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Identification of a developmentally regulated sialidase in *Eimeria tenella* that is immunologically related to the *Trypanosoma cruzi* enzyme

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Sporozoites and merozoites of three species of *Eimeria*, *E. tenella*, *E. maxima*, and *E. necatrix*, that cause diarrhea in chickens worldwide, were examined for their expression of sialidase (SA) activity. The enzyme was found in three species, and the activity of merozoites was 10–20 times higher than that of sporozoites. The enzyme was resistant to degradation by proteases that are normally present in the intestine, a site inhabited by the *Eimeria* parasites, and it was relatively resistant to heat, with optimum activity being at 40 °C, which is within the range of temperature in the chicken intestine (40–43 °C). *E. tenella* SA was immunoprecipitated by monoclonal and polyclonal antibodies raised against the *Trypanosoma cruzi* SA (TCSA), and enzyme activity was neutralized by these antibodies. *E. tenella* SA was identified by immunoblots as a doublet of molecular weight 190 000 and 180 000 using, as a probe, anti-TCSA antibodies and antibodies against a synthetic peptide (TR) derived from the long tandem repeat domain of TCSA. Binding of the monoclonal and polyclonal antibodies to *E. tenella* was completely blocked by TR, but not by an irrelevant peptide (BR). Therefore, *E. tenella* expresses a developmentally regulated SA that is structurally related to the *T. cruzi* counterpart. Because of the high SA activity in merozoites, and by analogy with other SA-producing microbes that inhabit mucin-rich epithelia, we suggest that the *Eimeria* SA plays a role in desialylating intestinal mucins to reduce viscosity of the local environment and thereby facilitate parasite migration. The enzyme could also play a role in host cell–parasite interaction.

Keywords: *Eimeria* spp; *Trypanosoma cruzi*; neuraminidase TCN-2

Abbreviations: SA sialidase (neuraminidase); Neu5Ac, *N*-acetylneuraminic acid; 4-MU-Neu5Ac, 2′-(4-methylumbelliferyl)-α-*N*-acetyl-D-neuraminic acid; BSA, bovine serum albumin; PBS, phosphate buffered saline; PMSF, phenylmethylsulfonyl fluoride; PNA, peanut agglutinin; Ab, antibody; TCN-2, monoclonal antibody to *T. cruzi* sialidase, anti-Ars, monoclonal antibody to *p*-azophenylarsonate. TCSA, *Trypanosoma cruzi* sialidase.

Introduction

Coccidiosis is a disease of the digestive tract of birds caused by protozoan *Eimeria* parasites. Nine species of *Eimeria* have been described in chicken, each infecting a specific site of the intestine [1, 2]. The infection produces diarrhea, the severity of which depends on the invading *Eimeria* species. *E. tenella* is the most pathogenic and economically important of the *Eimeria* parasites, followed by *E. necatrix* and *E. maxima* [1, 2]. *Eimeria* has a complex life cycle, most of which is inside intestinal epithelial cells. Infection starts when ingested sporulated oocysts release sporozoites in the intestine, where they invade epithelial cells, particularly those in the crypts. Once inside epithelial cells, sporozoites, transform into trophozoites, which multiply by schizogony to yield merozoites, which propagate the infection in the crypts [1, 2]. Asexual merozoites can differentiate to macrogametocytes and microgametocytes, to give rise to zygotes (sexual reproduction) and then unsporulated oocyts. Upon maturation, the unsporulated oocyts accumulate in the intestinal lumen and are gradually released in the feces.

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The interaction of *Emeria* with epithelial cells at the molecular level is still unknown. Also unknown is the mechanism underlying diarrhea and other alterations observed in infected chickens. In this report we identify and describe some properties of a sialidase (SA) found in three species of *Eimeria.* It is developmentally regulated, has highest enzymatic activity at the temperature of the chicken intestine (40 °C), and is resistant to degradation by intestinal proteases. The enzyme from *E. tenella* was identified as a doublet of 190000 and 180000 on the basis of its immunological cross-reactivity with the *Trypanosoma cruzi* SA (TCSA). Since the SA of some microorganisms, such as influenza virus [3] and *T. cruzi* [4], can mediate invasion of host cells and alter their functions, our findings raise the possibility that the *Eimeria* SA may play a similar role in coccidiosis.

