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POTENTIAL OF THE SULFOBETAINE DETERGENT ZWITTERGENT 3-12 AS A DESORBING AGENT IN BIOSPECIFIC AND BIOSELECTIVE AFFINITY CHROMATOGRAPHY

KARL H. NOLLSTADT*, YASHWANT D. KARKHANIS, MARK J. GNOZZIO, MARK St.J. CRANE, ANNE M. GURNETT, DENNIS M. SCHMATZ and MERVYN J. TURNER

Department of Biochemical Parasitology, Merck Sharp & Dohme Research Laboratories, P.O. Box 2000, Rahway, NJ 07065-0900 (U.S.A.)

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SUMMARY

The isolation in our laboratories of several antigens of interest from sporulated oocysts of *Eimeria* species by bioselective adsorption on matrices containing immobilized antigen-specific immunoglobulins IgG was initially unsuccessful. The preparations serving as source materials for these antigens contained low levels of the zwitterionic sulfobetaine detergent, Zwittergent 3-12. Since usually immunoaffinity processes are carried out in the presence of various detergents, we were surprised, subsequently, to find this detergent to be the cause of the problem in that it prevented antigen–antibody binding. These findings led us to study the potential role of Zwittergent 3-12 as an eluting agent from matrices holding bioselectively adsorbed materials. The results of seven case studies are presented in this paper and include experiments with β-D-galactosidase adsorbed biospecifically and bioselectively on matrices via either specific antibody or inhibitor analogue. In all cases, Zwittergent 3-12 proved to be an effective desorbing agent.

INTRODUCTION

Zwittergent 3-12, a zwitterionic sulfobetaine detergent, has been increasingly used in cell membrane protein extractions following its successful use by Gonenne and Ernst in 1978 [1]. Zwittergent 3-12 is a synthetic detergent with the following general structure

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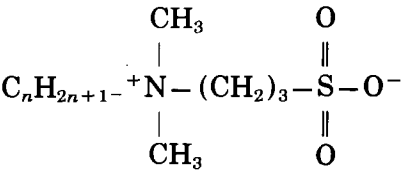

where $n=8$–16; in Zwittergent 3-12, $n=12$.

It is commercially available from Calbiochem. A lengthy bibliography is cited by the manufacturer highlighting the properties of this detergent as a membrane protein extractant [2]. In our work, to be described here, we have used Zwittergent 3-12 not as an extractant, but as an additive to the developing buffer in the molecular sieving operation of various protein antigens of interest on a column of Sephacryl S-200. We found that the presence of this detergent at levels of 0.1% (that is below its critical micelle concentration of 0.12%) resulted in reproducible fractionating patterns not attainable when this detergent was left out.

In the pursuit of development of a vaccine against coccidial infections induced by parasites of the genus *Eimeria*, several protein antigens were identified as potential vaccine candidates. Their isolation by both conventional and biospecific chromatography, and particularly the way in which Zwittergent 3-12 affects the latter, are the subject of this paper. In initial efforts to purify one of the antigen, pooled fractions from the Sephacryl S-200 column containing the antigen were applied directly to a biospecific matrix constructed with an immunoglobulin (IgG) fraction specifically directed to this antigen. No trace of the antigen was adsorbed. When the Zwittergent 3-12 was removed by dialysis, and the affinity chromatography step was repeated, the desired purified product was obtained using 3.5 *M* sodium thiocyanate as the eluent. These findings formed the basis for the use of Zwittergent 3-12 as an eluent in subsequent studies which are presented in this paper.

EXPERIMENTAL

Sporulated oocysts of *Eimeria* species were supplied as a 20-ml suspension in phosphate-buffered saline (PBS) containing approximately $7 \cdot 10^9$ oocysts per ml. Sporulated oocysts were isolated by the method of Schmatz et al. [3]. The suspension was made 0.1 m*M* phenylmethylsulfonyl fluoride (PMSF) (Sigma, MO, U.S.A.) and was sonicated in an ice bath until, by microscopic examination, no more than about 10% of intact sporozoites remained. The sporulated oocyst sonicate soluble fraction (SOSSF) was obtained by centrifugation of the sonicate at 30 000 *g* for 45 min at 4°C and collecting the supernatant. SOSSF contains an antigen designated A. Protein levels in SOSSF were between 2–3 mg/ml. The sporulated oocyst sonicate insoluble fraction (SOSIF), which contains antigens designated B and H, was washed three times with 40 ml of PBS.

Throughout the experiments described, protein was determined by the method of Lowry et al. [4].

