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# Developmentally regulated expression by *Trypanosoma cruzi* of molecules that accelerate the decay of complement C3 convertases

**(protozoan parasite/complement-mediated cytolysis)**

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**ABSTRACT** **We recently showed that culture-derived metacyclic trypomastigotes (CMT), but not epimastigotes (Epi), of the Miranda 88 strain of *Trypanosoma cruzi* evade lysis by the human alternative complement pathway because of inefficient binding of factor B to complement component C3b on the parasite surface. These results suggested that CMT and tissue-culture-derived trypomastigotes (TCT), which also activate the alternative pathway poorly, might produce a molecule capable of interfering with factor B binding to C3b. We now demonstrate that CMT and TCT lysates, as well as molecules spontaneously shed from CMT and TCT but not Epi, accelerate decay of $^{125}$I-labeled factor Bb from the alternative-pathway C3 convertase (C3bBb) assembled on zymosan or Epi and also accelerate decay of the classical-pathway C3 convertase (C4b2a) on sheep erythrocytes. Parasites metabolically labeled with [$^{35}$S]methionine spontaneously shed a limited number of radioactive components ranging in molecular mass from 86 to 155 kDa for trypomastigotes and 25 to 80 kDa for Epi. Decay-accelerating activity within supernatants is inactivated by papain and is coeluted with $^{35}$S-containing polypeptides on FPLC anion-exchange chromatography, suggesting that the active constituents are protein molecules. Molecules with decay-accelerating activity may explain the developmentally regulated resistance to complement-mediated lysis in infective and vertebrate stages of the *T. cruzi* life cycle.**

*Trypanosoma cruzi*, the causative agent of Chagas disease, is a hemoflagellate protozoan that cycles between blood-feeding reduviid insects and various vertebrate hosts including humans. The epimastigote (Epi), which is the major insect stage of the parasite, is highly sensitive to lysis in normal human serum by the alternative complement pathway (ACP). In contrast, the infective metacyclic trypomastigote (which develops in the vector), the vertebrate-blood-form trypomastigote, and the respective culture-derived representatives of these two stages, the CMT (culture-generated metacyclic trypomastigote) and TCT (tissue-culture-derived trypomastigote), are highly resistant to ACP-mediated lysis.

We have been studying the developmentally regulated mechanisms responsible for evasion of the ACP by the infective and vertebrate trypomastigote stages of the *T. cruzi* life cycle. Our studies have shown that upon incubation in human serum, complement-resistant CMT or TCT bind 5–8 times less complement component C3 or C9 than complement-sensitive Epi (1, 2). Furthermore, whereas C3 bound to Epi is mainly in the hemolytically active C3b form, CMT and TCT bear predominantly the hemolytically inactive iC3b fragment. In CMT, this difference appears to be due to inefficient binding of factor B to C3b on the surface of the parasite, such that binding of factor H to C3b is favored instead, resulting in subsequent factor I-mediated cleavage of C3b to iC3b. As a possible explanation of these parasite stage-dependent regulatory events, we now report that both CMT and TCT produce factors that interfere with B binding to C3b, thus diminishing the formation of C3 convertase. We also show that CMT and TCT shed substances into culture medium that accelerate the intrinsic decay of the alternative- and classical-pathway C3 convertases (C3bBb and C4b2a). Thus, *T. cruzi* may evade complement lysis by elaborating biologically active molecules analogous in their function to decay-accelerating factor (DAF), a substance that is thought to prevent lysis of vertebrate cells by inhibiting autologous complement activation (3, 4).

## MATERIALS AND METHODS

**Buffers.** The following buffers were used: isotonic Veronal-buffered saline (VBS: 5 mM Veronal/150 mM NaCl, pH 7.4) containing human serum albumin at 1 mg/ml and 4 mM $MgCl_2$ (VBSA-Mg); VBS containing 0.1% gelatin and either 1 mM $NiCl_2$ (GVBS-Ni) or 4 mM $MgCl_2$ (GVBS-Mg); low-ionic-strength ($\mu$ = 0.060) VBS containing dextrose, gelatin, 1 mM $MgCl_2$, and 0.15 mM $CaCl_2$ (DGVBS++); Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine and 1 mg of human serum albumin (DMEM-A) or 100 $\mu$g of ovalbumin (DMEM-O) per ml.