Materials and methods

Materials

2′-(4-Methylumbelliferyl)-α-*N*-acetyl-D-neuraminic acid (4-MU-Neu5Ac), soybean trypsin inhibitor, PMSF, leupeptin, pepstatin, trypsin, pepsin, pronase and protein A-Sepharose were purchased from Sigma Chemical Co., St. Louis, MO, USA; goat anti-mouse IgG-alkaline phosphatase conjugate was from Promega Corporation, Madison, WI, USA; and Nitroblue Tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate was from Research Organic, Cleveland, OH, USA. Peanut agglutinin (PNA) was purified as described earlier [5].

Parasites

Oocysts from *E. tenella* and *E. necatrix* were harvested from the cecae of infected 5-week-old chickens as previously described [6]. Briefly, after sporulation in 0.5% $K_2Cr_2O_7$, the oocysts were concentrated by centrifugation and sterilized by hypochlorite treatment for 30 min, followed by several washes with sterile deionized water. The sterile sporulated oocysts were then fractured by a Teflon tissue grinder to release sporocysts, which were then excysted *in vitro* [0.25% trypsin and 0.75% taurodeoxycholic acid in phosphate buffered saline (PBS), pH 7.8] to yield sporozoites. Sporozoites were separated from debris by passage through a cellulose column, adjusted to 1×10^7 per ml in water, lysed by sonication, and maintained frozen (−76 °C) in 1.0 ml aliquots until use. Second generation merozoites from *E. tenella, E. necatrix* and *E. maxima* were obtained from 4–5-week-old chickens that were inoculated with 10^5 oocytes *per os.* After five days the chickens were killed by cervical dislocation, the cecal pouches and small intestine removed and opened with scissors, and the content collected into a beaker containing warm RPMI 1640 without addition of serum or antibiotic. The bloody cecal content was emulsified with a small spatula and the debris allowed to settle for 2 min. The supernatant fluid was collected and centrifuged at 1500 × g for 10 min, the pellet resuspended in RPMI, washed twice by centrifugation as above and passed through a nylon wool column in a 10 ml syringe. The merozoites were washed off the column with RPMI, centrifuged at 1500 × g for 10 min, resuspended in 2 ml RPMI and spun on a discontinuous density gradient of isoosmotic Percoll in RPMI (layers of 10, 20, 30, 40, and 50% by vol; from top to bottom). Merozoites were concentrated in the 30%/40% interface and were largely free of host cell debris (< 5% epithelial cells). Epothelial cells from uninfected chickens were processes as for the purification of merozoites. Samples were lysed by sonication and maintained frozen (−76 °C) in aliquots of 1.0 ml until use.

SA activity

This was assayed in two ways. (i) PNA hemagglutination, which is based on the ability of PNA to agglutinate desialylated erythrocytes [7]. Briefly, 50 ml of 10% washed, glutaraldehyde-fixed chicken erythrocytes were incubated at 30 °C for 2 h with 50 ml of sporozoite or merozoite lysates in 200 µl of 0.2 M sodium acetate buffer, pH 5.0. The reaction was stopped by centrifuging the samples for 30 s in a microcentrifuge, washing the erythrocytes once in PBS-BSA (2 mg BSA per ml) and resuspending them in 250 µl PBS-BSA. Desialylation was assessed by titration with PNA. The enzyme unit was expressed as the reciprocal of the PNA agglutination titer h^{-1}. (ii) Fluorometric assay, which is based on the fluorescence produced by the specific hydrolysis of 4-MU-Neu5Ac [8]. Final concentration of 4-MU-Neu5Ac was 0.25 mM in the incubation mixture. The reaction was stopped by addition of 1.5 ml 0.5 M glycine-NaOH, pH 10.5, and the amount of fluorescence measured in a Perkin Elmer LS-5 fluorescence spectrophotometer with an excitation wavelength of 365 nm and an emission wavelength of 450 nm. An enzyme unit was defined as the amount of enzyme necessary to release 1 mmol sialic acid per min under the assay conditions. Boiled (100 °C, 5 min) samples were used as control in enzyme assays. The influence of pH on the SA activity was determined by both PNA and fluorometric assays in 0.2 M sodium acetate buffer, pH 3–5.5, and in 0.2 M sodium citrate buffer, pH 5–8; reaction was for 30 min at 40 °C. The influence of temperature (4, 25, 30, 37, 40, 42, 45 and 56 °C; 30 min) on enzyme activity was examined in 0.2 M sodium acetate buffer, pH 6.0, using PNA and fluorogenic assays. The requirement for divalent cations was assessed by incubating *Eimeria* lysates (20 µg) with EDTA (2, 5, 10, 20 mM in PBS, pH 7.0; 30 min at 40 °C) or by adding divalent cations (Ca^{++}, Mn^{++}, and Zn^{++}) at 0.1–5 mM, followed by determination of residual SA activity. Resistance or susceptibility of SA to proteolysis was determined by incubating merozoite and sporozoite lysates (20 µg) with either trypsin, pronase or pepsin (10, 20, 50 and 100 µg ml^{-1} in PBS, pH 7.2, for trypsin and pronase,

