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Kinetic Analyses of the Membrane-Bound Alkaline Phosphatase Activity of Hymenolepis diminuta (Cestoda: Cyclophyllidea) in Relation to Development of the Tapeworm in the Definitive Host

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The specific activities of the alkaline phosphatase (APase), type I phosphodiesterase and 5′-nucleotidase activities associated with the brush-border plasma membrane of the tapeworm, Hymenolepis diminuta, decrease significantly as the tapeworm grows and matures. Kinetic analyses of the APase activity associated with membrane preparations from whole 6-, 12-, and 18-d-old H diminuta, and individual pieces of 18-d-old H diminuta cut into ten pieces of equal length, failed to demonstrate qualitative changes in the APase activity. Therefore, the decreased specific activities are apparently due to changes in the ratios of enzymatically active to enzymatically inactive membrane proteins (ie, quantitative changes in the membrane proteins) which occur as the tapeworm grows.

Key words: Hymenolepis diminuta, tapeworm, plasma membrane, brush border, membrane-bound enzyme, alkaline phosphatase

A single mature (adult) tapeworm represents a continuous gradient of tissues in different developmental stages. The anterior or neck region consists of undifferentiated tissues which continue to form new proglottids throughout the tapeworm's life, and the posterior end consists of gravid proglottids which have ceased all reproductive activity and are filled with eggs; the remainder of the tapeworm's body (strobila) consists of proglottids in various stages of organogenesis and sexual maturation. Thus, tapeworms represent a unique model for the study of developmental changes in animals.

A previous paper from this laboratory [1] reported that the membrane-bound enzymatic activities of the brush-border plasma membrane of the tapeworm, Hymenolepis diminuta, decrease in specific activity as the proglottids age (mature), and that these changes are accompanied by qualitative and quantitative changes in the polypeptide composition of the membranes. The present study was undertaken to determine

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whether the decreases in membrane-bound enzymatic activities of different aged tapeworms are a result of qualitative or quantitative changes in the enzymatically active membrane proteins.

METHODS

H diminuta was reared in the beetle, Tenebrio molitor, and male Sprague-Dawley rats. Rats (80–100 gm) were either infected with 100 cysticercoids, and the tapeworms were removed 6 d postinfection (PI), or with 30 cysticercoids, and the tapeworms removed 12 to 18 d PI. A separate "Tris-disrupted fraction (TDF)" [2] (a fraction consisting of enriched brush-border plasma membrane) was prepared from whole 6-, 12-, and 18-d-old tapeworms and dialyzed against distilled water for 48 hr at 2°C. The protein concentration of each TDF was determined [3] using bovine albumin as a standard. Individual TDF preparations were also obtained from pieces of 18-d-old tapeworms. For these membrane preparations, 18-d-old tapeworms were cut into ten pieces of equal length and comparable pieces were pooled into a single sample. The TDF from each pooled sample was prepared and assayed for protein as above.

All TDF preparations were assayed for alkaline phosphatase (APase) activity using p-nitrophenyl phosphate (PNPP; Sigma 104 Substrate) as the substrate. Assays were conducted at 30°C in 200 mM Tris buffer containing 1 mM $MgCl_2$ and adjusted to the appropriate pH with HCl. The amount of TDF used in each assay was adjusted so the initial reaction rate ($\Delta A_{405} \cdot min^{-1}$) was linear for at least 2 min; specific activities were calculated in units of μmol p-nitrophenol liberated $\cdot$ mg^{-1} protein $\cdot$ min^{-1} [4]. Because the millimolar extinction coefficient (E^{mM}) of p-nitrophenol decreases as the pH of the assay medium decreases, specific activities were calculated using values of E^{mM} derived empirically (eg, values of E^{mM} for p-nitrophenol at 405 nm at pH 8.8, 8.0, and 7.4 were 17.5, 16.4, and 11.3, respectively). TDF preparations were also assayed, as previously described [5], for 5′-nucleotidase (NTase), type I phosphodiesterase (PDase), and ATPase activities using 5′-AMP, thymidine-5′-monophospho-p-nitrophenyl ester, and 5′-ATP, respectively, as substrates.