***T. cruzi* Strains.** The Miranda 88 (M88) clone of *T. cruzi* was obtained from J. Dvorak (National Institutes of Health). Epi and CMT were obtained as described (1). TCT (>90% pure) were grown in primary bovine embryo skin and muscle (BESM) cells cultured in RPMI 1640 (GIBCO) containing 2% fetal bovine serum, 2 mM L-glutamine, 100 units of penicillin per ml, and 100 $\mu$g of streptomycin per ml. Cultures were incubated in 175-cm$^2$ culture flasks at 37°C in a 95% air/5% $CO_2$ humidified atmosphere as described (5).

**Purification and Radiolabeling of Complement Components.** Human C4, C2, C3, factor B, and factor D were purified as described (1, 2, 6). Purified factor H was kindly provided by T. Gaither (Bethesda, MD). Human factor I and guinea pig C1 were purchased from Cordis Laboratories (Miami, FL). C3 and factor B were labeled with Na$^{125}$I (New England Nuclear) as reported (2). A partially purified preparation of the C3b

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Abbreviations: Epi, epimastigote(s); CMT, culture-generated metacyclic trypomastigote(s); TCT, tissue culture-derived trypomastigote(s); BESM cells, bovine embryo skin and muscle cells; ACP, alternative complement pathway; DAF, decay-accelerating factor; C3, complement component C3; C3b and iC3b, hemolytically active and inactive fragments of C3; B, complement factor B; Bb, activated fragment of B; $^{125}$I-, $^{125}$I-labeled; C3bBb, alternative-pathway C3 convertase; C4b2a, classical-pathway C3 convertase; ZC3b, zymosan bearing C3b; ZC3bBb, ZC3b bearing Bb; ZC3b($^{125}$I-Bb), ZC3Bb containing Bb derived from $^{125}$I-labeled B; EAC14b2a, antibody-sensitized sheep erythrocytes bearing C1, C4b, and C2a; CR1, C3b receptor.

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receptor (CR1) and monoclonal antibody 1B4, which recognizes CR1, were prepared as described (7) and were kindly provided by J. O'Shea (Bethesda, MD).

**Preparation of Zymosan-C3b for Assay of Decay Acceleration.** Zymosan-C3b (ZC3b) was prepared by modifications of published procedures (8). Zymosan at 10 mg/ml in GVBS-Ni was incubated at 37°C for 90 min with C3 (1 mg/ml), factor B (200 μg/ml), factor D (2 μg/ml), and C3 nephritic factor (20 μg/ml; kindly provided by R. McClean, Johns Hopkins University). ZC3b was washed in GVBS-Ni, incubated with factor B (200 μg/ml) and factor D (2 μg/ml) for 20 min at 37°C, then washed and incubated with C3 (1 mg/ml) for 30 min at 37°C. This procedure was repeated twice.

**Assay for Acceleration of ZC3b($^{125}$I-Bb) Decay or Inhibition of ZC3b($^{125}$I-B) Formation.** This assay was adapted from the procedure of Pangburn *et al.* (8). For experiments in which decay of $^{125}$I-labeled Bb ($^{125}$I-Bb) from ZC3b($^{125}$I-Bb) was to be assessed, ZC3b($^{125}$I-Bb) was formed by incubation of ZC3b (1.0 mg) with $^{125}$I-B (30 μg) and factor D (2 μg) in GVBS-Ni for 7 min at 37°C. The ZC3b($^{125}$I-Bb) was washed twice in cold VBS/10 mM EDTA, 100-μg aliquots were mixed at 0°C with 350 μl of unknown or buffer, and the mixtures were warmed to 22°C. At various times, triplicate samples (50 μl) were added to cold VBS/EDTA (750 μl) at 0°C and centrifuged at 12,500 × *g* for 30 sec at 4°C. Radioactivity in the pellet was measured with a gamma scintillation counter to determine $^{125}$I-Bb remaining bound. The $t_{1/2}$ for $^{125}$I-Bb release was calculated by linear regression and the slopes of the regression lines were compared by a modified Student *t*-test. The percent acceleration of decay was calculated as ($t_{1/2}$ buffer/$t_{1/2}$ experimental) × 100.

To measure inhibition of ZC3b($^{125}$I-B) formation, 100-μg aliquots (50 μl) of ZC3b in GVBS-Mg were mixed at 0°C with 2 μg of $^{125}$I-B and 50 μl of unknown or buffer. Samples were incubated at 37°C for 3 min and processed as described above to determine $^{125}$I-B bound. Nonspecific binding of $^{125}$I-B (<5% of total bound) was assessed using tubes in which either a 200-fold excess of unlabeled factor B was added or in which zymosan not bearing C3b was used.