The B and H antigens were extracted from SOSIF by reduction and carboxymethylation using a modification of the method of Stone and Smith [5]. Briefly, washed SOSIF was suspended in 60 ml of 5 *M* guanidine·HCl–0.5 *M* Tris–HCl, pH 8.6, at room temperature. The suspension was then made 40 m*M* in dithiothreitol (DTT) and kept at room temperature for 24 h with mild agitation. The suspension was then centrifuged at 30 000 *g* for 60 min at 4°C, and the supernatant was concentrated to 20 ml by ultrafiltration on an Amicon YM-10 membrane (Amicon, Danvers, MA, U.S.A.). The concentrate was made 170 m*M* in iodoacetic acid (BDH, Poole, U.K.). The pH was adjusted to 8.6 immediately with 3 *M* Tris base and the system was kept at room temperature and in the dark for 2 h, followed by dialysis against 50 m*M* NH_4HCO_3–0.1 m*M* PMSF–0.02% sodium azide for 48 h (several changes of the permeate). During dialysis, some insoluble matter formed which was removed by centrifugation at 30 000 *g* for 30 min at 4°C. The retentate constituted the reduced and carboxymethylated soluble fraction (RCSF), containing on the average 400 μg/ml protein in a total of about 30 ml.

RCSF was applied to a column (100 cm×2 cm) of Sephacryl S-200 (Pharmacia, Piscataway, NJ, U.S.A.) equilibrated in running buffer, 50 m*M* NH_4HCO_3–0.1% m*M* PMSF–0.1% zwittergent 3-12–0.02% sodium azide. In each operation, a total of 60 fractions (5.5 ml) was collected, 4 fractions per hour. Between 8 and 10 ml of RCSF were applied to the column. The antigens of interest were identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting using specific antisera raised against the recombinant fusion proteins designated as A, B and H. The cloning and biological testing of these antigens, and the production and characterization of antisera specific for them, will be described elsewhere. Appropriate fractions were pooled, concentrated to approximately 10 ml by ultrafiltration (Amicon YM-10 membrane) and treated with two volumes of acetone at −20°C for 24 h to precipitate the antigen and separate it from Zwittergent 3-12 (which stays in solution). After collection of the precipitate by centrifugation and a wash in acetone–water (75:25), the precipitate was redissolved in column wash (0.1 *M* borate pH 8.0–0.5 *M* NaCl–0.02% sodium azide–0.1 m*M* PMSF), ca. 12 ml, to constitute the charge to the biospecific matrix.

Rabbit antisera for immunoblots (Western blots), from which the IgG fraction was isolated for the construction of biospecific matrices, were kindly furnished by Stefan Galuska of Merck Sharp and Dohme Research Labs. (Rahway, NJ, U.S.A.). To isolate the IgG fraction, ca. 5–7 ml of serum were brought to 45% saturation with respect to ammonium sulfate, and after stirring for 2 h in an ice-bath, the salted-out fraction was collected by centrifugation at 30 000 *g* for 15 min at 4°C. The insoluble fraction was resuspended in 5–7 ml of 25 m*M* Tris–HCl–35 m*M* NaCl, pH 8.8, and dialyzed against this buffer over-

night. The retentate was then slowly passed over a 5-ml bed of DEAE–Tris–acryl M (Reactif IBF) previously equilibrated in this buffer. The pass through containing the IgG including a 10-ml buffer wash was diafiltered and concentrated to 5–7 ml (Amicon YM-30 membrane) with coupling buffer (0.2 *M* $NaHCO_3$–0.5 *M* NaCl, pH 8.5). It is essential that in the diafiltration step Tris is eliminated as it would otherwise compete with IgG in the subsequent coupling step to the matrix.

Biospecific affinity matrices were prepared based on the method of Hearn et al. [6]. The 1,1-carbonyldiimidazole-activated support is available commercially under the trade name Reactigel (Pierce, Rockford, IL, U.S.A.). The advantage of this support is the formation of a very stable and uncharged N-alkyl carbamate bond on reaction with a free amino group of the ligand [7]. Reactigel was used in accordance with the manufacturer's suggested procedure. Briefly, 5 ml of an acetone suspension (50% bed volume) of Reactigel were transferred into an Econocolumn (Bio-Rad Labs.) and drained free of acetone. IgG was then introduced as a solution of 12 mg per 8 ml of coupling buffer (0.2 *M* $NaHCO_3$–0.5 *M* NaCl, pH 8.5). The column was sealed, mounted on a rocking platform and kept at 5 °C overnight. The column was drained and washed with 5 ml of coupling buffer. Quenching was done by suspending the support in 10 ml of coupling buffer containing 50 μl of aminoethanol and placing the column on a rocking platform for 4 h at room temperature. The matrix was then washed with 10 ml of coupling buffer, 6 ml of 3.5 *M* sodium thiocyanate and finally with 10 ml 'column wash' (0.1 *M* borate buffer pH 8.0–0.5 *M* NaCl–0.02% sodium azide–0.1 m*M* PMSF).

