

ANTIGENIC ENZYMES OF *SCHISTOSOMA MANSONI*: POSSIBLE USE FOR IMMUNODIAGNOSIS

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Different enzymes of Schistosoma mansoni are recognized by IgG antibodies present in the sera of infected human patients. The antigenicity of these enzymes suggests their possible use in immunodiagnostic assays that would take advantage of their activities.

Various *Schistosoma mansoni* enzymes have been previously reported to be immunogenic. The enzymatic activities were revealed in antigen-antibodies complexes obtained after immunoelectrophoresis of adult worm homogenates against rabbit sera hyperimmunized with this preparation (P. Tran Van Ky et al., 1967, *Ann. Inst. Pasteur*, 112: 763-771).

The *S. mansoni* hemoglobinase, the main thiol acidic proteinase present in the adult worm blind gut, was found to be antigenic in mice (A. M. Deelder et al., 1977, *Exp. Parasitol.*, 40: 189-197) and it is able to induce a rapid histaminic response in the skin of various animal species and in man (A. W. Senft & S. Maddison, 1975, *Am. J. Trop. Med. Hyg.*, 24: 83-89; A. W. Senft et al., 1979, *Parasite Immunol.*, 1: 79-89).

Two malate dehydrogenase (MDH) isoenzymes were defined as major *S. mansoni* adult antigens specific for the *Schistosoma* genus; the pI 7.1 isoenzyme was very immunogenic (D. Bout et al., 1978, *Immunochem.*, 15: 633-638). This enzyme has been detected in circulating immune complexes in human schistosomiasis and was present as an excretory and/or secretory (ES) product in adult worm incubates (D. Bout et al., 1977, *J. Immun. Meth.*, 15: 1-8; J. P. Rotmans, 1978, *Exp. Parasitol.*, 46: 49-58). The antigen is located in the superficial cellular layer of the gut in adult schistosomes and anti-MDH antibodies appear early in human infections (Bout et al., 1978).

The IgG fraction purified from mice chronically infected with *S. mansoni* inhibited the alkaline phosphatase (AP, pH 9.5) activity of a detergent-solubilized tegumental membrane enriched (TME) fraction from adult worms by up to 25% but did not effect the AP isolated from host (mouse, hamster) liver. The IgG fraction purified from normal mice or mice infected with highly irradiated (20 krd) cercariae had no inhibitory effect on the enzymes. An IgG fraction prepared against mouse liver 5'-nucleotidase had no effect on the parasite AP (5'-nucleotidase) although these antibodies inhibited mammalian membrane-bound 5'-nucleotidase enzyme (I. M. Cesari et al., 1981, *Biochem. J.*, 198: 467-473).

A neutral peptidase from the larval and adult stages of *S. mansoni* was antigenic; the immunity against the enzyme was expressed in rats towards day 28 after infection and cross reacted with *S. haematobium* (M. Damonville et al., 1982, *Mol. Biochem. Parasitol.*, 6: 265-275). An acidic, thiol-dependent proteinase from *S. mansoni* eggs (miracidia) was shown to be immunogenic in animals infected with this parasite (M. H. Dresden, 1982, *Acta Leidensia*, 44: 81-99). ELISA and Western blot analysis showed also that sera from infected mice and patients with schistosomiasis reacted with a cercariae proteinase of Mr 30000 (S. Pino-Heiss et al., 1986, *Am. J. Trop. Med Hyg.*, 35: 536-543).

The reported data indicate that some enzyme components or their subunits are probably excreted and/or secreted by schistosomes into the host blood circulation at certain time of their development and are able to induce anti-enzyme antibodies.

Although the worms have to adapt after transference from *in vivo* to a medium *in vitro* which in general can be considered sub-optimal, some of the ES components produced *in vitro* may correspond to ES products released *in vivo*. However, the rate and quality of ES products release may vary from an *in vitro* to an *in vivo* condition. Adult *S. mansoni* worms maintained *in vitro* in a Minimum Essential Medium (I. M. Cesari et al., 1983, *Exp. Parasitol.*, 56: 119-128) up to 16 hours at 37 °C excreted and secreted to the medium various polypeptides in the range of Mr 15-100 kD (I. M. Cesari, 1985, p. 64-67, In *Simposium de Bioquimica de Parasitos*, USB, Caracas). Among the ES products it was possible to detect the presence of hydrolases like acid phosphatase (AcP, pH 5.2), the hemoglobinase (HGase, pH 3.8) and membrane-associated alkaline activities like the AP, phosphodiesterase (PDE, pH 9.5), Ca-stimulated ATPase (pH 9.5) and leucine aminopeptidase (LAP, pH 8.0). The enzymes were detected as ES products after 3 hours of incubation *in vitro* and their specific activities were much increased after 16 hours of incubation suggesting that they were accumulated in the medium by the living worms (Cesari, 1985). The AP, PDE and ATPase are integral glycoproteins of the tegumental double surface membrane (DSM) (I. M. Cesari & P. Santos, 1974, *Proc. 3rd. Int. Congr. Parasitol.*, 3: 1481-1482; G. Payares et al., 1984, *Parasitol.*, 13: 343-360; F. H. Pujol, 1985, p. 105, *M. Sc. Thesis*, IVIC, Caracas); the AP exposes probably a glycosylated site on the parasite surface whereas the active site seems to be buried in the internal part of the membrane (Pujol, 1985). The continuous turnover and shedding of the DSM (J. R. Kusel et al., 1975, *Parasitol.*, 71: 247-259; R. A. Wilson & P. E. Barnes, 1977, *Parasitol.*, 74: 61-71) is probably the mechanism by which particulated antigens from this membrane are put into circulation *in vivo* or are released *in vitro* (Kusel et al., 1975). On the other hand, the soluble and particulated ES products that are present in the parasite blind gut may enter the host blood circulation or may be released *in vitro* by regurgitation; this process occurs regularly every 3-4 hours to eliminate residual products of the host hemoglobin and plasma components digestion (J. P. Rotmans et al., 1981, *Exp. Parasitol.*, 52: 171-182). The numerous eggs that become trapped in the host tissues release also soluble, enzyme-rich (Y-X. Zu & M. H. Dresden, 1986, *J. Parasitol.*, 72:

