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Isozyme patterns of *Schistosoma japonicum* and *S. mansoni*

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ABSTRACT

Isozyme patterns of six enzymes, glucose-6-phosphate dehydrogenase, glucosephosphate isomerase, hexokinase, malate dehydrogenase, 6-phosphate dehydrogenase and phosphoglucomutase were examined in electrophoresed homogenates of adult male worms of *Schistosoma japonicum* and *S. mansoni*. In general, enzyme patterns obtained from the parasite homogenates differed from that of host (mouse) blood and muscle, indicating that electrophoretic patterns from parasite extracts are most probably of parasite origin. Adult male and female *S. mansoni* worms yielded identical patterns. However, all six enzyme patterns showed distinct differences between *S. japonicum* and *S. mansoni*. These results suggest that *S. japonicum* is clearly distinguishable from *S. mansoni* at the molecular level.

INTRODUCTION

Electrophoretic procedures have been successfully used to differentiate the species of *Schistosoma*, especially those of African origin (COLES, 1970; ROSS, 1976; ROSS *et al.*, 1978; WRIGHT & ROSS, 1980). On the other hand, only a minor study has been made of the patterns of *S. japonicum* (YAN *et al.*, 1976).

In this study, starch gel electrophoresis was used for the purpose of studying the taxonomic relationships between a Japanese strain of *S. japonicum* and a Puerto Rican strain of *S. mansoni*. The results are discussed in respect of previous studies in isoenzyme patterns of *S. mansoni* (COLES, 1970, 1971a, b; WIUM-ANDERSON & SIMONSEN, 1974).

MATERIALS AND METHODS

Strains of *S. japonicum* from Yamanashi in Japan and of *S. mansoni* from Puerto Rico were used. Mice were exposed to infections of 100 to 200 cercariae by immersing the tails for one to two hours. Six to seven weeks after infection, the animals were killed and the adult worms were recovered by the isotonic saline perfusion technique (RADKE *et al.*, 1961). All the worms were washed with physiological saline, grouped according to sex, and stocked in a deep freezer at −80°C until required for electrophoresis. The 30 to 40 adult worms of each species were homogenized in 0·1 M phosphate buffer (pH 7·5) and the extracts obtained were applied to electrophoresis, carried out using the starch gel as described previously (AGATSUMA & SUZUKI, 1980, 1981). In this study, six enzymes, glucose-6-phosphate dehydrogenase, glucosephosphate isomerase, hexokinase, malate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphoglucomutase, were examined. Conditions of electrophoresis and the staining methods for all the enzymes, except malate dehydrogenase, have been described previously (AGATSUMA & SUZUKI, 1980, 1981). Malate dehydrogenase was studied by the methods described by SHAW & PRASAD (1970).

RESULTS

As shown in Fig. 1, host (mouse) blood and muscle enzymes gave patterns which were generally very different from those of either parasite species. Thus, it can be assumed that enzyme patterns obtained from parasite extracts are probably clearly of parasite origin. Male and female enzyme patterns were compared in *S. mansoni* and no