or in sodium acetate buffer, pH 4.0, for pepsin; reactions were for 1 h at 37 °C) and then with protease inhibitors (soybean trypsin inhibitor, 10 μg ml^{-1}; iodoacetamide, 1 mM and pepstatin, 5 μM), followed by measurement of enzyme activity using the fluorogenic assay. Controls were parasite samples treated as above, except for the presence of the proteases. These proteasees were also tested on TCSA using trypomastigote lysates as source of enzyme [5]. For inhibition of SA activity, merozoyte lysate (10 μg) was incubated for 15 min at room temperature with varying amounts of TCN-2 or anti-Ars, and the mixture assayed for residual SA activity by the PNA or fluorogenic methods.

Synthetic peptides

Peptides corresponding to the TCSA gene sequences [8] ^{25}Y-S-V-D-D-G-E-T-W-E^{34} (BR) and ^{641}D-S-S-A-H-G-T-P-S-T-P-A^{652} (TR) were prepared by solid-phase synthesis on the Milligen 9400 synthetizer using Fmoc (9-fluorenylmethyl-carbonyl) chemistry at the Protein Chemistry Facility (Tufts University Medical School). They were deprotected and cleaved from the resin with trifluoroacetic acid and then identified by amino acid sequencing. A terminal cysteine residue was added to the carboxyl terminal each of each peptide to allow coupling to keyhole limpet hemocyanin (KLH) and ovalbumin, performed as described by Sambrook *et al.* [10] using *m*-maleimidobenzoic acid *N*-hydroxysuccinimide ester as a coupling agent.

Antibodies

Immunofluorescence, immunoblot and immunoprecipitation experiments were performed using a monoclonal antibody (IgG_{2a}) raised against TCSA (TCN-2) [11]. An isotype matched monoclonal antibody generated against the hapten *p*-azophenylarsonate (Ars) was used as negative control [12]. Anti-TR antibodies were generated by injecting KLH-TR into Balb/C mice as described [13].

Immunoblot analysis

Sporozoite and merozoite lysates (10 μg) were electrophoresed on 10% SDS-polyacrylamide gel in the presence or absence of 2% β-mercaptoethanol, transferred to nitrocellulose paper, and allowed to react with TCN-2, as described previously [11]. Lysates of *T. cruzi* trypomastigotes were used as positive control. Inhibition of antibody binding was performed by incubating TCN-2 with either 4.4 mg ml^{-1} ovalbumin-TR or ovalbumin-BR for 1 h at room temperature, prior to the reaction with the nitrocellulose strips.

Immunoprecipitation of SA activity

Immunoprecipitation was performed by incubating 10 μg merozoite lysate with 25 μg of TCN-2 or anti-Ars in 100 μl PBS, pH 7.2, for 1 h at 4 °C, followed by addition of 20 μl of 30% protein A-Sepharose, and incubation overnight at 4 °C. After centrifugation in a microfuge for 3 min, the supernatants were collected and the pellets washed twice in PBS. Measurements of SA activity in the pelleted beads and in the supernatants were performed using the 4-MU-Neu5Ac method.