507-511) ES products that stimulate the host cellular and humoral immune responses (S. R. Smithers & M. J. Doenhoff, 1982, p. 527-607. In S. Cohen, K. S. Warren, *Immunology of Parasitic Infections*. Blackwell, London).

Some of the enzymatic activities were tested for their capacity to be recognized and captured as antigens by antibodies in the sera from infected human patients. Using the immunosorbent method of Damonville et al. (1982), the IgG fraction of infected sera was adsorbed on protein-A Sepharose beads that were then incubated with the antigenic parasite preparation and later with a specific substrate. Since the ES products are released in small amounts and are difficult to obtain, other enzyme-rich parasite preparations like 1% Triton X-100 solubilized TME fraction (Cesari et al., 1981), butanolic extracts (I. M. Cesari, 1973, *Acta. Cient. Venez.*, 24 Supl. 1: 10) or a soluble egg antigen (SEA) preparation (D. L. Boros & K. S. Warren, 1970, *J. Exp. Med.*, 132: 488-507) were also used in the assays (Pujol, 1985).

Preliminary experiments indicate that IgG antibodies binding AP, PDE, Ca⁺⁺-stimulated ATPase and AcP of the detergent-solubilized TME fraction and the LAP activity from the SEA preparation are present in sera of patients infected with *S. mansoni* (Fig.). The HGase from the ES products of adult worms was negative in this IgG-dependent assay. However, as stated previously, this enzyme seems to induce an IgE response in infected humans and animals (Senft & Maddison, 1975; Senft et al., 1979) and then it would not be detectable by this assay.

Minimal activity for AP and relatively low levels of activity for the other enzymes were seen with the pool of uninfected sera or sera from humans infected with other parasites (Fig.). Non-specific adsorption of enzymes to the protein-A beads was not detected and capture of enzymatically active circulating IgG-enzyme complexes from the infected sera by the beads was not observed. The results would indicate that the enzymes are probably circulating antigens responsible for an important part of the host humoral response.

Upon washing, the immunoadsorbed antigen-IgG-protein A-Sepharose beads could be reused several times for the same or for other enzyme

assays suggesting the independence of the enzyme-antibody systems tested.