The column charge to the biospecific matrices contained less than 5.0 mg protein in 10 ml appropriate buffer, usually column wash whenever the previous operation was a Sephacryl S-200 operation. Other samples were made 0.5 *M* in NaCl. All samples were additionally made 0.2% in *n*-octylglucoside, 0.1 m*M* in PMSF and 0.02% in sodium azide. The charges were continuously recycled over the matrix by use of a suitable peristaltic pump, usually for 18 h at room temperature. After draining out the column charge, the matrix was washed with 2×5 ml of column wash and 2×5 ml of 10 m*M* Tris–HCl, pH 8.0.

The matrix was then eluted by gravity passage of 10 ml of 10 m*M* Tris–HCl, pH 8.0, made 0.1% in Zwittergent 3-12 (Tris–Zwittergent buffer, TZW) (single cycle, unless indicated otherwise in the captions). The same volume was used of eluents containing lithium diiodosalicylate (LIS), where applicable. Eluates were dialyzed against 50 m*M* NH_4HCO_3 and concentrated to 3–5 ml by ultrafiltration (Amicon YM-10 membranes).

The matrices were regenerated by washing with 10 ml of TZW containing 35 m*M* LIS, 2×5 ml of 20 m*M* Tris–HCl, pH 8.0, and 10 ml of column wash and were stored in this buffer at 4 °C.

SDS-PAGE was carried out in a Protean II chamber (Bio-Rad Labs.) according to the manufacturer's instructions which are based on the method of

Laemmli [8]. The patterns were visualized by either silver staining [9] or with a 0.25% solution of Coomassie brilliant blue R in fixing solution (10% acetic acid–50% methanol). Gels were destained in 10% methanol–7% acetic acid. For the molecular weight range of the *Eimeria* antigens, 12.5% polyacrylamide gels were used. For β-D-galactosidase, 7.5% gels were appropriate. The A antigens and β-galactosidase were applied in reducing sample buffer (RSB: 4 *M* urea–2% SDS–10% glycerol–0.05% bromothymol blue–4% mercaptoethanol). The B and H antigen, already reduced and carboxymethylated, were applied in non-reducing sample buffer (NRSB same as RSB but without mercaptoethanol). Amounts of proteins applied to lanes are indicated in the captions.

For visualization of the antigens by Western blotting, transfer of mobilized proteins from gel slabs to nitrocellulose was made in a transblot chamber (Bio-Rad Labs.) according to the manufacturer's instructions and based on the method of Towbin et al. [10]. Best transfers were obtained overnight at 4°C applying 20 V. Nitrocellulose sheets were obtained from Schleicher and Schuell (Keene, NH, U.S.A.). Detection of transblotted proteins was realized by the use of double antibody (goat anti-rabbit) with coupled alkaline phosphatase according to the method of Blake et al. [11].

RESULTS AND DISCUSSION

Four membrane-bound and two cytoplasmic protein antigens from the sporulated oocysts of the parasitic protozoan *Eimeria* were purified by biospecific adsorption. Invasive parasites are contained within sporocysts which in turn are found within the sporulated oocysts. The membrane-bound protein antigens are associated with the sporozoites. The isolated antigens are trivially named the A antigen from *E. tenella* (see Fig. 2), the B antigen from *E. tenella* (see Fig. 3), *E. acervulina* (see Fig. 5) and *E. maxima* (see Fig. 7) and the H antigen from *E. tenella* (see Fig. 4). Fusion proteins A, B and H (in addition to other fusion proteins not described here) were found to give significant in vivo protection against coccidial infection [12]. These fusion proteins were purified by conventional means in our laboratories (to be published elsewhere) and were used in the production of specific antisera. From these, the IgG fraction was isolated for subsequent immobilization on matrices.

The native B and H antigens are membrane-bound and thus are not solubilizable by sonication in the absence of detergent (Fig. 1). In contrast, the native A antigen is found in the sonicate supernatant (Fig. 1). Fig. 1 summarizes the isolation steps of the antigens of interest. The A antigen is found in the sonicate soluble fraction, while the B and H antigens had to be extracted from the sonicate insoluble fraction by reduction with DTT in guanidine and by carboxymethylation.