ELISA

Microtiter plates were coated with 25 μg merozoite lysate in sodium carbonate–bicarbonate buffer (pH 9.6) by incubation overnight at 4 °C. Plates were washed twice in PBS-Tween 20 (0.05%), blocked with 200 μl 1% denaturated BSA at 37 °C for 1 h, followed by the addition of 100 μl TCN-2 culture supernatant that had been previously incubated with ovalbumin-TR or ovalbumin-BR at the concentrations indicated in Fig. 8. Following washing, the plates were incubated with 100 μl 1:7500 dilution of an alkaline-labeled goat anti-mouse IgG (Promega Corporation, Madison, WI, USA). After 1 h of incubation at 37 °C and washing as above, bound antibodies were detected by addition of 100 μl of the substrate *p*-nitrophenyl phosphate (1 mg ml^{-1}) in diethanolamine buffer, pH 9.8. After 30 min the reaction was stopped with 100 μl 3M NaOH, and the absorbance measured at 405 nm using an automated ELISA reader (Biotek Instruments, Model EL-308). The results are expressed as percentage of inhibition of TCN-2 binding to the *E. tenella* SA.

Protein determination

Protein concentration was determined using the dye-binding method of Bradford, with BSA as standard [14].

Results

SA activity in sporozoites and merozoites of *E. tenella* was first measured using erythrocytes as substrate and assessing desialylation by PNA hemagglutination. The results showed that chicken or human erythrocytes were desialylated by merozoites, which were 20–30 times more active than sporozoites. These findings were confirmed using a quantitative SA assay, which is based on the hydrolysis of the synthetic substrate 4-MU-Neu5Ac. As shown in Fig. 1 and Table 1, SA activity of *E. tenella* merozoites was nearly 20 times that of the corresponding sporozoites and about 30 times that of *T. cruzi* (Silvio X-10/4 strain) trypomastigotes (not shown). *E. maxima* and *E. necatrix* also expressed SA activity, albeit to a lower extent than in *E. tenella* (Table 1). In *E. necatrix*, as in *E. tenella*, the activity of merozoites was higher than that of sporozoites. The material collected from normal chicken intestinal lavage at the Percoll 30/40% interface had no detectable SA activity.

Optimum temperature for the SA of the three *Eimeria* species was 40 °C (Fig. 2). Activity was reduced by only 15% at 45 °C, and by 80% at 56 °C (Fig. 2), whereas the activity of TCSA was virtually abolished by short incubations (e.g., 5 min) at 45 °C (data not shown). Reduction of SA activity in the lysates of *E. tenella* merozoites was reversible, because, if samples that had been incubated for 30 min at 56 °C were re-equilibrated at 40 °C, enzyme activity was fully restored

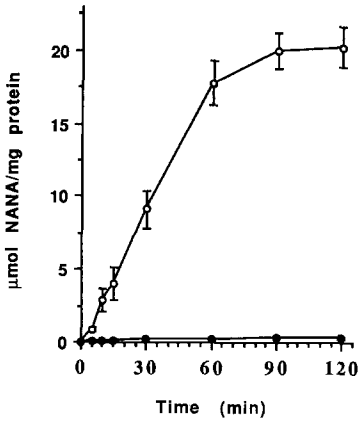

Figure 1. Kinetics of *E. tenella* SA activity. Lysates of merozoites (open circles) and sporozoites (solid circles) were assayed for SA activity using 4-MU-Neu5Ac as substrate. Reactions were at 40 °C in 0.2 M sodium acetate buffer, pH 5.5.

Table 1. Specific SA activity in sporozoite and merozoite lysates of *E. tenella*, *E. maxima* and *E. nexatrix*[a].

	Sporozoites	*Merozoites*
E. tenella	16.2 ± 1.5 [6]	296.0 ± 8.0 [6]
E. maxima	10.2 ± 1.3 [4]	ND
E. necatrix	16.6 ± 1.6 [4]	56.0 ± 0.4 [4]

[a] Results are based on the 4MU-Neu5Ac assay. Values represent mU mg^{-1} ± SD [number of determinations]. ND, not determined. The numbers of assays are indicated in brackets. Under the same conditions, the activity of lysates of *T. cruzi* trypomastigotes (Silvio X-10/4 strain) was 10.8 mU mg^{-1}.