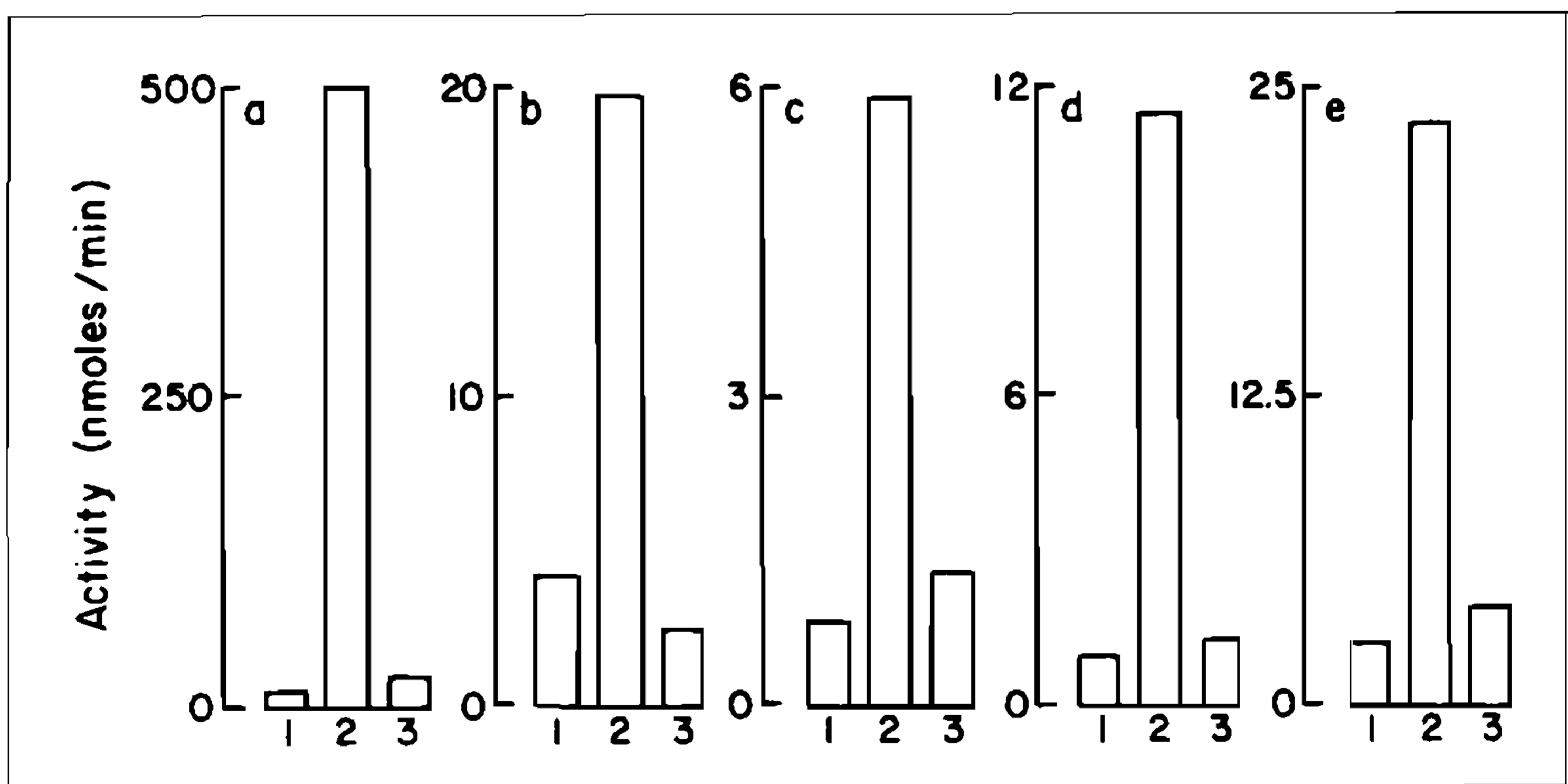
From these results, the AP emerges as a first candidate for possible use in immunodiagnose. The search for anti-AP IgG antibodies is actually being done in a more simple direct enzyme immunoassay consisting in the previous adsorption of the IgG fraction from sera on protein A – coated polyvinyl microtiter plates (S. J. Freeman et al., 1984, *Anal. Biochem.*, 141: 248-252; F. H. Pujol & I. M. Cesari, in preparation). Preliminary results using butanolic extracts of adult schistosomes (rich in AP) as antigenic preparation and a high number of sera tested individually indicate that sensitivity and specificity in this assay are higher than 90% (Pujol & Cesari, in preparation).

The principal advantage in assays for the search of anti-enzymes antibodies would lie in the fact that pure antigen preparations are not required and it allows the detection of the immune response to one antigen (a given antigenic enzyme) as it were pure and independently from other antigens or antigenic enzymes. A limitation of the assay would occur when

antibody inhibits the activity of the enzyme antigen. If this does not occurs, the degree of sensitivity with a determined enzyme might be limited by the genetic capacity of the host to respond to the available enzyme epitopes, the amount of enzyme captured, the turnover number of the enzyme.

Antigenic enzymes may help to develop improved methods of immunodiagnose for schistosomiasis and perhaps for other parasitic diseases. In this respect, appropriate longitudinal studies are needed on the time of appearance of specific anti-enzyme antibodies during infection and/or disappearance after chemotherapy.

Requisites for the use of antigenic enzymes in direct assays would be: a) that the enzymes be present only in the parasite and absent in the mammal host; b) that they be sufficiently different in antigenic structure or in assay conditions (substrate concentration, ionic strength, pH) to the host enzymes; c) that the antigen-antibody complex formation do not completely interfere with the binding of substrate to the enzyme.



Immunoabsorption of antigenic enzymes from *Schistosoma mansoni*. Sera (50 μ l) were incubated with protein A-Sepharose (5 mg dry weight) equilibrated with phosphate-buffered saline (PBS) pH 7.4 at 4 °C for 2 hr. After several washings with PBS, 4-25 μ g of ES, detergent-solubilized TME fraction or SEA were added to the IgG-coated beads and incubated overnight at 4 °C with constant mixing. The immune complexes bound to the beads were washed with PBS and incubated with the appropriate buffered 1 mM enzymatic substrate solution. a: AP (TME, p-nitrophenyl phosphate); b: PDE (TME, thymidine-5'-monophospho-p-nitrophenyl ester); c: Ca⁺⁺ stimulated ATPase (TME, vanadate-free ATP); d: AcP (TME, p-nitrophenyl phosphate); e: LAP (SEA, leucine p-nitroanilide). 1: pool of ten sera from healthy donors; 2: pool of ten sera from *S. mansoni* infected patients; 3: pool of ten sera from patients infected with other parasites (schistosomiasis haematobia, trichuriasis, fascioliasis, ascariasis, taeniasis, cysticercosis, leishmaniasis, malaria).