SHORT COMMUNICATION

Cyclic 3′-5′ Guanosine Monophosphate-dependent Activity in *Leishmania amazonensis*

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Although there are some data concerning the nitric oxide and the cyclic 3'-5'guanosine monophosphate (cGMP) signaling pathway in trypanosomatids, there is no report about the cGMP-dependent enzymatic activity identification. In this sense, a cGMP dependent activity was detected on soluble fraction from Leishmania amazonensis *promastigotes with a high metacyclic level. This information is valuable in order to explore the metabolic pathway of G kinase protein in this parasite.*

Key words: *Leishmania amazonensis* - cyclic 3'-5'-guanosine monophosphate - metacyclogenesis

Leishmania spp. are evolutionary ancient protozoans that cause a spectrum of diseases ranging from asymptomatic to lethal ones. Despite the fact that 12 million new cases of leishmaniasis occur each year, current options for disease control and treatment are limited (Modabber 1987). A more effective and less toxic treatment, as well as a protective vaccine, is needed to manage these diseases.

In mammalian, protein kinases are a family of enzymes that participate in a wide number of processes as metabolism, gene expression, growth and cell differentiation, but their precise functions are not well defined (Vo et al. 1998). Protein kinases in parasites have been described (Ulloa et al. 1988, Gómez et al. 1989,1999, Farber et al. 1997, Flawiá et al. 1997, Shalaby et al. 2001). In *Leishmania* evidences indicate that the parasite possesses the capability of regulating the properties and function of, not only its own cell surface macromolecules, but also those of host cells through phosphorylation and desphosphorylation reactions (Glew et al. 1988, Vannier-Santos et al. 2002).

Considering the importance of the signaling pathway between cells and also the fact that little has been described about the phosphorylation activity associated to the cyclic 3'-5' guanosine monophoshate (cGMP) in *Leishmania* spp. our study was intended to evaluate this enzymatic activity in promastigotes of *L. amazonensis*.

The enzymatic activity dependent on cGMP was detected in soluble fraction (FS) from *L. amazonensis* promastigotes (MHOM/BR/77/LTB0016, kindly provided by leishmaniasis laboratory of the Instituto Oswaldo Cruz, Rio de Janeiro. The parasites were maintained by continuous passage in Balb/c mice. Amastigotes isolated from infected foot were propagated as promastigotes in Schneider's insect medium supplemented with 10% fetal calf serum. Parasites were routinely sub-cultured every 72 h and freshly transformed virulent promastigotes were obtained. The FS, supplemented with inhibitor of proteases, was separated by a fast protein liquid chromatography system on MONO Q column (HR 5/5) with a linear gradient of 0-400 mM NaCl in 25 mM Tris/HCl pH 7.4 (Patel & Diamond 1997). Different peaks were obtained and the enzymatic assay was determined by measuring ^{32}P incorporation from [γ-^{32}P] ATP as described previously (Wolfe et al. 1989). Activity was detected in a peak eluted with 0.14 M of NaCl and the activity was higher in the presence of the cyclic nucleotide (10 µM cGMP). The increase of the activity was statistically significant ($p = 0.0255$) when compared with the reaction in the absence of cGMP. The activity associated to cGMP was significantly reduced ($p = 0.0077$) in the presence of 10 µg/ml of Arg-Lys-Arg-Ala-Arg-Lys-Glu, a synthetic peptide inhibitor of the cGMP-dependent protein kinase, PKG (Figure). Previous studies have demonstrated that AMP-dependent protein kinase (PKA) and PKG have similar catalytic activities, suggesting that high homology exists among the catalytic domain of these two enzymes (Yan et al. 1996). In this work 200 µg/ml of an specific PKA inhibitor, PKAI (TTYADFIAS GRTGRRNAIHD) was added to the reaction mixture, to eliminate any contribution from its activity to the kinase activity investigated.

As far as we know, this is the first report about the cGMP dependent activity in *Leishmania* spp. but the biological significance of the above mentioned data remains to be defined. New knowledge about enzymes, proteins and other macromolecules that are part of important metabolic ways for the parasite could supply information about their function and the role in the life cycle or in the infectivity of the protozoa.

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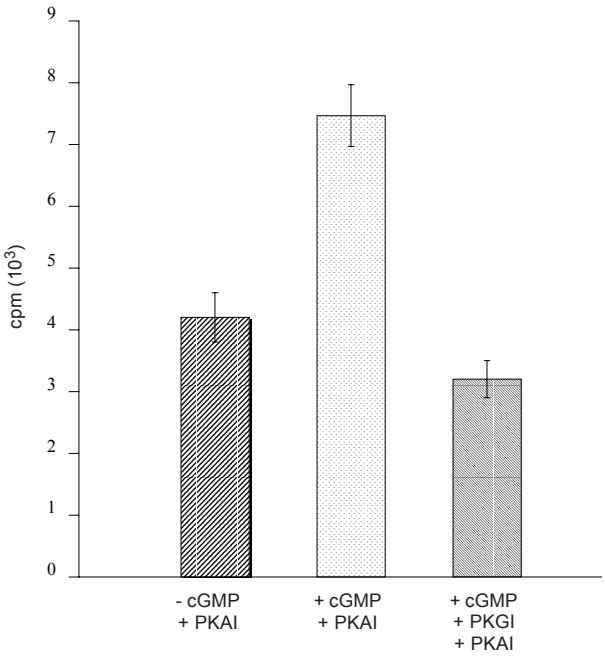

Cyclic 3'-5'-guanosine monophosphate (cGMP)-dependent enzymatic activity of the peak obtained with the linear gradient by ion exchange chromatography. The enzymatic activity was measured in absence ( ) and in the presence ( ) of cGMP. A synthetic peptide inhibitor of the protein kinase (PKG) (PKGI,) was also evaluated. In all experiments a PKAI was included. Results shown here represent a means of three experiments.

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