**Assay for Acceleration of EAC14b2a Decay.** This assay was adapted from the procedure of Nicholson-Weller *et al.* (3). Human C2 (10 sites per cell) was added to antibody-sensitized erythrocytes bearing complement components C1 and C4b (EAC14b) in DGVBS++ (9). Cells were incubated at 30°C for 10 min and then washed in ice-cold GVBS containing 10 mM EDTA. These EAC14b2a cells were aliquoted in 50-μl portions, mixed for 10 min at 0°C with 100 μl of either unknown or buffer at 0°C, and then warmed to 22°C. Aliquots were removed at various times for determination of residual C4b2a sites after addition of guinea pig serum diluted 1:10 in 10 mM EDTA (9).

**Assay for Cofactor Activity for Cleavage of C3b to iC3b.** We tested the capacity of substances present in *T. cruzi* supernatants to serve as cofactors for factor I-mediated cleavage of C3b to iC3b (10), using an assay (11) that measures the release of $^{125}$I-C3c from $^{125}$I-iC3b by trypsin. In brief, sheep erythrocytes bearing $^{125}$I-C3b were prepared, then incubated with factor I (150 ng/ml) and either buffer, various concentrations of unknown, or factor H (2.4 μg/ml or 0.24 μg/ml) for 10 min at 37°C. Cells were then treated with trypsin (10 μg/ml) for 3 min at 30°C, the reaction was stopped by addition of soybean trypsin inhibitor (50 μg/ml), and the amount of $^{125}$I-C3c released was determined by gamma scintillation counting. Only in the situation in which *T. cruzi* products contain cofactor activity for I-mediated cleavage of C3b to iC3b would C3c release be detected with trypsin treatment.

**Assay for Acceleration of $^{125}$I-Bb decay from EpiC3b($^{125}$I-Bb).** Epi bearing C3b were prepared as described (2) and were converted to EpiC3b($^{125}$I-Bb) by incubation of EpiC3b ($5 \times 10^7$) with $^{125}$I-B (50 μg) and factor D (1 μg) in VBSA-Mg for 5 min at 37°C. EpiC3b($^{125}$I-Bb) were washed twice in VBSA-Mg and aliquoted at 0°C into triplicate tubes to which was added 500 μl of buffer or unknown. Samples (200 μl) were collected at 0°C and then the mixtures were warmed to 22°C. After various times aliquots (200 μl) were centrifuged and pellets were subjected to gamma counting to determine residual $^{125}$I-Bb bound.

**Treatment of TCT Supernatants with Immobilized Papain.** TCT supernatants (100 μl) in phosphate-buffered saline (17 mM phosphate/150 mM NaCl) were incubated for 30 min at 37°C with 1, 5, or 25 μl (0.1, 0.5, or 2.5 units) of papain immobilized on agarose (Sigma). After incubation, supernatants were assayed for capacity to accelerate decay of EAC14b2a as described above.

**Metabolic Labeling of Epi, CMT, and TCT and Preparation of Parasite Lysates and Supernatants.** Epi, CMT, and TCT were intrinsically labeled with [$^{35}$S]methionine. Parasites were washed twice in DMEM-A without methionine and resuspended ($10^8$ parasites per ml) in the same buffer. [$^{35}$S]Methionine (New England Nuclear; 50 μCi/ml final concentration; 1 μCi = 37 kBq) was added, and the mixtures were incubated for 2 hr at 37°C. Cells were washed three times in DMEM and resuspended ($10^8$ per ml) in DMEM-O for collection of supernatant samples. The mixtures were incubated for an additional 4-hr period at 37°C in DMEM-O, and parasite pellets were collected by centrifugation at 12,500 × *g* for 5 min. Parasites remained >90% viable during the 4-hr incubation, as determined by assessment of motility and shape. Both supernatant and pellet were saved, after addition of *p*-nitrophenyl-*p'*-guanidinobenzoate (25 μM) (Sigma) and leupeptin (10 μg/ml) to the supernatant sample. Samples for NaDodSO$_4$/PAGE were prepared by solubilizing ≈5 × $10^6$ Epi, CMT, and TCT and 25 μl of their respective supernatants in NaDodSO$_4$/PAGE sample buffer. In each case, the entire sample was applied to the gel lane.

