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Preliminary studies on the purification and biochemical characterization of cyclooxygenase from *Setaria digitata*

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Global elimination strategy of lymphatic filariasis, which is endemic in over 80 countries, is based on a limited number of therapeutic agents: diethylcarbamazine (DEC), ivermactin and albendazole. To achieve total eradication of the disease, it is essential to develop new macrofilaricidal drugs or drugs to permanently inhibit microfilariae production. A novel approach in this respect is identifying parasite specific enzymes as targets to new drugs. Cyclooxyganase (COX) enzyme is the regulatory enzyme of the committed step in the biosynthesis of prostaglandins and thromboxanes. These products of the COX enzyme have been speculated to play an important role in the host-parasite interactions. And therefore might be a potential target for drug development. Since *Wuchereria bancrofti* cannot be cultured, the phylogenetically related cattle filarial parasite *Setaria digitata* was used in the purification and biochemical characterization of a COX enzyme(s). Crude COX enzyme extract prepared from the solubilization of microsomal vesicles obtained from 10,000 g centrifugation of homogenized parasitic material was directly subjected to fast protein liquid chromatography (FPLC) on an anion exchange column. Active fractions were further purified isocratically by FPLC on a gel filtration column. We managed to achieve approximately 89 fold purification of the Cox enzyme with a specific activity of 2377.9 nmol hydroperoxide reduced min/mg with H_2O_2 as substrate by utilizing NNN'N'-tetramethyl-p-phenylenediamine (TMPD) as the chromogenic cosubstrate. K_m and V_{max} were studied utilizing arachidonic acid, the specific substrate for the COX enzyme(s) and the approximate K_m value for peroxidase activity for arachidonic acid was found to be 3.75 µM with V_{max} being 12.50 µM/min.

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