To determine the maximum specific activities (V_{max}) and apparent Michaelis constants ($K_{m\ app}$), initial velocities (V_0) of substrate (PNPP) hydrolysis were determined using at least 10 substrate concentrations between 0.01 and 1 mM; data were plotted as V_0 vs $V_0 \cdot [S]^{-1}$, and lines were fit to the data by regression analyses. Apparent inhibitor constants (K_i') were determined by measuring the effects of increasing concentrations of inhibitor on the hydrolysis of a low concentration of substrate (ie, 0.1 mM PNPP). These experiments were run at pH 8.8 using at least seven different inhibitor concentrations, and data were plotted as the reciprocal of the fractional inhibition (i^{-1}) versus the reciprocal of the inhibitor concentration ($[I]^{-1}$). Lines were fit to these data by regression analyses and K_i' values calculated as described previously [1,6].

RESULTS

The specific activities of APase, PDase, and NTase associated with the brush-border plasma membrane of whole tapeworms decreased dramatically as the tapeworms matured (Fig. 1). Similar patterns of enzymatic activity were noted when

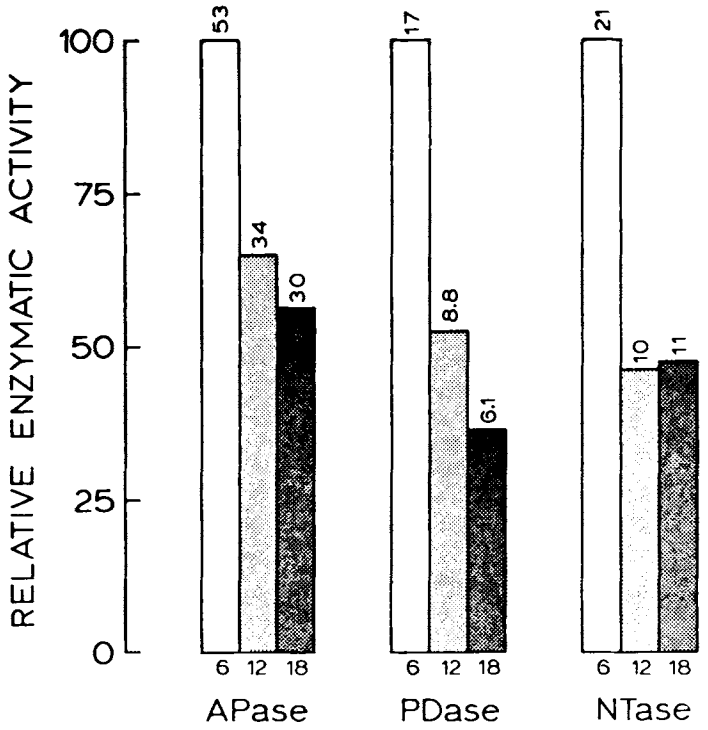

Fig. 1. Alkaline phosphatase (APase), phosphodiesterase (PDase), and 5′-nucleotidase (NTase) activities of the brush-border plasma membrane of whole 6-, 12-, and 18-d-old Hymenolepis diminuta. The number over each bar represents the specific activity in units of μmol product (p-nitrophenol or P_i) liberated · mg^{-1} · min^{-1}, and the relative enzymatic activities were calculated as a percentage of the membrane preparation with the highest specific activity. Values represent the means of triplicate assays.

membrane preparations of individual pieces of 18-d-old H diminuta were assayed (Fig. 2); APase, PDase, and NTase activities were highest in the most anterior sections of 18-d-old tapeworms, and the activities decreased in the more posterior sections of the tapeworm's strobila.