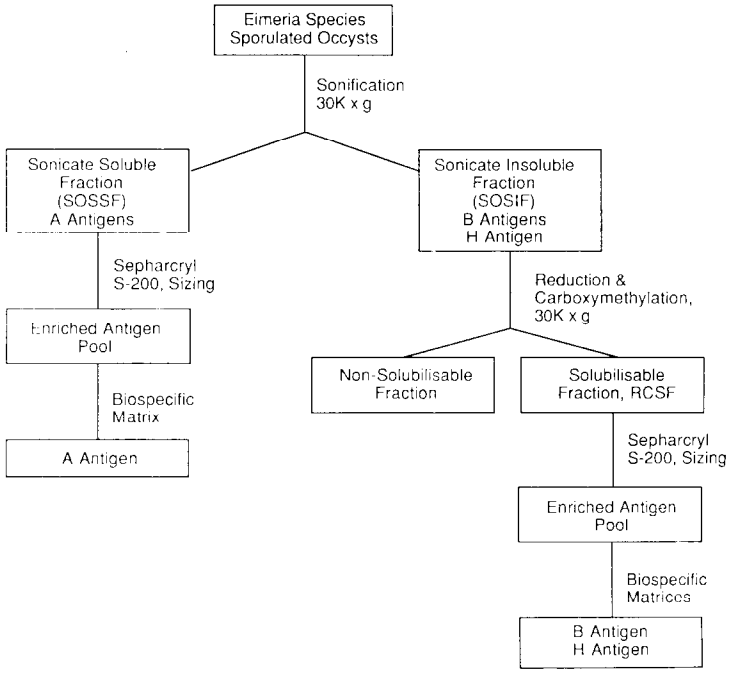

Fig. 1. Flow diagram of isolation steps: localization of antigens.

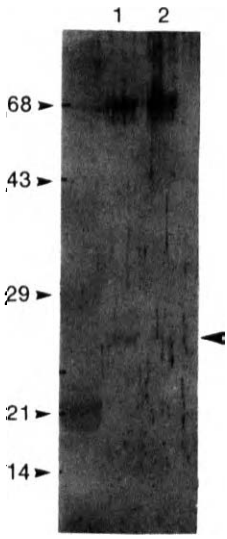

Fig. 2. Western blots of the affinity-purified A antigen of *E. tenella*. A prepurified pool of this antigen was passed over a matrix containing immobilized anti-A antigen IgG. The source serum for this IgG was used in the blotting and identification of the A antigen (ca. 24 kD). Elution was apparently complete with TZW (lane 1). Subsequent elution with 3.5 *M* sodium thiocyanate did not result in release of additional material (lane 2). Attempts to obtain silver-stained SDS-PAGE patterns of this A antigen were unsuccessful. Samples were applied in RSB.

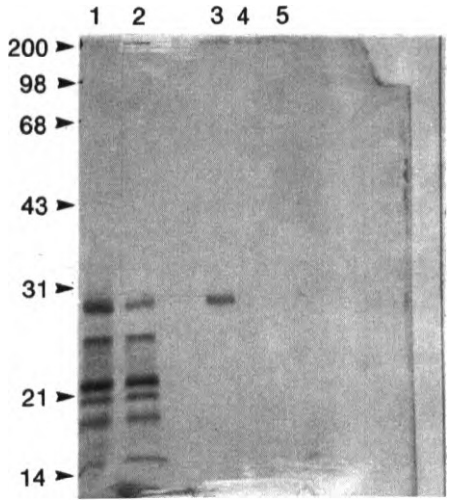

Fig. 3. Silver-stained SDS-PAGE pattern of the affinity-purified B antigen of *E. tenella*. The prepurified pool containing this antigen was applied to a matrix constructed with the IgG fraction of specific antisera raised against the cloned B antigen. Lane 1 represents the charge to the matrix and lane 2 the final flow-through fraction. Lanes 3, 4 and 5 represent the materials eluted with TZW, TZW containing 5 m*M* LIS and TZW containing 35 m*M* LIS, respectively. Charge and flow-through were applied at 3 μg per lane, eluted fractions at 1 μg per lane or equivalent volumes. Samples were applied in NRSB.