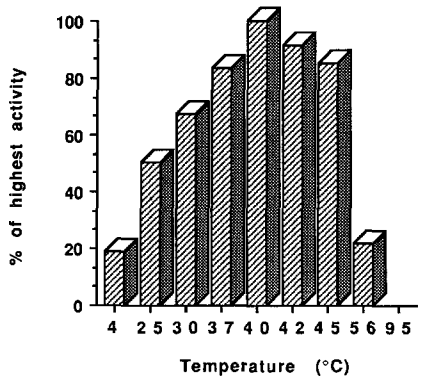

Figure 2. Influence of temperature on the SA activity of *E. tenella*. Merozoite lysates were incubated in 0.2 M sodium acetate buffer, pH 5.5, at the indicated temperatures for 15 min followed by the addition of 4-MU-Neu5Ac as specified in the Materials and methods section. Similar results were obtained with lysates of sporozoites.

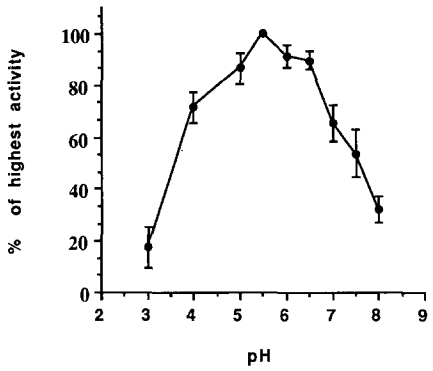

Figure 3. Influence of pH on the SA activity of *E. tenella*. Results are expressed as % of the highest activity. Reactions were incubated at 40 °C for 30 min at the indicated pHs. The activity of merozoites was identical to that of sporozoites.

(data not shown). The optimum pH for the *E. tenella* SA (merozoite and sporozoite) was 5.5 (Fig. 3), and for the *E. necatrix* and *E. maxima*, 6.0 (data not shown). Addition of EDTA (1–20 mM) and divalent cations (Ca^{++}, Mn^{++}, and Zn^{++}; 0.1–5 mM) to the reaction mixtures did not alter enzyme activity.

Since *Eimeria* thrives in the intestine, where trypsin and other proteases are abundant, it was of interest to determine the susceptibility of the *Eimeria* SA to proteolysis. As shown in Fig. 4, trypsin did not alter the activity of the *E. tenella* SA, in contrast with the almost complete abolition of the SA activity of *T. cruzi*, a parasite that does not inhabit the intestine. The *E. tenella* SA was also resistant to chymotrypsin and pronase, but not to pepsin, an enzyme present in the gastric lumen where *Eimeria* does not live (Fig. 4). Incubation of trypsin, chymotrypsin, and pronase with merozoite extracts did not alter proteolytic activity towards gelatin (data not shown), thus excluding the presence of significant protease inhibitors in the merozoite extracts.

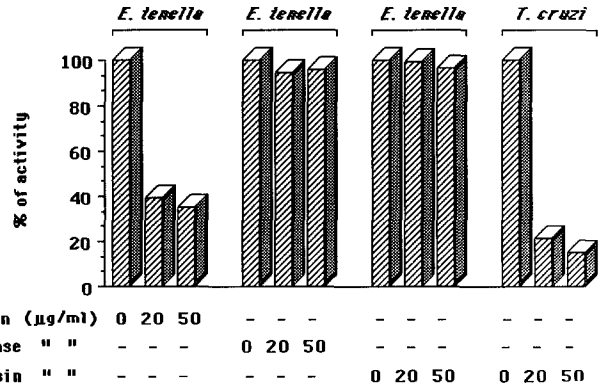

Figure 4. Influence of proteases on the SA activity of *E. tenella*. Trypsin, pepsin and pronase were incubated with lysates of merozoites at the indicated concentrations at 40 °C for 1 h. Protease inhibitors were then added and residual neuraminidase activity evaluated using the fluorometric assay. Results with chymotrypsin were similar to pepsin and pronase.

