Comparative biochemical characterisation and inhibitory profiling of cattle tick, human, bovine and mosquito Flavin Adenine Dinucleotide sub-domains.

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Abstract:

The Southern Cattle Tick, *Rhipicephalus microplus*, is a significant pest of tropical and sub-tropical regions of the world, who's ectoparasitism by hematophagy results in health detriments to domestic cattle because of anaemia and transmission of erythroparasites. Subsequently, this results in severe economic losses to farmers, many of whom are in developing regions and rely on their cattle for food security. These issues are additionally exacerbated by resistance in tick populations to active ingredients of common pesticides/acaricides. One of the mechanisms underlying resistance is an increased rate of acaricide inactivation/metabolism through upregulation of detoxifying enzymes such as cytochrome P450s (CYPs). In addition to their role in detoxification, CYPs also are essential for many endogenous reactions such as hormone biosynthesis. All CYPs require a single redox partner cytochrome P450 oxidoreductase (POR) to facilitate the supply electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to the enzyme to catalyse the monooxygenation of a substrate. Considering this fact, it is reasoned that inhibition of POR function would consequently shut down all CYP catalysed reactions, resulting in multi-system failure within an organism. One of the first steps necessary to use this approach successfully in the control of acaricide resistance ticks, is determining if there are biochemical differences between host and parasite POR isoforms that can be exploited for the development of specific inhibitory agents. Therefore, the aim of this work was to express and biochemically characterise POR from *R. microplus*, as well as the domestic cow, *Bos taurus,* in terms of their kinetic parameters for NADPH and the inhibitory activity of various adenine nucleotide analogues. To extend the value of this study, POR from the malarial mosquito, *Anopheles gambiae*, and from *Homo sapiens* were included. Previous work had indicated reduced expression of a soluble membrane truncated full-length POR from *R. microplus*, therefore a decision was made to express and characterise the conserved flavin adenine dinucleotide (FAD) binding domains from all species, which can accept electrons from NADPH and facilitate transfer to pseudoredox partners such as potassium

ferricyanide. All purified FAD binding domains displayed a characteristic flavin oxidoreductase spectrum with absorbance peaks at ~379 nm and ~454 nm. Michaelis-Menten constants for NADPH as a substrate were calculated as 35.98, 62.57, 66.2 and 110.18 µM respectively for RmFAD, BtFAD, AnFAD and HFAD. The turnover, K_{cat}, for NADPH was calculated as 0.1, 0.62, 0.96 and 1.71 sec^{-1} respectively for RmFAD, BtFAD, AnFAD and HhFAD. IC_{50} (Half maximal Inhibitory Concentration) values obtained for the adenosine analogues 2'-AMP and $NADP^+$ were 3.5 mM and 190.4 µM for RM FAD; 4.7 mM and 119.6 µM for BtFAD; 3.58 mM and 149.7 µM for AnFAD and finally, 14.38 mM and 209.5 µM for HFAD. An IC_{50} value for 2', 5'-ADP could only be determined for AnFAD, 5.61 mM. Comparison of the kinetic and inhibitory profiles across all four species identified potential biochemical differences between host and parasite for both *R. microplus* - *Bos taurus* and *Anopheles gambiae* - *Homo sapiens* pairings, and that from the adenine analogue inhibitory data, that as expected, *R. microplus* and *Anopheles gambiae* are more similar biochemically to each other than to their respective hosts. These findings, therefore, support the potential of POR as a target for the rational design of safer and more potent insecticides/acaricides against parasite populations that have developed metabolic resistance through modification of CYP activity.