that a serine protease(s) activity (unrelated to MIC2 processing) may indeed be required for efficient invasion of host cells.

In an experiment to determine the pH optimum for proteolytic processing of MIC2, we found that MPP2 was inactive below pH 6.25 (Fig. 4B). MPP2 activity also appeared to be inhibited at pH > 8.5, although the total amount of released MIC2 also declined above this pH. These results suggest that MPP2 is likely a neutral protease.

CytD Enhances Proteolytic Processing of MIC2—Previously, we demonstrated that the posterior re-localization of MIC2 occurs in the absence of host cells and is blocked by CytD (18), suggesting that MIC2 is associated with the actin cytoskeleton of the parasite. To determine if CytD affects MIC2 processing, we preincubated extracellular tachyzoites with CytD before preparing an ESA fraction from the treated parasites. As shown in Fig. 5, CytD treatment resulted in preferential accumulation of the smallest released form of MIC2 (MIC295) in the ESA fraction. CytD did not directly alter migration of MIC2 on SDS-polyacrylamide gels since addition of CytD to ESAs prepared from untreated tachyzoites failed to produce the same effect (data not shown). A possible explanation for these results is that MPP2 may be restricted to the apical surface of the tachyzoite and that CytD treatment traps surface MIC2 in the same vicinity, thereby facilitating processing before release into the supernatant.

MIC2 Is Proteolytically Processed at Its N Terminus—As described above, two species of MIC2 (MIC2115 and MIC2110) were detectable on the surface of apically attached parasites. Also, since MIC2110 has an intact C terminus, it is likely the product of N-terminal proteolytic processing of MIC2115. Since the size differential between the surface forms of MIC2 (~5 kDa) is the same as that between the secreted forms (MIC2100 and MIC295), we hypothesized that MIC295 is also a product of N-terminal processing. To test this hypothesis, we determined the N-terminal sequences of MIC2115 and the secreted forms of MIC2 using large-scale ESA preparations and a subcellular fraction of tachyzoites that is enriched in micronemal antigens (Fig. 6A). Unambiguous sequences were obtained for each species of MIC2, and these were found to match sequences in the N-terminal region of MIC2 (Fig. 6B) (14). An 8-residue N-terminal sequence (SIVDALRK) from cellular MIC2 (MIC2115) matched a sequence adjacent to the predicted secretory leader peptide, indicating that cellular MIC2 is not secondarily processed during transit through the secretory pathway or storage in micronemes. These data are consistent with earlier data from pulse-chase metabolic labeling experiments showing that MIC2 does not change in size for at least 1 h after it is synthesized (26). The same N-terminal sequence (SIVDALRK) was also obtained from the largest released form (MIC295), enriched by ALLN treatment, confirming that this form corresponds to MIC2100 minus the C-terminal domain. The smallest released form (MIC295, CytD-enriched) yielded an N-terminal sequence (SIVDALRK) that began 38 amino acids downstream of the C-terminus of cellular MIC2 (MIC2110). A mixture of sequences (one primary and one secondary) was obtained from the species of MIC2 present in the 95–98-kDa
region of ESA prepared in the absence of drug. No obvious tertiary or quaternary sequences were observed during analysis of the 95–98-kDa species, suggesting that two incompletely processed species predominated. Since their production was inhibited by ALLN, these species (MIC2<sup>95–98</sup>) are likely the result of N-terminal processing by MPP2. Taken together, these results suggest that MPP2 cleaves MIC2 at several sites in its N terminus, culminating in the production of MIC2<sup>95</sup>, which is the fully processed species. The functional significance of MPP2 processing of MIC2 is unclear since only a fraction of the secreted MIC2 is N-terminally processed. Furthermore, chymostatin or ALLN failed to significantly reduce tachyzoite invasion of host cells in vitro (data not shown). Further experiments will be required to determine whether N-terminal processing of MIC2 has a subliminal effect on invasion, perhaps by increasing the affinity of MIC2 for receptors or by altering its interaction with other parasite proteins.

We demonstrate here that MIC2 is modified by two parasite-derived proteases, MPP1 and MPP2, following its secretion from the micronemes. MPP1 and MPP2 are likely active on the surface of the parasite based on the following observations. 1) In tachyzoite lysates, MIC2 is a single species of 115 kDa, suggesting that MIC2 is not proteolytically modified during storage in micronemes. 2) On the surface of apically attached tachyzoites, N-terminal truncation of MIC2 reduces its size by ~5 kDa, which is consistent with modification by MPP2. 3) Kinetic analysis (data not shown) of MIC2 species in ESA suggests that MPP2 does not act upon MIC2 after it is released from the parasite. 4) The C-terminal domain of MIC2 is not detectable on the released forms of MIC2 (MIC2<sup>95–100</sup>), suggesting that MPP1 acts upon MIC2 coincident with its release from the tachyzoite.