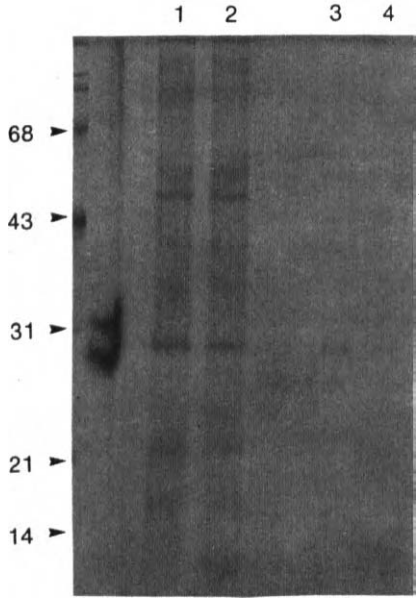

Fig. 4. Silver-stained SDS-PAGE pattern of the affinity-purified H-antigen of *E. tenella*. The solubilized fraction from SOSIF (see Fig. 1) was directly applied to the affinity matrix without prior prepurification on Sephacryl S-200. The matrix was constructed with the IgG fraction of specific antisera raised against the cloned H antigen. Lanes 1 and 2 represent the charge and flow-through, respectively, at 3 μg per lane. Lanes 3 and 4 represent the materials eluted with TZW and TZW containing 5 m*M* LIS, respectively. The charge to lane 3 was 1.0 μg and the volume equivalent to lane 4. Samples were applied in NRSB.

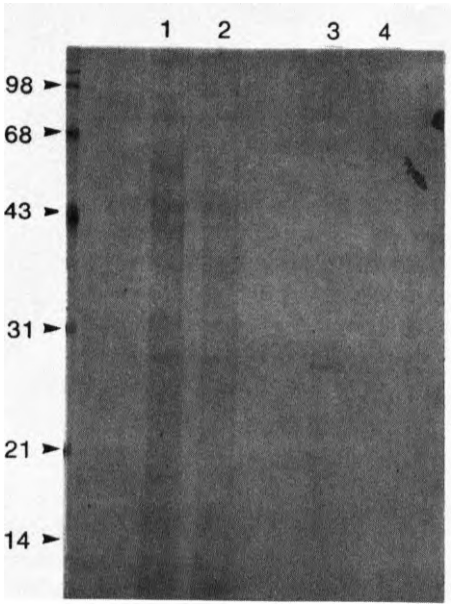

Fig. 5. Silver-stained SDS-PAGE pattern of the affinity purified B antigen of *E. acervulina*. The prepurified pool containing this antigen was applied to a matrix constructed with the IgG fraction of specific antisera raised against the B antigen of *E. tenella*. Lane 1 represents the charge to the matrix and lane 2 the final flow-through fraction. Lanes 3 and 4 represent materials eluted with TZW and TZW containing 5 m*M* LIS, respectively. Charge and flow-through were applied at 3 μg per lane, eluted fractions at 1 μg per lane or an equivalent volume. Samples were applied in NRSB.

Isolation of the A antigen from E. tenella

SOSSF containing the A antigen was applied to the Sephacryl S-200 column and the collected fractions were monitored for the antigen by Western blotting using antisera specific to the A clone fusion protein. The A antigen has an apparent molecular weight of about 24 kD. Active fractions were pooled and prepared for application to the affinity matrix as described under Experimental. Elution was carried out first with TZW, then followed by 3.5 *M* sodium thiocyanate. The eluates were examined by Western blotting but only the TZW eluate contained the bulk of the A antigen with only a trace amount in evidence in the thiocyanate eluate (Fig. 2). This indicates that TZW is a highly effective desorbing agent even with a polyclonal IgG population on the matrix.

Isolation of the B antigen from E. tenella

As shown in Fig. 1, the B antigen is located in SOSIF. The antigen was solubilized for affinity chromatography by reduction and carboxymethylation in the presence of guanidine. The solubilized antigen in RCSF was then prepurified by molecular sizing on Sephacryl S-200, and the collected fractions

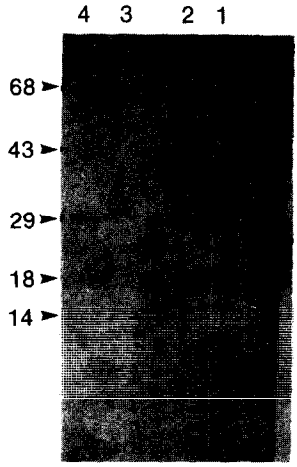

Fig. 6. Western blots of the affinity-purified B antigen from *E. acervulina*, as an extension of data shown in Fig. 8. Included here is a desorption attempt with 0.2% *n*-octylglucoside in 10 m*M* Tris-HCl, pH 8.0 (lane 3), followed by elution with TZW (lane 4). Lanes 1 and 2 represent matrix charge and flow-through, respectively. The matrix was a construction of immobilized anti-B antigen fusion protein IgG. The source serum for this IgG was used in developing the blots. Charge and flow-through were applied at 10 μg per lane, the eluates at 3 μg per lane or an equivalent volume. There was no evidence of elution of antigen with *n*-octylglucoside at this level. The results obtained here were the basis of carrying out all our affinity operations in the presence of 0.2% of this neutral detergent. Samples were applied in NRSB.

containing the B antigen were identified by Western blotting following SDS-PAGE. Active fractions were pooled and prepared for application to the affinity matrix as already described. The results of biospecific adsorption/desorption are shown in Fig. 3. The effectiveness of TZW alone as an eluent stands out strikingly even with a polyclonal IgG population (lane 3), inasmuch as additional levels of LIS to the eluting TZW had no effect on eluting additional antigen (lanes 4 and 5).