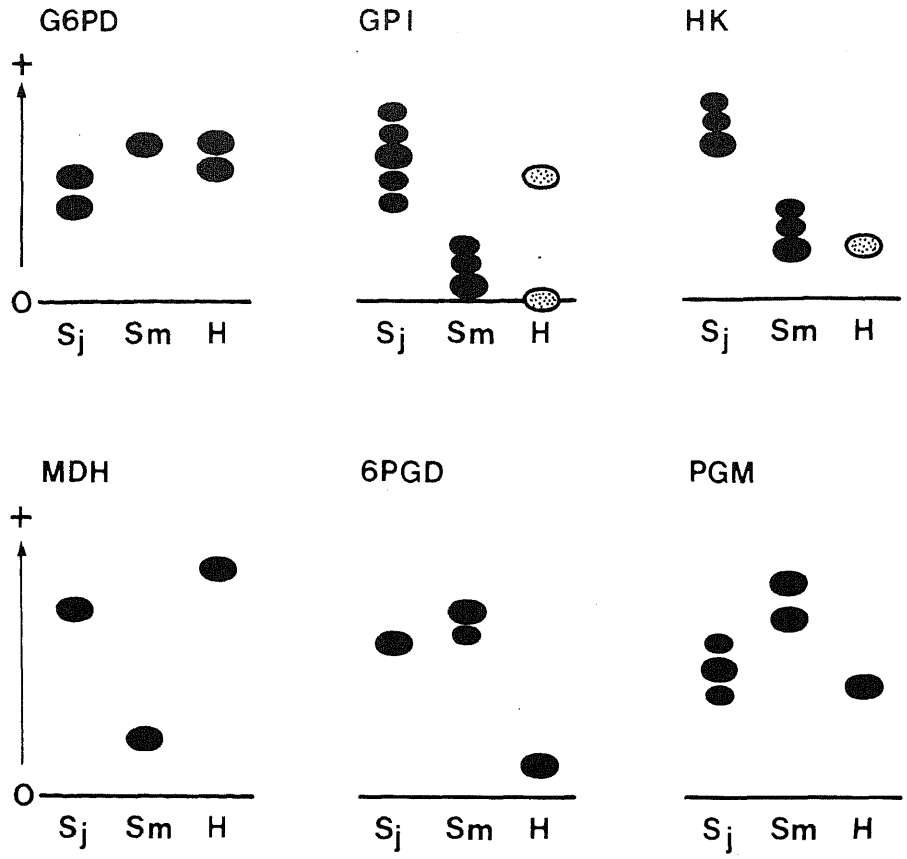

FIG. 1. Diagrammatic representation of electrophoretic patterns of six enzymes in *Schistosoma japonicum* and *S. mansoni*. o; origin. Sj; *S. japonicum*. Sm; *S. mansoni*. H; host.

differences were found between them. Therefore, only males of each species were examined in this study. In all cases the two species of schistosomes could be clearly distinguished by their isoenzyme patterns.

Glucose-6-phosphate dehydrogenase (G6PDH)

In *S. japonicum*, two bands were identified, whereas in *S. mansoni* only a single band was detected, this having migrated faster anodally than either band of *S. japonicum*.

Glucosephosphate isomerase (GPI)

In *S. japonicum*, five bands were detected and, of these, the middle band was most strongly stained. In contrast, three bands were found in *S. mansoni* of which the least mobile was most strongly stained. There was no overlap in electrophoretic mobilities of the various GPI isoenzymes of the two schistosome species.

Hexokinase (HK)

Both schistosome species gave similar three-banded isoenzyme patterns but with different mobilities. Thus, in each species, the greatest activity was in the band with slowest migration. As with GPI, the *S. japonicum* isoenzymes migrated faster than those of *S. mansoni*, with a clear separation between the two species.

Malate dehydrogenase (MDH)

In each species a single band was detected, but with a marked difference in electrophoretic mobility.

6-phosphogluconate dehydrogenase (6PGD)

S. japonicum showed a single band of activity, whereas *S. mansoni* possessed two bands, of which the fastest was the more strongly stained. The mobilities were different in the two species.

Phosphoglucomutase (PGM)

In *S. japonicum*, three bands were detected, the middle band being the most strongly stained, whereas in *S. mansoni*, two bands were found, each of which stained equally.

DISCUSSION

COLES (1970) stated that the presence of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase may represent a functional pentose pathway. In this study, these two enzymes were detected in both species, suggesting that this pathway occurred. COLES (1970) found G6PDH enzyme to be a multiple form in *S. mansoni* from Africa, but CONDE-DEL-PINO *et al.* (1968) observed only a single band in Puerto Rican strain of *S. mansoni*. Results from the present study were similar to those of CONDE-DEL-PINO *et al.* (1968).

WIUM-ANDERSEN & SIMONSEN (1974) studied GPI in the sporocysts of *S. mansoni* using starch gel electrophoresis, and a single band moving slowly towards the cathode. Whether the difference from the present result was due to differences in materials or methods used in the two studies is unknown. Striking differences of GPI patterns were obtained here between *S. japonicum* and *S. mansoni*. SOUTHGATE *et al.* (1980) observed patterns of GPI in *S. bovis* similar to our present results for *S. mansoni*, rather than for *S. japonicum*, although different methods of electrophoresis were used.

Several sources of information are available on MDH in *Schistosoma* species (CONDE-DEL-PINO *et al.*, 1966, COLES, 1971a, b; ROSS, 1976; YAN *et al.*, 1976; SOUTHGATE *et al.*, 1980). CONDE-DEL-PINO *et al.* (1966) found two isozymes of malate dehydrogenase in *S. mansoni* from Puerto Rico, whereas COLES (1970) found either two or four isozymes in African *S. mansoni*. Furthermore, COLES (1971a) reported that significant differences occur between male and female, but CONDE-DEL-PINO *et al.* (1966) did not find these differences. On the other hand, YAN *et al.* (1976) found distinct differences among strains and between sexes in malate dehydrogenase of *S. japonicum*. In this study, however, only a single band was detected in both species.

Recently it has been shown by enzyme analysis that natural hybrids between different species of *Schistosoma* occur (WRIGHT & ROSS, 1980). In the light of the present results, using these enzyme systems, we may be able to study the occurrence of hybrids between *S. japonicum* and other species of *Schistosoma*.

Comparisons between a variety of species of *Schistosoma* have been made using methods of electrophoresis (ROSS, 1976; ROSS *et al.*, 1978; WRIGHT & ROSS, 1980). COLES (1970) compared six enzymes between *S. mansoni* and *S. haematobium* and showed that four of these, were clearly distinctive between the species. In the present study, all six enzymes revealed considerable differences between *S. japonicum* and *S. mansoni*. The results suggest that *S. japonicum* is clearly separated from *S. mansoni* at the molecular level. This is supported by YOSHIMURA (1969) who investigated the protein component of both species and demonstrated that each species is represented by characteristic patterns.

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