**Separation of CMT and TCT Supernatants by FPLC Anion-Exchange Chromatography.** Supernatants from [$^{35}$S]methionine-labeled CMT and TCT were dialyzed at 4°C versus 20 mM Tris/100 mM NaCl/1% betaine, pH 8.0, and then applied at a flow rate of 1.0 ml to a Mono Q HR5 column (Pharmacia) attached to a Beckman model 421 high-performance liquid chromatograph. Fractions of 0.5 ml were collected. A NaCl gradient from 100 mM to 1000 mM was developed over 20 min, followed by isocratic elution at 1.0 M NaCl. Samples were assayed for $A_{280}$, $^{35}$S counts, and capacity to accelerate decay of EAC14b2a, as described above.

**NaDodSO$_4$/PAGE and Fluorography.** Samples were electrophoresed in either 5–15% or 7.5% polyacrylamide gels with NaDodSO$_4$, using the buffer system of Laemmli (12) as reported earlier (2). Gels were impregnated with EN-$^3$HANCE (Dupont) and dried with a gel drier (Bio-Rad) for fluorography at −70°C with Cronex x-ray film and Du Pont Quanta III intensifying screens.

## RESULTS

**Identification of Decay-Accelerating Activity in CMT and TCT but not in Epi.** In initial experiments, we tested Epi, CMT, and TCT lysed by repetitive freezing and thawing or by detergent solubilization for capacity to accelerate decay of $^{125}$I-Bb from the alternative-pathway C3 convertase on zymosan, ZC3b($^{125}$I-Bb). Freeze/thaw lysates of CMT as well as whole, freeze/thaw, and detergent-solubilized TCT but not Epi accelerated decay of $^{125}$I-Bb in comparison to buffer alone (Table 1). Decay-accelerating activity in detergent-solubilized TCT was partially inhibited by protease inhibitors, suggesting that proteolytic cleavage of the ligands was occurring. As expected, CR1 had decay-accelerating

Table 1. Decay-accelerating activity in Epi, CMT, and TCT

| Sample | % acceleration of ZC3b($^{125}$I-Bb)decay |
|---|---|
| Epi | |
| Whole parasites | 5 ± 2 ($n$ = 3) |
| Freeze/thaw lysate | 3 ± 7 ($n$ = 4) |
| Detergent lysate | 0 ± 2 ($n$ = 2) |
| + EDTA, NPGB, and leupeptin | 0 ± 5 ($n$ = 2) |
| CMT freeze/thaw lysate | 40 ± 9 ($n$ = 2) |
| TCT | |
| Whole parasites | 32 ± 7 ($n$ = 3) |
| Freeze/thaw lysate | 42 ± 7 ($n$ = 3) |
| Detergent lysate | 81 ± 6 ($n$ = 2) |
| + EDTA, NPGB, and leupeptin | 53 ± 2 ($n$ = 2) |
| CR1 | 56 ($n$ = 1) |
| CR1 + anti-CR1 1B4 | 20 ($n$ = 1) |

Parasites ($10^6$) were either suspended in 500 μl of GVBS-Ni, or repetitively frozen and thawed and then suspended in 500 μl of GVBS-Ni, or solubilized for 2 hr at 4°C in 500 μl of GVBS-Ni containing 2% (vol/vol) Nonidet P-40. For some experiments, EDTA (10 mM), *p*-nitrophenyl-*p'*-guanidinobenzoate (NPGB, 50 μM), and leupeptin (10 μg/ml) were added to detergent lysates. Samples were tested for capacity to accelerate decay of $^{125}$I-Bb from ZC3b($^{125}$I-Bb). Values, calculated as described in *Materials and Methods*, represent mean ± SD for $n$ experiments.

activity, and this activity was partially blocked by the anti-CR1 monoclonal antibody 1B4.

**Determination of Decay-Accelerating Activity in Epi, CMT, and TCT supernatants.** Because of the complexity of the parasite lysates as well as the complicating protease activity in the lysates, we sought another source for decay-accelerating activity. Supernatants from CMT and TCT, but not from Epi, accelerated decay of $^{125}$I-Bb from the nickel-stabilized C3 convertase, ZC3b($^{125}$I-Bb) (Fig. 1). For TCT supernatants, this effect was dose-related. Supernatants collected from BESM cells, washed free of medium and incubated for 4 hr at 37°C, had no activity. Acceleration of decay by CMT and TCT supernatants was not due to proteolysis of ligands since (*i*) activity was not inhibited by protease inhibitors, which were routinely added to supernatant samples, and (*ii*) NaDodSO$_4$/PAGE analysis of released $^{125}$I-Bb and of bound $^{125}$I-C3b incubated for 2 hr at 37°C with TCT supernatants revealed no evidence for proteolytic cleavage (data not shown).