Isolation of the H antigen from E. tenella

Fig. 1 shows that the H antigen also is located in SOSIF along with the B antigen. Again, solubilization was necessary for application to the affinity system. This was accomplished by the same method described for the B antigen. Following solubilization, an aliquot of RCSF was directly applied to the appropriate affinity matrix, without prior fractionation on Sephacryl S-200. The results of this operation are given in Fig. 4. With the exception of a trace amount, the antigen was elutable with TZW only, even though a polyclonal IgG matrix was used (lanes 3 and 4). Although the apparent molecular weight (28 kD) is nearly identical to that of the B antigen, they are totally different proteins

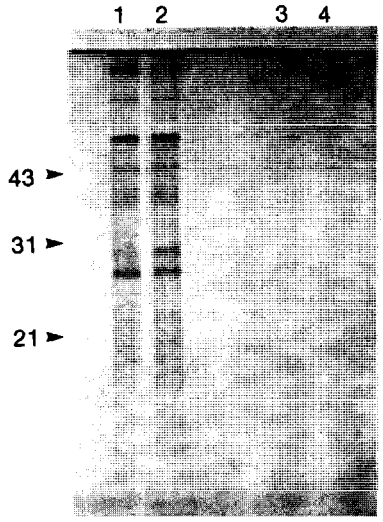

Fig. 7. Silver-stained SDS-PAGE pattern of the affinity purified B antigen of *E. maxima*. The solubilized fraction from SOSIF (see Fig. 1) by use of guanidine·HCl, DTT and carboxymethylation was directly applied to the affinity matrix without prior prepurification on Sephacryl S-200. The matrix was constructed with the IgG fraction of specific antisera raised against the B antigen of *E. maxima*. Lane 1 represents the charge to the matrix and lane 2 the final flow-through fraction. Lane 3 represents materials eluted with TZW and lane 4 the material eluted with TZW containing 5 m*M* LIS. Elution was complete without this added reagent. Charge and flow-through were applied at 3.0 μg per lane, the eluates at 1.0 μg per lane or an equivalent volume.

based on the lack of cross-reactivity between the two antigens and lack of homology in their cDNA deduced amino acid sequences (unpublished data).

Isolation of the B antigen from E. acervulina

The antigen was isolated by solubilization from SOSIF, prepurified on Sephacryl S-200 and by biospecific adsorption/desorption as described above for the B antigen from *E. tenella*. The results of the affinity purification are summarized in Fig. 5. Lane 3 shows the antigen as eluted with TZW only. Adding LIS to the eluting buffer resulted in no further release of antigen from the polyclonal IgG matrix (lane 4). In a separate biospecific purification experiment with this antigen, it was established that the neutral detergent *n*-octylglucoside at 0.2% did neither prevent adsorption nor act as an eluent (Fig. 6). It was the result from this particular experiment which made us decide to routinely add this detergent to all our affinity operations at 0.2%.

Isolation of the B antigen from E. maxima

The isolation of this antigen was carried out by a method already discussed for the B antigen from *E. tenella* and *E. acervulina*. However, prepurification