The pH optimum, V_{max}, and $K_{m\ app}$ for APase activity were determined in membrane preparations from whole tapeworms of different ages and pieces of 18-d-old tapeworms; the latter two parameters were determined at pH 8.8 (the pH optimum for APase activity) and 8.0, because these parameters change dramatically as a function of pH [6]. The data (Figs. 3, 4, Table I) demonstrated clearly that the V_{max} of the APase activity decreased as the tapeworm aged, while the pH optimum and $K_{m\ app}$ remained unchanged. Additionally, the values of K_i' for P_i, 5′-AMP, and 5′-ATP as inhibitors of the APase activity were similar in membrane preparations from whole tapeworms of different ages and individual pieces of 18-d-old tapeworms (Table II).

DISCUSSION

Decreases in the specific activities of membrane-bound enzymes might result from either a change in the properties of the enzymatically active membrane proteins

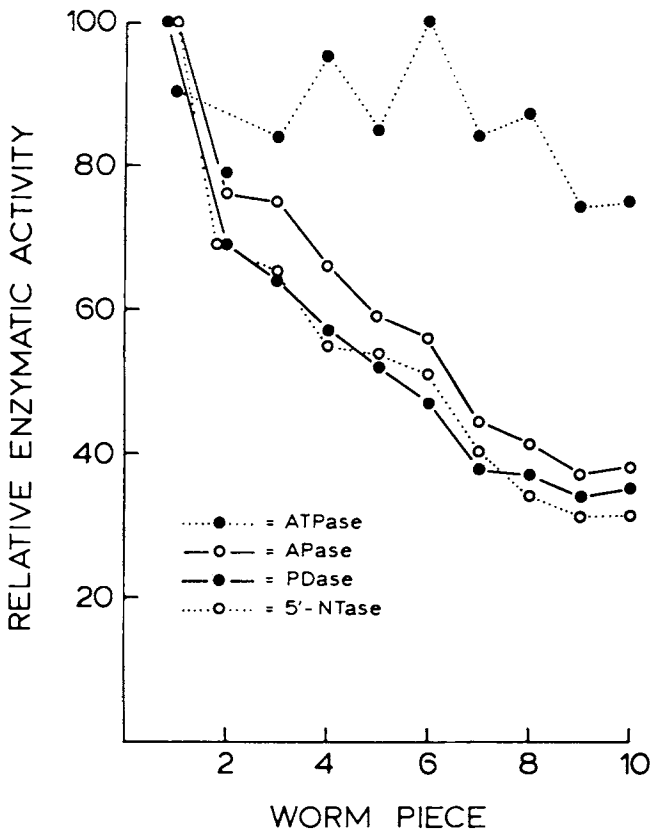

Fig. 2. APase, PDase, NTase (5′-NTase), and ATPase activities of the brush-border plasma membrane of individual pieces of Hymenolepis diminuta which had been cut into 10 pieces of equal length (piece 1 = anterior end of the tapeworm). Relative enzymatic activities were calculated as in Figure 1. Values represent the means of triplicate assays.

(ie, a qualitative change) or a decrease in the amount of enzymatically active membrane proteins relative to enzymatically inactive membrane proteins (ie, a quantitative change). The data presented in the report indicate that the membrane proteins responsible for APase activity do not change qualitatively; neither the pH optimum nor the affinity of the enzymatically active membrane proteins for substrates and inhibitors change as the tapeworm matures. Rather, the data support the hypothesis that the ratio of enzymatically active to enzymatically inactive membrane proteins changes as the tapeworm matures.

It is not clear at this time whether the quantitataive changes in the membrane proteins are a result of (1) a decrease in the absolute amount of enzymatically active membrane proteins, (2) an increase in the absolute amount of enzymatically inactive membrane proteins, or (3) a combination of 1 and 2 above. The mechanism by which these quantitative changes are manifested could be elucidated, at least in part, if the enzymatically active membrane proteins could be isolated, purified, and quantified. However, previous attempts at isolating and purifying the enzymatic activities