**Inhibition of ZC3b($^{125}$I-B) Formation by Epi, CMT, and TCT Supernatants.** We next examined whether supernatants from CMT, TCT, or Epi inhibited formation of magnesium-stabilized ZC3b($^{125}$I-B), since our previous experiments had shown that B bound inefficiently to C3b on CMT. Epi, CMT, and TCT supernatants inhibited ZC3b($^{125}$I-B) formation by 9%, 96%, and 79%, respectively. When supernatants were diluted 1:1 in buffer, these values changed to 8%, 48%, and 31%. This inhibition of ZC3b($^{125}$I-B) formation by CMT and TCT supernatants was not due to proteolysis of ligands, since NaDodSO$_4$/PAGE of ZC3b and $^{125}$I-B incubated for 60 min at 37°C with supernatants showed no cleavage fragments.

**Acceleration of EAC14b2a Decay.** We then tested whether Epi, CMT, and TCT supernatants would accelerate decay of the classical-pathway C3 convertase, C4b2a. CMT and TCT but not Epi supernatants accelerated decay of C4b2a (Fig. 2). In the case of TCT supernatant, this effect was dose-related. Supernatants from uninfected BESM cells had no activity (data not shown).

**Cofactor Activity for Cleavage of C3b to iC3b.** The capacity for CMT, TCT, and Epi supernatants to serve as cofactors for cleavage of C3b to iC3b by factor I was tested. No cofactor activity was demonstrated for CMT, TCT, or Epi supernatants when compared to buffer alone, in contrast to factor H, which had significant cofactor activity at low concentrations (Table 2).

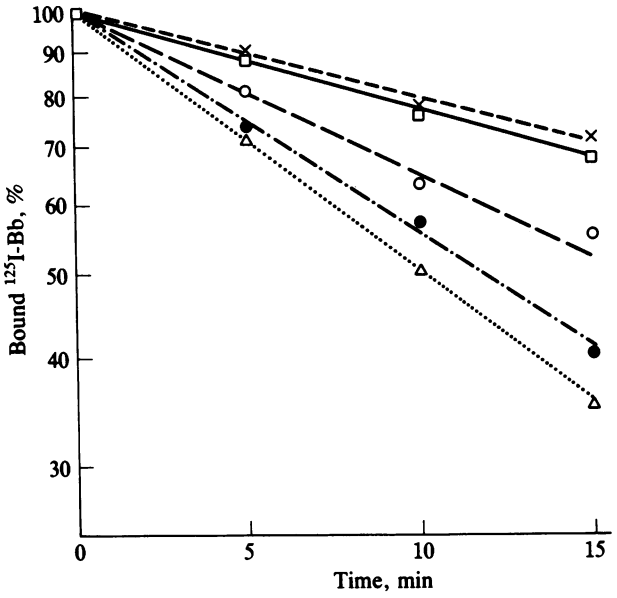


FIG. 1. Acceleration of alternative-pathway C3 convertase decay on zymosan by supernatant samples from Epi, CMT, and TCT. Supernatant samples shed from Epi, CMT, and TCT were collected after incubation of unlabeled parasites at $10^8$ per ml for 4 hr at 37°C in DMEM-O. Protease inhibitors (*p*-nitrophenyl-*p'*-guanidinobenzoate and leupeptin) were added, and supernatants were dialyzed extensively against GVBS-Ni. Neat or diluted supernatants were added to ZC3b($^{125}$I-Bb) and the rate of release of $^{125}$I-Bb was determined. Data shown are from a representative experiment. Each condition was repeated at least twice, and in general experiments were done 6–8 times. Slopes for CMT (△, $P < 0.001$), TCT (●, $P < 0.001$), and TCT (1:1) (○, $P < 0.01$) supernatants were significantly different from slopes for Epi supernatant (×) and buffer (□).