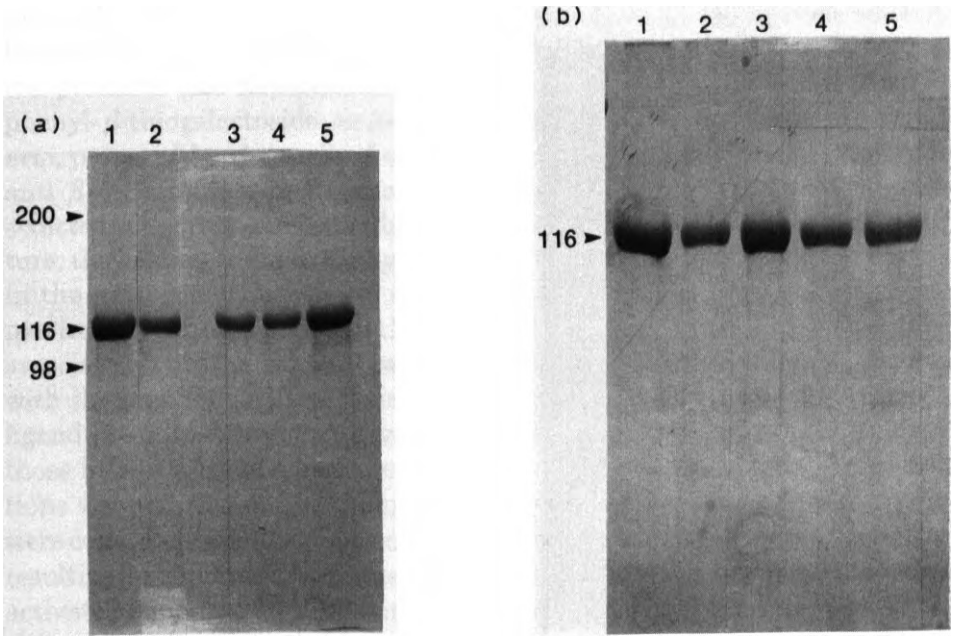

Fig. 8. (A) Coomassie Brilliant Blue-stained SDS-PAGE pattern of biospecific adsorption/desorption of β-galactosidase on a matrix constructed with rabbit anti-β-galactosidase IgG (commercially procured, see Experimental). This figure presents the first phase of the study. Lanes 1 and 2 represent the charge to the matrix and the flow-through, respectively. The charge contained 2.0 mg of β-galactosidase per 6 ml, of which 30 μg (90 μl) were applied per lane, with an equivalent volume applied to lane 2. Elution was carried out with 10 ml TZW containing 0.0, 5 and 35 m*M* (lanes 3, 4 and 5, respectively). The volume applied to lanes 3, 4 and 5 were equivalent to that of the column charge. Samples were applied in RSB. (B) Coomassie Brilliant Blue-stained SDS-PAGE pattern of biospecific adsorption/desorption of β-galactosidase on the same matrix as described in (A). This figure represents the second phase of the study where it was attempted to shift the elution pattern shown in (A) by recycling 10 ml TZW over the matrix three times. Again, lanes 1 and 2 represent the charge and the flow-through, respectively. Lane 3 represents the β-galactosidase released by recycling. Lane 4 represent additional material released when a fresh 10-ml volume of TZW was passed over the column without recycling. Finally, lane 5 represents material released when the eluent was additionally charged with 35 m*M* LIS. Amounts charged to lanes as described in (A).

Sephacryl S-200 was omitted. Virtually the same results were obtained as with the other B antigens: total release of the antigen from the matrix with TZW alone (Fig. 7, lane 3). No additional antigen was released from the polyclonal IgG matrix by TZW, supplemented with 5 m*M* LIS (lane 4).

As shown, it was possible to isolate all of the protozoan antigens of interest by desorption from their respective biospecific matrices with TZW alone. Desorption was virtually complete in all five cases, as LIS added to this eluting system (TZW) at 5 m*M* (Figs. 3, 4, 5 and 7) or 3.5 *M* sodium thiocyanate (Fig. 2) failed to produce additional desorbed products.

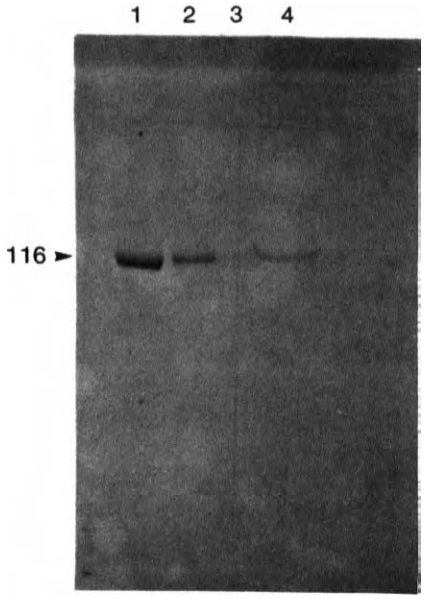

Fig. 9. Coomassie Brilliant Blue-stained SDS-PAGE pattern of bioselective adsorption/desorption of β-galactosidase. The matrix contained an immobilized inhibitor analogue, *p*-aminophenyl-β-thiogalactoside (commercially procured, see Experimental). The matrix was made bioselective in the presence of high salt levels (0.5 *M* NaCl) in the charge, thus suppressing the anion-exchange effect of the matrix. Lanes 1 and 2 represent the charge and flow-through, respectively. Elution of the adsorbed enzyme was carried out with TZW and with TZW supplemented with 0.5 *M* NaCl and the eluted material applied to lanes 3 and 4, respectively. The charge to the matrix contained 1.0 mg of β-galactosidase in 6 ml of 0.1 *M* borate buffer–0.5 *M* NaCl, adjusted to pH 8.1. Aliquot samples of charge and flow-through were desalted with two volumes of acetone ($-20\,^{\circ}$C, 24 h). The precipitate was reconstituted in reducing sample buffer (0.6 ml). A 15-μg amount (Lowry protein) of the charge was applied to lane 1 and equivalent volumes of the flow-through to lane 2 and of the eluates to lanes 3 and 4.