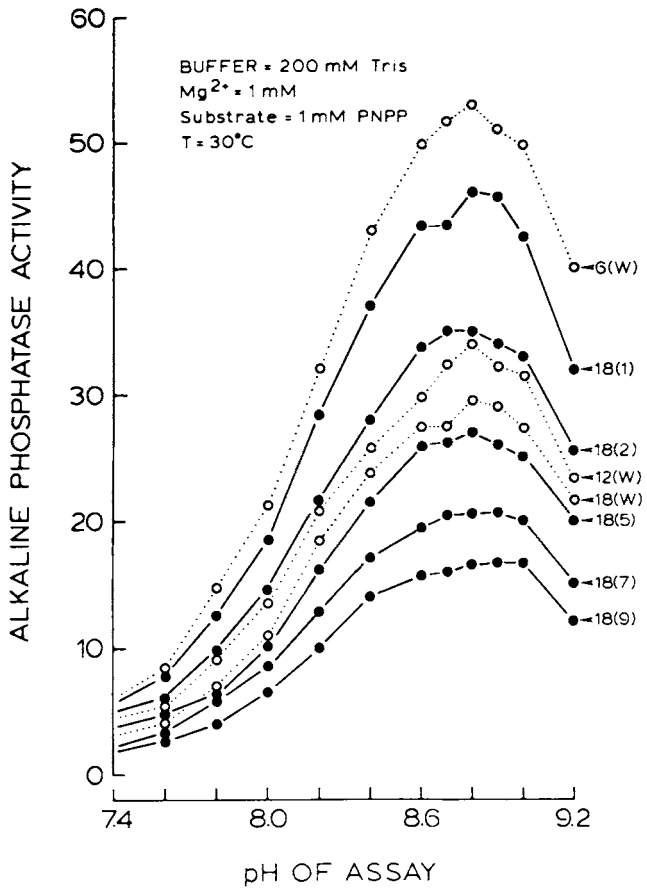

Fig. 3. APase activity, as a function of pH, of the brush-border plasma membrane of whole 6-, 12-, and 18-d-old Hymenolepis diminuta [6(W), 12(W), and 18(W), respectively], and 18-day-old tapeworms cut into 10 pieces of equal length [18(1) through 18(10), where 18(1) = anterior end of the tapeworm]. For purposes of clarity, pH profiles for pieces 18(3, 4, 6, 8, and 10) have been omitted. APase activities were calculated as in Figure 1, and points represent the means of triplicate assays.

associated with the brush-border plasma membrane of H diminuta have been unsuccessful [6].

In addition to quantitative changes in the brush-border membrane proteins noted in the current and a previous study [1], qualitative changes also occur. Such qualitative changes are suggested indirectly by the alterations in membrane transport constants (K_t) for amino acids, monosaccharides, and nucleosides during growth and maturation of H diminuta [7–11], and directly by analyses of the brush-border membrane proteins by electrophoresis [1].

The processes leading to membrane biogenesis and turnover in tapeworms, although not elucidated completely, appear similar to those occurring in vertebrate cells [11,12]. Additionally, the composition and biochemical properties of the tapeworm's brush-border plasma membrane change as the tapeworm matures or ages,