**Acceleration of $^{125}$I-Bb Decay from EpiC3b($^{125}$I-Bb).** To test decay-accelerating activity on a biologically relevant surface, we tested whether supernatants would accelerate decay of $^{125}$I-Bb from EpiC3b($^{125}$I-Bb). Both CMT and TCT supernatants substantially accelerated $^{125}$I-Bb decay from EpiC3b-($^{125}$I-Bb) (Fig. 3), in comparison to a minor effect of Epi supernatants.

**Treatment of TCT Supernatants with Immobilized Papain.** TCT supernatants were treated with immobilized papain and then were assayed for the capacity to accelerate decay of EAC14b2a. Samples treated with 0.1, 0.5, and 2.5 units of papain accelerated decay by 36%, 11%, and 8%, respectively, in comparison to 89% acceleration of decay by untreated supernatants and 6% acceleration by buffer incubated with immobilized papain. This result provides evidence that protein molecules within the TCT supernatant contain decay-accelerating activity.

**CMT and TCT Selectively Shed a Restricted Population of High Molecular Mass Molecules into the Supernatant.** The material released from $^{35}$S-labeled Epi, CMT, and TCT was compared with metabolically labeled whole parasites by NaDodSO$_4$/PAGE. Labeled parasites yielded a large number of bands ranging from <20 to >250 kDa (Fig. 4). However, when labeled parasites were incubated at 37°C in buffer for 4 hr, both CMT and TCT selectively and spontaneously shed labeled molecules ranging from 86 to 155 kDa but enriched in components of 86–98 kDa. Epi also shed labeled components, but these were generally smaller than those released by trypomastigotes. Minimal shedding of $^{35}$S-labeled constituents from uninfected BESM cells was observed during a 4-hr incubation at 37°C (data not shown).

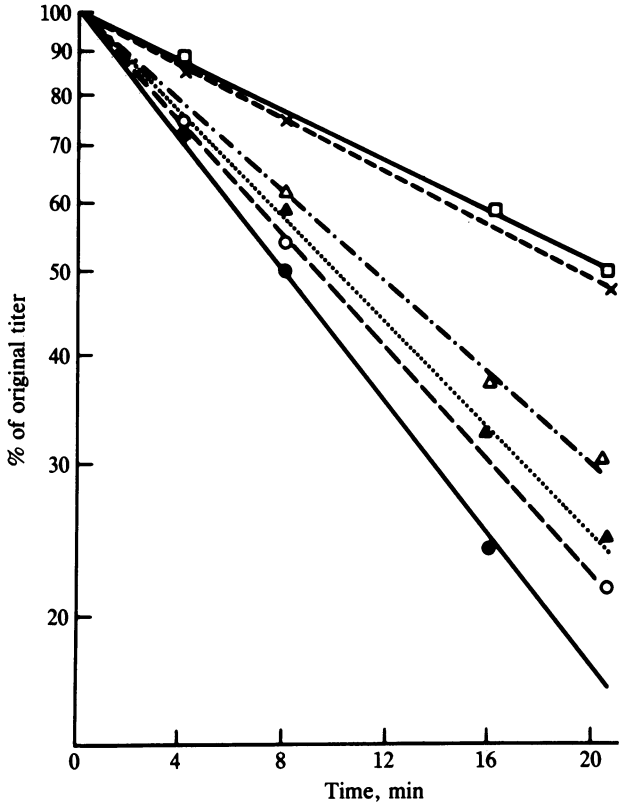


FIG. 2. Acceleration of classical-pathway C3 convertase decay on sheep erythrocytes by supernatant samples from Epi, CMT, and TCT. Sheep erythrocytes bearing C14b2a were incubated at 22°C with neat or diluted supernatant samples from Epi, CMT, or TCT collected as described in the legend to Fig. 1 and dialyzed against DGVBS++. The rate of decay of the C14b2a complex was determined by hemolytic assay. Data are from a representative experiment that was repeated three times. CMT (▲, $P < 0.001$), TCT (●, $P < 0.001$), and TCT (1:1) (○, $P < 0.005$) supernatants significantly accelerated decay in comparison to Epi supernatant (×) and buffer (□). △, TCT supernatant diluted 1:3.