Based on the above evidence, we constructed a model that depicts the most likely genesis of each of the MIC2 species observed (Fig. 7). We propose that cellular MIC2 (MIC2<sup>115</sup>) is mobilized from the micronemes to the tachyzoite surface membrane, where it experiences one of two alternative fates. If cellular MIC2 immediately encounters MPP2, MPP2 cleaves its N terminus to generate the predominant surface form of MIC2 (MIC2<sup>110</sup>). Subsequent C-terminal cleavage of surface MIC2 by MPP1 then generates the smallest released form of MIC2 (MIC2<sup>95</sup>). Alternatively, if cellular MIC2 encounters MPP1 first, MPP1 cleaves the C terminus of MIC2, generating the largest released form of MIC2 (MIC2<sup>100</sup>). However, the presence of multiple species of MIC2 (MIC2<sup>95–100</sup>) in the culture supernatants indicates that N-terminal processing by MPP2 is often incomplete at the time of C-terminal cleavage by MPP1. We observed that CytD, which prevents the posterior re-localization of MIC2 (18), enhanced N-terminal processing of MIC2.

**Fig. 6.** Proteolytic processing of the N terminus of MIC2. A, shown is a Coomassie Blue-stained SDS-polyacrylamide gel of an enriched microneme fraction (Mic. Prep.) and concentrated ESAs prepared from tachyzoites treated with Me<sub>2</sub>SO (DMSO; solvent control), ALLN, or CytD. In parallel, equivalent samples were transferred to polyvinylidene difluoride membranes, and bands a–d were excised and subjected to N-terminal sequence analysis by Edman degradation. B, N-terminal sequences are indicated by solid lines under the deduced amino acid sequence of MIC2. Two sequences (b<sup>1</sup> and b<sup>2</sup>) were detected in band b (encompassing the 95–98-kDa size range). The signal peptidase cleavage site is indicated with a vertical arrow, and the secretory leader peptide is demarcated with a dashed line. The start of the I-domain (i-dom.) is indicated with a horizontal arrow, and the first 7 residues of the I-domain are in boldface. C, depicted is a schematic of the processed forms of MIC2. a–d correspond to the species shown in A. M-dom, M-domain; TM, transmembrane domain; C-dom, C-terminal domain.

**Fig. 7.** Schematic model of proteolytic processing events involving MIC2 on the surface of the tachyzoite. Evidence described herein suggests that cellular MIC2 (MIC2<sup>115</sup>; second from the right) can experience one of two fates. In one fate (depicted on the left), MIC2<sup>115</sup> immediately encounters MPP2, which cleaves the N terminus (for clarity, only one of three or more cleavage sites is shown), producing the major surface form, MIC2<sup>110</sup>. Subsequently, MIC2<sup>110</sup> encounters MPP1, which cleaves the C terminus, producing the smallest released form (MIC2<sup>95</sup>). In the alternative fate (depicted on the right), MIC2<sup>115</sup> first encounters MPP1, which cleaves the C terminus, yielding the smallest released form (MIC2<sup>95</sup>). Domains of MIC2 are represented as follows: I-domain, green sphere; M-domain, blue cylinder; C-terminal domain, green U-shape.
by MPP2. This indicates that MPP2 may be restricted to the apical surface of the parasite since CytD treatment would effectively trap MIC2 with MPP2, thereby increasing the likelihood of N-terminal processing. Although it remains uncertain where MPP1 is located, the observation that surface MIC2 retains its C-terminal domain as it translocates rearward during parasite invasion suggests that MPP1 may cleave MIC2 on the posterior surface of the parasite. However, MPP1 may not be strictly limited to the posterior surface since CytD treatment does not affect MPP1-mediated release of MIC2 from the parasite in the absence of host cells (e.g. Fig. 5).

Together with this work, earlier studies suggest that proteolytic processing may be a common mechanism for releasing adhesive proteins from invasive apicomplexan parasites. For example, the C-terminal cytoplasmic domain of Plasmodium knowlesi DBP-1 (Duffy-binding protein-1) is proteolytically removed upon release of the DBP-1 ectodomains into the culture medium (27). Also, the 235-kDa erythrocyte-binding antigen of Plasmodium yoelii is a transmembrane protein that is proteolytically released upon release into the culture supernatant (28). Finally, MSP-1 (major surface protein-1) of Plasmodium falciparum is proteolytically released from the surface of merozoites during invasion of erythrocytes (29). Since adhesive proteins play a key role in attachment and invasion of target host cells, inhibition of these processing events may therefore inhibit parasite invasion, offering a potential target for therapeutic intervention. Future studies involving the molecular characterization of parasite-derived proteases such as MPP1 and MPP2 should shed light upon the precise role of proteases in invasion of host cells by T. gondii.

Acknowledgments—We thank Amy Crawford and Gale Sherman for excellent technical assistance. We also gratefully acknowledge Drs. Dan Goldberg, Gary Ward, Kami Kim, Michael Blackman, and Eleanor Hoff for critically reading this manuscript prior to submission. Additionally, we thank Drs. John Boothroyd, Alan Johnson, and Joshua Sanes for generously providing antibodies used in this study and Dr. Kami Kim for many helpful suggestions.

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Vern B. Carruthers, Gale D. Sherman and L. David Sibley

*J. Biol. Chem.* 2000, 275:14346-14353.
doi: 10.1074/jbc.275.19.14346

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