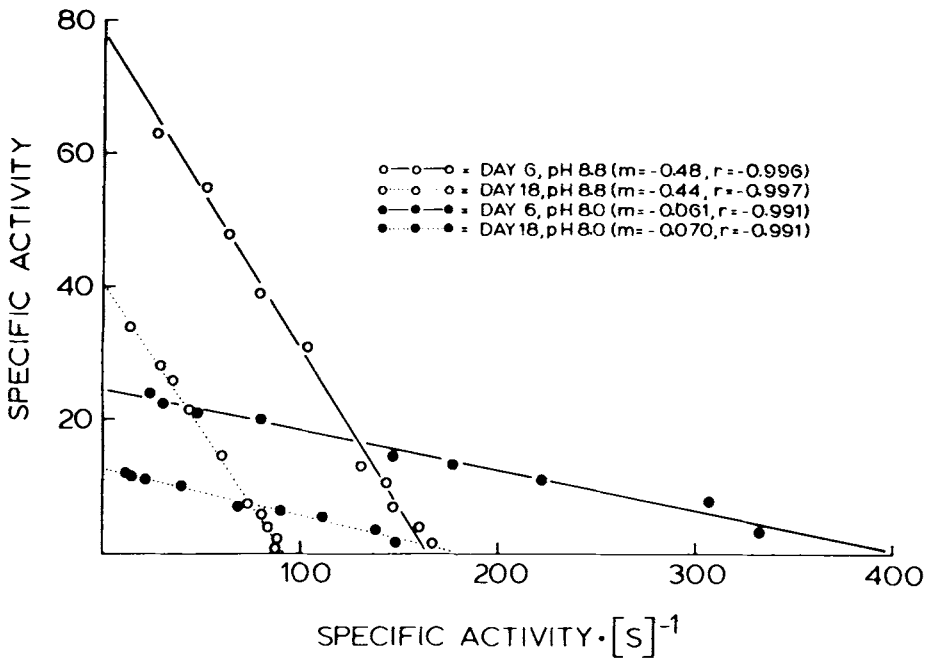

Fig. 4. An example of the graphic analysis used to determine maximum specific activities (V_{max}) and apparent Michaelis constants ($K_{m\ app}$) of the APase activity associated with the brush-border plasma membrane of Hymenolepis diminuta. In this type of analysis, V_{max} = the Y-intercept and $K_{m\ app}$ = −(m). The data presented represent membrane preparations from whole 6- or 18-d-old tapeworms, and APase activities at pH 8.8 and 8.0 (see figure legend). Values represent the means of triplicate assays.

TABLE I. A summary of the Kinetic Parameters Describing APase Activity Associated With the Brush-Border Plasma Membrane of Whole 6-, 12-, and 18-d-Old Hymenolepis diminuta, and Individual Pieces of 18-d-Old Tapeworms*

	pH 8.8		pH 8.0	
	V_{max}	$K_{m\ app}$	V_{max}	$K_{m\ app}$
6(W)	78.4	0.480	24.6	0.061
12(W)	44.1	0.473	14.4	0.068
18(W)	40.7	0.439	12.7	0.070
18(1)	64.0	0.479	16.7	0.062
18(2)	44.4	0.479	13.2	0.068
18(3)	42.8	0.460	13.4	0.066
18(4)	36.3	0.445	12.5	0.063
18(5)	34.4	0.553	ND[a]	ND[a]
18(6)	33.1	0.437	11.9	0.063
18(7)	27.4	0.504	ND[a]	ND[a]
18(8)	25.1	0.459	9.1	0.073
18(9)	21.3	0.488	ND[a]	ND[a]
18(10)	20.1	0.469	8.9	0.094

*Abbreviations as in Figure 3. Maximum specific activities (V_{max} = μmol · mg^{-1} · min^{-1}) and $K_{m\ app}$ (mM PNPP) were calculated as demonstrated in Figure 4; $r \leq -0.98$ in all cases.
[a]ND, not determined.

TABLE II. A Summary of Apparent Inhibitor Constants (K_i' = mM inhibitor) for P_i, 5'-AMP and 5'-ATP Acting as Inhibitors of the Membrane-Bound APase Activity of Whole 6-, 12-, and 18-d-old Hymenolepis diminuta, and Individual Pieces of 18-d-old Tapeworms*

	Inhibitor		
	P_i	5'-AMP	5'-ATP
6(W)	0.752	0.446	1.119
12(W)	0.801	0.486	1.439
18(W)	0.940	0.451	1.021
18(1)	0.852	0.550	1.016
18(2)	0.849	0.645	1.217
18(3)	0.945	0.614	1.008
18(4)	0.829	0.470	1.028
18(5)	0.900	0.475	ND[a]
18(6)	0.894	0.445	1.143
18(7)	0.915	0.448	ND[a]
18(8)	0.861	0.553	0.919
18(9)	0.704	0.405	0.951
18(10)	0.904	0.529	1.398

*Abbreviations as in Figure 3. Values for K_i' were calculated using the graphic analysis described in the Methods section and the V_{max} values listed in Table I.
[a]ND = not determined.

and similar changes are demonstrable in the cell membranes of diverse animal groups [13]. Thus, tapeworms may provide an excellent and unique model system for the study of age-related changes in membrane structure, function, biogenesis, and turnover.

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