**Fractionation of CMT Supernatant by FPLC Anion-Exchange Chromatography.** We next fractionated $^{35}S$-labeled CMT supernatant by FPLC ion-exchange chromatography on a Mono Q column (Fig. 5). CMT supernatant gave one major and several minor peaks of $^{35}S$ counts. A single peak of protein comigrated with ovalbumin, which had been added to the supernatant after collection. Decay-accelerating activity comigrated with the major $^{35}S$ peak, although incomplete

Table 2. Cofactor activity of Epi, CMT, and TCT supernatants for cleavage of C3b to iC3b

| Sample | % release of $^{125}I$-C3c by trypsin* |
|---|---|
| Epi supernatant | 1 ± 2 |
| CMT supernatant | 3 ± 3 |
| TCT supernatant | 6 ± 5 |
| Concentrated 3-fold | 1 ± 1 |
| Diluted 1:1 | 4 ± 4 |
| Diluted 1:5 | 9 ± 9 |
| Diluted 1:25 | 6 ± 10 |
| Factor H | |
| 2.4 μg/ml | 83 ± 6 |
| 0.24 μg/ml | 66 ± 2 |

Parasite supernatants or factor H were incubated with EAC14b2a-($^{125}I$-C3b) and factor I. Cells were washed and then incubated with trypsin (10 μg/ml), and the release of $^{125}I$-C3c by trypsin was determined. Only if supernatant samples contain cofactor activity for I-mediated cleavage of C3b to iC3b will C3c release be detected with trypsin treatment.

*% release in comparison to buffer; results are mean ± SD for 3 experiments.

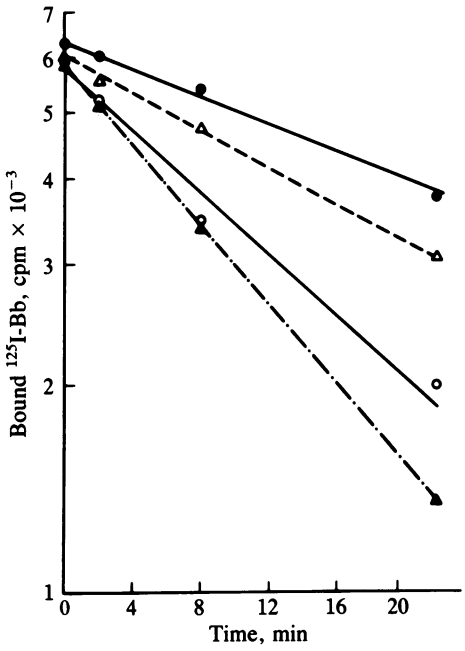


FIG. 3. Acceleration of alternative-pathway C3 convertase decay on Epi by supernatant samples from Epi, CMT, and TCT. EpiC3b-($^{125}I$-Bb) were incubated at 22°C with buffer (●) or with supernatant samples from Epi (△), CMT (○), or TCT (▲) collected as described in the legend to Fig. 1 and dialyzed against GVBS-Mg. The rate of release of $^{125}I$-Bb was measured. Data shown are the mean for two experiments. The curve for CMT supernatant was significantly different ($P < 0.01$) from the curves for Epi supernatant and buffer but not from the curve for TCT supernatant. The $t_{1/2}$ values are 29.2 min (buffer), 23.7 min (Epi), 12.8 min (CMT), and 10.2 min (TCT).

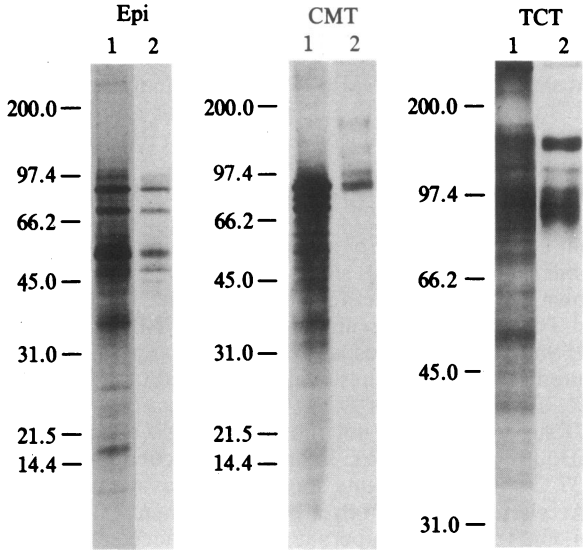


FIG. 4. Analysis of labeled molecules shed from Epi, CMT, and TCT. Epi, CMT, and TCT were metabolically labeled by incubation of purified parasites in [$^{35}S$]methionine in DMEM-A without methionine. Parasite pellets (lanes 1) and components spontaneously shed during incubation at 37°C for 4 hr in DMEM (lanes 2) were analyzed by $NaDodSO_4$/PAGE followed by fluorography. Epi and CMT samples were electrophoresed in 5–15% gradient polyacrylamide gels; TCT samples were analyzed in a 7.5% polyacrylamide gel. Positions and sizes (kDa) of standard proteins are shown to the left of each fluorograph.