The mechanism by which Zwittergent 3-12 acts as a desorbing agent is not known. It appears not to involve a simple detergent effect, since *n*-octylglucoside at double the concentration level (0.2%) had no influence on adsorption/desorption. The fact that the matrices were constructed with polyclonal IgG indicates that it is not an effect on a single epitope. Perhaps Zwittergent 3-12 interacts with protein surfaces, thus affecting the charge distribution on the surface. In preliminary and unrelated studies we have shown that Zwittergent 3-12 (at 150 μM) uniquely interacts with certain enzymes, an event of potentially therapeutic value (unpublished data). These events may be related mechanistically to the properties of Zwittergent 3-12 which are the subject of this paper.

Studies with β-D-galactosidase were included in this investigation to deter-

mine what effect, if any, Zwittergent 3-12 may have on this enzyme when it is bioselectively and biospecifically adsorbed. The matrices used were commercial products (see Experimental) and contained either immobilized *p*-aminophenyl-β-thiogalactoside, an inhibitor analogue, including a ten-carbon spacer arm, prepared by the method of Steers, Jr. et al. [13], or immobilized rabbit anti-β-galactosidase IgG. According to the supplier, the matrices were constructed with cyanogen bromide-activated supports. Due to their isourea structure, they act as anion exchangers, unless the affinity operation is carried out in the presence of high ionic strength [14]. This anion exchanger effect is particularly pronounced with the inhibitor analogue, since the positive charge associated with the isourea linkage remains localized. It is less pronounced with the immobilized IgG, because of charge delocalization over the protein ligand [14]. Results with β-D-galactosidase and Zwittergent 3-12 were exactly those to be expected under the conditions just described. The affinity operations were carried out in the presence of 0.5 *M* NaCl, whether the matrices were constructed with 1,1-carbonyldiimidazole-activated supports (Reactigel) resulting in a charge-free immobilized ligand [7] or with cyanogen bromide-activated supports. Results with β-D-galactosidase clearly show that biospecific adsorption on the IgG matrix occurred and the elution could be achieved with TZW (Fig. 8A). However, elution was less effective and recycling the eluent was necessary to effect a greater degree of product release. Alternately, complete desorption could be accomplished with 35 m*M* LIS (Fig. 8B) by the method of Struck et al. [15] designed for the affinity purification of β-D-galactosidase fusion proteins.

The anion effect of the bioselective matrix incorporating *p*-aminophenylthio-β-D-galactoside became apparent when attempting to desorb β-D-galactosidase with TZW. Fig. 9 shows that the enzyme was effectively adsorbed, even in the presence of 0.5 *M* NaCl. Because of the presence of salt, the enzyme was bioselectively rather than ionically bound to the matrix. Subsequent desorption attempts with TZW, however, were not successful until TZW was supplemented with 0.5 *M* NaCl. The observations can easily be explained as the result of using a matrix which is both an anion exchanger and a bioselective system. We think that the enzyme was indeed released under the influence of TZW from its bioselective binding site, but was then held ionically until the addition of sodium chloride to TZW.

In six out of the seven cases discussed here, Zwittergent 3-12 was found to be a very effective desorbing agent. Zwittergent 3-12 was less effective in desorbing biospecifically held β-D-galactosidase; but in this case we did not investigate whether or not continued recycling of the eluent might have resulted in complete desorption, eventually.

In conclusion, our major objective was the identification and isolation of potentially useful candidates for a coccidiosis vaccine. In using affinity processes to these ends, we have gathered the data presented here as a spin off

which we deem useful for others engaged in affinity processes. It is our feeling that we have merely scratched the surface of the potential of Zwittergent 3-12 in affinity applications, but particularly in the bioselective purification of enzymes. In this area, a mild eluting agent as Zwittergent 3-12 in lieu of substrate, coenzyme or inhibitor analogues should be highly acceptable. One only needs to consider their cost and the fact that these agents must yet be separated from the eluted enzyme.

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