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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (10): 1703-1705, 2004
ISSN 1028-8880

Fractionation of Lizard *Leishmania promastigote* Protein

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Abstract: The objective of this study was fractionation of Lizard *Leishmania promastigote*. Mass culture of lizard *Leishmania promastigote* was provided. Fractionation was carried out by ammonium sulfate precipitation and size exclusion chromatography. The 19.2, 21.5, 25, 30, 35, 64 and 78 kDa fractions were found and they used for vaccination against mouse leishmaniasis.

Key words: lizard leishmania, fractionation, size exclusion chromatography

INTRODUCTION

Although drug therapeutic still in use has some good effects in leishmaniasis, but appearance of new cases of drug resistance, caused the world hygiene to get nervous about the treatment and start designing new vaccines which offer long lasting prevalence to leishmania infection[1].

There were some procedures for control of leishmaniasis like: leishmanization[2], use of non pathogenic *Leishmania* strains[3], vaccination by killed[4] or attenuated *Leishmania promastigote*[5] and use of membrane lipophosphoglycan[6], autoclave promastigotes[7] and oral vaccine[8].

This research was planned to fractionate lizard *Leishmania promastigote* protein by ammonium sulfate precipitation and size exclusion chromatography in order to apply them for vaccination against *Leishmania major*.

MATERIALS AND METHODS

Parasite: A lizard *Leishmania promastigote* has been isolated from Shahrood region of Iran through lizard heart blood culture on NNN medium[9]. Promastigotes were grown on NNN medium and mass culture was provided in $RPMI_{1640}$ medium supported by 20% fetal calf serum.

Crude antigen preparation: To prepare the crude extract of the proteins, A mass culture of promastigotes was accomplished and continuous freeze/thaw actions followed by a final sonication.

Ammonium sulfate precipitation: The extract was fractionated applying different concentrations of ammonium sulfate in the order of 10, 20, 30, 40, 50, 60, 70 and 80%[10]. The concentration of the salt was determined according to the standard table[10], considering the total volume of the crude extract in every different step. The salt and obtained extract were mixed together properly at low temperature (0°C) and then centrifuged. The pellets of each step and the supernatant of the final step were dialyzed against phosphate buffered saline (pH = 7.4) at 4°C to remove the accompanying salt of the proteins extracted. Finally, the molecular weights of different fractions were determined using SDS-PAGE[11].

Chromatography: The dialyzed ammonium sulfate precipitation proteins were fractionated by size exclusion chromatography through sephadex gel. Then the optical density (OD) of all different fractions was determined at 280 nm (same molecular weight on SDS-PAGE were mixed) and the final concentration of proteins in each fraction assigned by Bradford standard test. These fractions of protein will be used as antigen (Table 1).

RESULTS AND DISCUSSION

Two fractions of 25 and 35 kDa were isolated by ammonium sulfate precipitation process and five fractions of 19.2, 21.5, 30, 64 and 78 kDa by chromatography process (molecular weights are determined using SDS-PAGE (Table 1).

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Table 1: Fractionation of lizard leishmania promastigotes protein by size exclusion chromatography and their molecular weights

Protein fraction name.	Protein fraction MW (dalton)
F1	78000
F2	64000
F3	30000
F4	35000
F5	19200
F6	21500

Recently the numerous active strategies have lead to research on leishmania vaccines[12-15] and TDR has decided to produce an appropriate vaccine against leishmaniasis.

In this research in order to fractionate and detection of antigenic details, Gel filtration method was used. It supposed that this method is an appropriate replacement for polyacrylamide gel electrophoresis in order to provide nearly leishmania antigenic components[17].

By definition in gel filtration we may detect enormous amount of an antigen, depending on column size, every time. Whereas in electrophoresis method in order to having enough amount of an antigen we have to do this protocol several times. Not only later technique spends more time and is more expensive, but also it has lower sensitivity and specificity related to changing percentage of stacking gel, buffer pH or electricity current pulses. All of these disorders may influence pattern of electrophoretic mobility and isolation of component. Isolated bands in this method may lack antigenic activity, because of protein denaturation reagents like SDS and 2 Mercaptoethanol. Components, which gained from gel filtration technique, are appropriate and useable in laboratory cell culture that has been led to proliferative responses and determination of excreted cytokines in cell culture medium. It is related to not being any SDS or nonpolymerized acrylamide substances.

This study detected seven protein fractions of lizard *Leishmania promastigotes*, including, 19.2, 21.5, 30, 64, 78, 25 and 35 kDa which the last two fractions have been sedimented by ammonium sulfate precipitation.

There are similar studies to the present research like gp63 (glycoprotein), which Murry *et al.*[18] detected this glycoprotein during purification of proteins of *L. major* by using Triton X-114, a non-anionic detergent. Cardoso isolated 7 fractions include of 42, 46, 63, 66, 73, 87, 97 and 160 kDa of American *Leishmania* by polyacrylamide gel electrophoresis and electro elution and tried to vaccinate the C57BL/10 mice with them[17]. Malekzadeh *et al.*[19] isolated fractions of promastigote forms of *Leishmania major* include: 40-60, 60-80 and 80-100 KD proteins and used for vaccination against *Leishmania major*. During the study on *Leishmania major* antigenic components.Hejazi *et al.*[16] detected 26 bands(10-112 KD) by column chromatography with cephacryl and SDS-PAGE. He isolated 18, 40, 53, 71, 80, 252 kDa proteins by native PAGE and obtained the membrane antigens includhng: 20 kDa by triton X-114 and ultra filtration.

ACKNOWLEDGMENT

This research was supported by WHO grant No. T5/72/6.

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