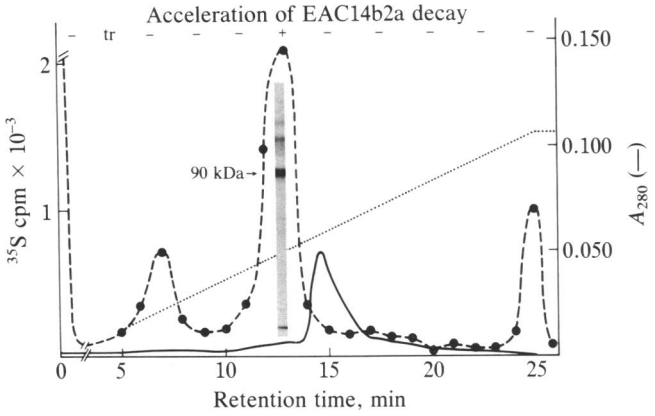


FIG. 5. FPLC anion-exchange chromatography of supernatant from [$^{35}$S]methionine-labeled CMT. Supernatant was applied at a flow rate of 1.0 ml/min to a Mono Q column in 20 mM Tris/100 mM NaCl/1% betaine, pH 8.0. A NaCl gradient from 100 mM to 1000 mM was developed (·····) and column fractions were analyzed for $A_{280}$ (—), $^{35}$S counts (●), capacity to accelerate decay of EAC14b2 (+, −), and NaDodSO$_4$/PAGE profile (*Inset*; arrow indicates 90-kDa band).

separation of the broad 86- to 98-kDa band from higher molecular mass constituents was achieved in this pool. This result provides further evidence that $^{35}$S-labeled proteins within CMT supernatant have decay-accelerating activity.

## DISCUSSION

We have shown that CMT and TCT of *T. cruzi* produce factors that interfere with formation and accelerate decay of the alternative- and classical-pathway C3 convertases. These molecules, which are spontaneously shed by CMT and TCT but not Epi during incubation in culture, may be responsible for the developmentally based resistance of trypomastigotes to direct complement killing. These released factors accelerate decay of the C3 convertase assembled on Epi (Fig. 3), the most biologically relevant surface in this system.

The functional properties of the *T. cruzi* decay-accelerating molecules are similar to those of several known regulatory molecules of the human complement cascade. The molecules from *T. cruzi* accelerate decay of both the classical and alternative C3 convertases and inhibit formation of the ACP C3 convertase but lack cofactor activity for cleavage of C3b to iC3b by factor I. Both CMT and TCT supernatants have decay-accelerating activity for extrinsic targets (13) such as zymosan or Epi. Although the functional activity of the CMT and TCT supernatants is therefore somewhat more analogous to DAF (3, 4, 8, 13) than to other complement regulatory molecules (factor H, C4-binding protein, CR1), further comparison must await identification and characterization of the responsible molecule(s) produced by CMT and TCT.

The CMT and TCT supernatant materials in which we detected decay-accelerating activity were found to contain a highly restricted set of methionine-labeled polypeptides ranging in size from 86 to 155 kDa, but enriched for proteins of 86–98 kDa that were absent from Epi supernatants (Fig. 4). We demonstrated previously (14) that Pronase treatment, which renders CMT susceptible to lysis by normal human serum and enhances binding of factor B to C3b on parasites of this stage, removed a set of molecules of similar size (90 and 115 kDa) from the surface of labeled organisms. Although we currently have no evidence that CMT supernatant molecules with decay-accelerating activity are identical, we have found that the functional activity in CMT supernatant is coeluted with $^{35}$S-containing molecules when the supernatant is separated by FPLC ion-exchange chromatography (Fig. 5). Furthermore, papain treatment of TCT supernatants removes decay-accelerating activity, suggesting that the responsible molecules are proteins. Recently, Kipnis *et al.* (15) showed that supernatants from Y-strain TCT that have been heat-shocked at 45°C contain decay-accelerating activity for the classical-pathway C3 convertase. However, it is unclear whether this activity is related to the shed constituents described in this report, since it was not released at 37°C and since production of the activity by metacyclic trypomastigotes was not tested.

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