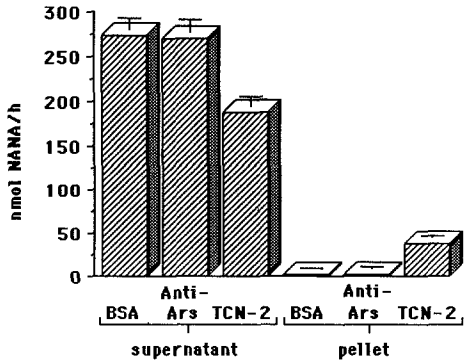

Figure 5. Immunoprecipitation of *E. tenella* SA by TCN-2. Immunoprecipitation was performed as described in the Materials and methods section. Enzyme activity was assayed by the 4-MU-Neu5Ac method at 40 °C for 30 min.

E. tenella SA was readily recognized by monoclonal and polyclonal antibodies raised against TCSA, as directly demonstrated by immunoprecipitation experiments. For this purpose, TCN-2, an anti-TCSA monoclonal antibody, was mixed with merozoite lysates followed by protein A-Sepharose and centrifugation, to precipitate the immunocomplexes. As shown in Fig. 5, TCN-2 depleted SA activity from solution, whereas an isotype-matched control antibody (anti-Ars) did not. In addition, SA activity was present in the immunoprecipitates (pellets) produced by TCN-2 but not in the immunoprecipitates brought down by anti-Ars. These results were confirmed by a different experimental approach, namely inhibition of enzyme activity by the monoclonal antibody. As can be seen in Fig. 6, TCN-2 inhibited about 50% of the activity of *E. tenella* merozoites, whereas the isotype-matched antibody was ineffective. Similar results were obtained with a rabbit polyclonal antibody against the TCSA [15] (data not shown).

The reaction of TCN-2 with *E. tenella* SA allowed identification of the enzyme by immunoblot analysis. By this criterion, the enzyme was composed of two broad bands of 190000 and 180000 (Fig. 7, lane A). Addition of the

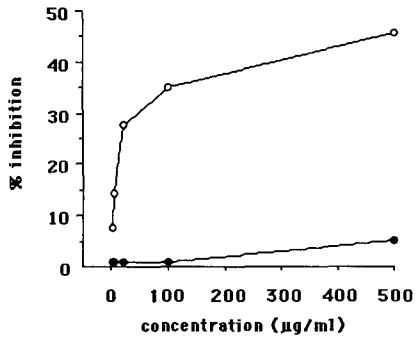

Figure 6. Specific inhibition of *E. tenella* SA activity by TCN-2. Inhibition of SA activity by varying concentrations of TCN-2 (open circles) and anti-Ars (solid circles) was measured by the PNA method.

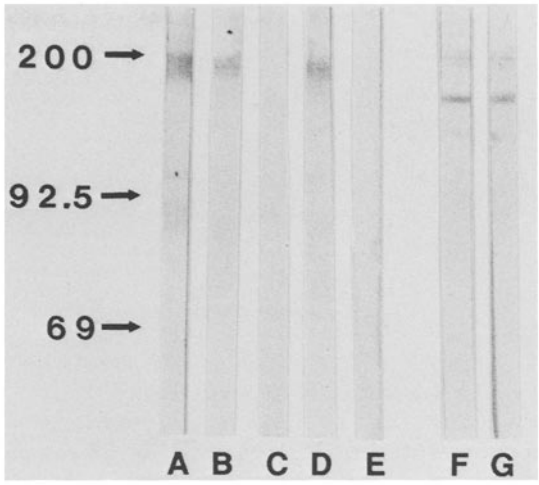

Figure 7. Identification of the *E. tenella* SA by immunoblotting. Lysates of merozoites were run on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with TCN-2 in the absence (lane A) or presence of peptides TR (lane C) and BR (lane D), or with anti-TR antibodies in the absence (lane B) or presence (lane E) of TR. *T. cruzi* trypomastigotes probed with TCN-2 (lane F) and anti-TR (lane G) antibodies served as positive controls. The samples shown above were run in the absence of 2-β-mercaptoethanol, but identical results were obtained in the presence (2%) of the reducing agent. Arrows indicate molecular masses in kDa. TCN-2 and anti-TR antibodies identified bands of identical mobility in lysates of *E. tenella* sporozoites.

