BIOLOGICAL ACTIVITIES OF PROTEIN ISOLATED FROM *LONOMIA OBLIQUA* HEMOLYMPH

Mendonça, RZ[1] Souza, APB[1]; Maranga, L[2]; Peixoto CC[2]; Moraes, RHP[1]; Silva PI[1] ; Pereira, CA[1] and Carrondo, MJT[2]
(1) Laboratório de Imunologia Viral, Instituto Butantan, Av. Vital Brasil 1500, Sao Paulo, Brasil; fax:(55) (011) 3726.1505;
e-mail: zucatelli@uol.com.br (2) Instituto de Biologia Experimental e Tecnologica (IBET), Oeiras, Portugal
Keywords: hemolymph, Sf-9, biologycal activities

Introduccion: Four decades after Grace's first addition of hemolymph to insect cell culture media we are still learning and understanding the extraordinary properties of this supplement. Recently several proteins with biotechnological interest were seem to be present in insect hemolymph as cell growth factor , protector factor and antimicrobicide agents. This represent a challenge and an opportunity for future research with the system.

Material and Methods

Spodoptera frugiperda (Sf-9) cells were grown in 100 ml schott flasks containing Grace's medium (Gibco) supplemented with 10% fetal bovine serum (FBS). The cultures were incubated at 28ºC using a shaker at 100 rpm.

Hemolymph feeding : Hemolymph of *Lonomia obliqua* was collected from sixth-instar larvae after setae cut-off. The collected hemolymph was centrifuged and the supernatant was filtered with 0.2 µm membrane filter and used afterwards for medium supplementation (1, 2 or 5%)

Analytical procedures: Samples were obtained daily and the cell concentration was measured by hematocytometer cell counts and cell viability was evaluated by trypan blue exclusion dye. Nutrients concentration were determining enzymatically using a YSI biochemical analyzer model 2700by.

Hemolymph fractionation by chromatography: Total and fractions of hemolymph, were loaded on "Superdex 75", "superdex peptide HR", "Resource Q", "Resource S", and reverse phase column (Amersham Pharmacia Biotech). The purified fractions and sub-fractions were applied at SDS-PAGE electrophoresis for analysis and added to Sf-9 cell cultures for cell activity studies.

SDS-PAGE Electrophoresis: SDS-PAGE Electrophoresis of each fraction or sub-fractions were carried out using 9% polyacrylamide separating gels (400 mA).

Results and discussion: A potential enhancing and protector factor of Sf-9 cell growth from *Lonomia obliqua* hemolymph were isolated and characterised. The addition of hemolymph to the medium induced high levels of cell growth reaching a maximum cell concentration 2-fold higher than the control. These actions were much dependent of the hemolymph concentration. Hemolymph was too very effective in enhancing Sf-9 cell culture under conditions of 5% FBS. It was also observed that while low hemolymph concentration has a positive effect in cell growth, high hemolymph concentration show a deleterious effect. One novel protein that converts sucrose to glucose was too isolated. Various concentrations of hemolymph were tested and the glucose concentration was determined. Addition of hemolymph to the medium induced high levels of glucose production. The sucrose (present in Grace's medium) to glucose conversion was linearly dependent of the hemolymph concentration. Sucrase (sucrose hydrolase) activity was higher in culture medium supplemented with salt solution (Na^+, Ca^{2+}, Mg^{2+}) being this effect more intensive with Na^+ salt. This could be due to the cations be necessary for the enzyme activity. The glucose production was linearly dependent of the hemolymph concentration, reaching a value of 10 mmol L^{-1} day^{-1} at 10% of hemolymph concentration. One third protein present on hemolymph showed a beneficial effect in insect cell cultivation since that the viability was maintained for longer periods of time after hemolymph supplementation maybe due to a potential anti-apoptotic effect. Some small peptides (10-15 a.a.) were seem also to have a potent antimicrobial effect against micrococcus luteus

Conclusion:

The date obtained in this study showed that cell culture supplementation with hemolymph can be an important tool to obtain high cell culture densities. The supplementation of the culture medium by hemolymph enhanced insect cell culture growth twice and longevity of viability for more than 4 days. The proteins of interest were effectively resolved by gel filtration and ion-exchange chromatography. The sucrase activity was clearly identified and isolated and a potent antimicrobial agent was identified and isolated.

Acknowledgements

The authors acknowledge and appreciate the financial support received for a Post-Doctorate fellowship to R.Z.Mendonça from FAPESP (01-09040-8) and to L.Maranga from Fundação para a Ciência e Tecnologia - Portugal (PRAXIS XXI/BD/16136/98).

References: Kanaya T, Kobayashi J. Purification and characterization of an insect haemolymph protein promoting in vitro replication of the Bombyx mori nucleopolyhedrovirus. J Gen Virol. 2000 Apr;81 Pt 4:1135-41.

Kim EJ, Rhee WJ, Park TH. Isolation and characterization of an apoptosis-inhibiting component from the hemolymph of Bombyx mori.. Biochem Biophys Res Commun. 2001 Jul 13;285(2):224-8.

Rhee WJ, Kim EJ, Park TH. Kinetic effect of silkworm hemolymph on the delayed host cell death in an insect cell-baculovirus system Biotechnol Prog. 1999 Nov;15(6):1028-32.

Rhee WJ, Park TH.Silkworm hemolymph inhibits baculovirus-induced insect cell apoptosis. Biochem Biophys Res Commun. 2000 Apr 29;271(1):186-90.

Woo SD, Kim WJ, Kim HS, Choi JY, Jin BR, Kang SK. Effect of silkworm hemolymph on the expression of E. coli beta-galactosidase in insect cell lines infected with recombinant baculoviruses. Mol Cells. 1997 Aug 31;7(4):572-4.