ABSTRACT

Yellow-related proteins (YRPs) form an abundant protein family, whose members were found in salivary gland transcriptomes of all sand fly species studied up to date. This protein family belongs to the group of MRJP/Yellow proteins occurring in insects and some other organisms. Though sharing similar folding as a six-bladed β-propeller comprising central tunnel, MRJP/Yellow proteins adopted different functions. The structure and biogenic amine-binding property described for the sand fly salivary YRPs was based on a single study conducted on *Lutzomyia longipalpis*.

In the present work, we have modelled the structures of 32 salivary YRPs belonging to 13 different sand fly species. We have shown the general structural similarity of these proteins along with both inter- and intra-specific differences in surface charge, tunnel parameters and in amino acids composition of the amine-binding motif. These modifications indicated divergence in function of individual YRPs, which was experimentally verified in the second project focused on identification of the amine-binding properties of YRPs in two important vectors of *Leishmania*; *Phlebotomus perniciosus* and *P. orientalis*. In each species, two YRPs differ in affinities for biogenic amines serotonin, histamine and catecholamines. However, in both sand fly species the highly abundant YRPs (rSP03B and rPorASP4, respectively) were demonstrated to bind with high-affinity serotonin. This suggested their potential ability to facilitate inhibition of the vasoconstriction and platelet aggregation at the fly feeding site.

Apart from their effect on haemostatic responses, the very same YRPs of *P. perniciosus* and *P. orientalis* were shown to elicit antibody response in bitten hosts, which can be used as a marker of sand fly exposure. In *P. perniciosus*, we have applied the recombinant YRP (rSP03B) to screen for canine exposure in a longitudinal field study in Italy. We have detected a high correlation between the canine anti-rSP03B antibody response and the response against the whole salivary glands. The kinetics of antibody response measured by both antigens revealed a comparable pattern related to the annual activity of *P. perniciosus*. Protein rSP03B was therefore shown as a valid tool to screen for canine exposure. Similarly, in *P. orientalis* a recombinant YRP (rPorASP4) was shown strongly antigenic for humans exposed to this sand fly in Sudan and Ethiopia. The antibody response against rPorASP4 correlated well with the response against the whole salivary glands. Combination of this protein with another antigen (antigen 5-related protein) significantly increased the performance of the test, and therefore represents the best tool to screen for human exposure to *P. orientalis* in Eastern Africa.