reducing agent 2-β-mercaptoethanol did not alter the mobility of the doublet. The specificity of TCN-2 binding to *E. tenella* SA was demonstrated by inhibition of antibody–antigen interaction with peptide haptens. For this purpose we used the synthetic peptide TR, which was derived from the long terminal tandem repeat of the TCSA [9], and recently shown to contain the TCN-2 epitope [13] and the irrelevant peptide BR. When coupled to ovalbumin, peptide TR effectively inhibited TCN-2 binding to *E. tenella* (Fig. 7, lane C), whereas BR-ovalbumin was ineffective (Fig. 7, lane D). Furthermore, antibodies raised in mice against peptide TR reacted with *E. tenella* merozoites, producing a pattern similar to that of TCN-2 (Fig. 7, lane B). The reaction of this polyclonal anti-TR antibody, like that of TCN-2, was blocked by the homologous TR-ovalbumin (Fig. 7, lane E). As expected, the reaction of the anti-TR antibodies with *T. cruzi* lysate was similar to that of TCN-2 (Fig. 7, lanes F and G, respectively). Both monoclonal (TCN-2) and polyclonal (anti-TR) antibodies recognized additional weak low molecular weight bands (not clearly discernible in Fig. 1) in the *E. tenella* lysates, but the migration of these bands was not consistent from experiment to experiment, and may represent degradation products. Nevertheless, the immunoblots clearly demonstrate that *E. tenella* SA, like the *T. cruzi* counterpart, is polymorphic.

Specific binding of TCN-2 to lysates of *E. tenella* merozoites was also demonstrated by ELISA. As with the

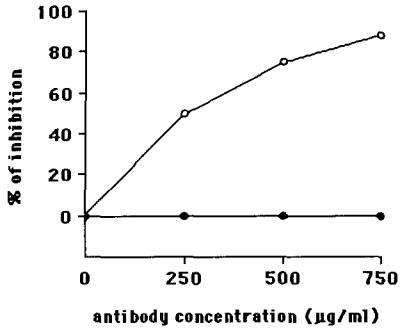

Figure 8. Inhibition of TCN-2 binding to *E. tenella* by ELISA. Binding of TCN-2 to microtiter plates coated with *E. tenella* merozoite lysates was performed in the presence of various concentrations of soluble TR (open circle), or BR (solid circle). The results shown are from representative experiments, and expressed as % inhibition of antibody binding by TR and BR.

immunoblots, TCN-2 binding was inhibited in a dose-dependent manner by the TR peptide but not by the BR (Fig. 8).

Discussion

Our results confirm and extend those of Farooqui and co-workers [16] who found SA activity in *E. tenella* sporozoites. However, these authors did not assay for SA in the merozoite stage of *E. tenella* nor in the developmental forms of other *Eimeria* species. The results presented here also show SA activity in *E. tenella* sporozoites, but it is only about 5% of that of merozoites. In addition, SA was expressed in two additional species of *Eimeria*, namely *E. necatrix* and *E. maxima*. Optimum catalytic activity was at 40 °C, which is within the range of the normal temperature in chicken intestine [17]. Another adaptation of *Eimeria* SA to the site of infection was its resistance to degradation by trypsin and chymotrypsin, two enzymes normally present in the environment where *Eimeria* thrives. In addition to these two proteases, pronase was also without effect on the *E. tenella* SA. But, *Eimeria* SA was not universally resistant to proteolysis, as it was destroyed by pepsin, a protease present in an organ (stomach) not inhabited by the parasite. Resistance to proteolysis is probably important in allowing the SA to be active *in situ*, which normally contains high concentrations of trypsin. Indeed, erythrocytes, arterial and venous endothelial cells, and epithelial cells of the intestine of chicken infected with *E. tenella* are heavily desialylated, presumably by the parasite SA (J. Alroy, V. Goyal, R. G. Strout, and M. E. A Pereira, unpublished).

E. tenella SA is structurally related to TCSA. This conclusion was based on immunoblot analysis using monoclonal and polyclonal antibodies directed against a peptide deduced from the sequence of the tandem repeat domain of the TCSA [9]. Such analysis revealed a cross-reactivity of anti-TCSA antibodies with *E. tenella* lysates, identifying a doublet of 190 000, and 180 000 in both merozoite and sporozoite forms. Evidence that these bands represent the *Eimeria* SA was provided by experiments demonstrating that anti-TCSA antibodies immunoprecipitated *E. tenelle* SA and inhibited its activity.

We have shown that the cross-reactive antibodies are specific for the dodecamer D-S-S-A-H-G-T-P-S-T-P-A [16], that is repeated many times in the TCSA [9]. Therefore, the binding of these antibodies (TCN-2 and anti-TR) to merozoites and sporozoites implies that *E. tenella* SA has a structure similar to the dodecamer, namely, that it is rich in Ser, Thr, and Pro. This structure may be conserved in the SA of other pathogenic protozoa such as *Plasmodium falciparum*, whose infection in man elicits antibodies that recognize that tandem repeat domain of TCSA (J. S. Mejia, R. P. Prioli, and M. E. A. Pereira, unpublished), and *Acanthamoeba castellani*, whose SA is recognized by TCN-2 and anti-TR antibodies [18]. However, this inference needs to be confirmed by comparison of the primary sequence of TCSA with that of the other pathogenic protozoan parasites. For this purpose, TCN-2 and other anti-TCSA antibodies should be valuable tools to screen expression libraries to identify the genes encoding the SAs of the other pathogens, as was the case for the *T. cruzi* enzyme.

What role could *Eimeria* SA play in the pathogenesis of coccidiosis? One possibility is that it may facilitate migration of merozoites in the mucin-rich lumen of the intestine. High viscosity is the most striking and physiologically relevant property of mucins, which function as lubricants in the lumen of various organs of mammals [19]. Viscosity is also dependent on the sialic acid content of mucins because, if they are desialylated by SA digestion, their viscosity is sharply reduced [20]. Thus, a possible scenario includes merozoites releasing SA in the intestine, where it will desialylate mucins present in the intestinal juice, thereby reducing viscosity and enhancing the ability of the parasite to migrate in the lumen, in its way to invade neighboring enterocytes. Resistance to degradation by intestinal proteases and optimum catalytic activity at 40 °C should enhance the activity of *Eimeria* SA.

But while desialylated mucins might provide an adequate environment for the movement of merozoites in the intestinal lumen, it may not be as good a lubricant because of its reduced viscosity, which could result in dysfunctions of the gastrointestinal tract, like, for example, diarrhea. In addition to mucin, functional alteration of the intestine may be enhanced by the desialylation of the enterocytes (J. Alroy, V. Goyal, R. G. Strout, and M. E. A. Pereira, unpublished). Reduced viscosity of mucins induced by SA, as a way to promote parasite survival in the host, is thought to occur in some infections of the respiratory tract such as those produced by influenza virus [21], *Pseudomonas aeruginosa* [22] and *Streptococcus pneumoniae* [23].

Another role for the *Eimeria* SA would be in parasite attachment to host cells. This could be accomplished in two

ways. First, if host cell sialic acid is a recognition determinant for parasite attachment and penetration, SA, by desialylating plasma membrane glycoconjugates, should remove the recognition site, and therefore reduce invasion of the host cell, as is the case with influenza virus [3] and *T. cruzi* [24]. Second, if host cell sialic acid does not function directly as a ligand for parasite-host cell interaction, it might nevertheless be involved in attachment by masking underlying recognition structures. In this case, the endogenous *Eimeria* SA would remove sialic acid and expose host cell ligands, thereby promoting attachment and invasion, as in *Entamoeba histolytica*, that attaches to cells by interacting with Gal/GalNAc determinants [25], which are normally internal in sugar chains and linked to terminal sialic acid residues [26]. Results with *E. meleagrimitis* provide experimental support for the first hypothesis, because pretreatment of primary cultures with exogenous bacterial SA resulted in significant reduction of sporozoite penetration of the host cells [27]. However, further experiments are needed to ascertain the role of the endogenous *Eimeria* SA in infection. These experiments should be greatly facilitated by the antibodies used in this study, since they react with and inhibit the activity of the *Eimeria* enzyme.

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