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ISOLATION AND DETERMINATION OF PROTEIN FRACTIONS OF *Trichophyton mentagrophytes* Var.*erinacei* USING GEL FILTERATION CHROMATOGRAPHY

Afrah Z. Risan,

Chemistry Department, Faculty of Science,Basrah University

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Summary

The protein in broken-cell extract from *Trichophyton mentagrophytes var.erinacei* was isolated *and* fractionated using sephadex G-100 gel filtration technique. On ultra-violet absorption of the isolated fractions was measured at 280 nm. the molecular weight of these fractions was calculated and found to be four fractions ranging :102,000,220,000,425,000 and 610.000 Daltons. Amino acid analyses showed a predominance of the following: glutamic acid ,glycine ,proline,valine,methionine and cyctine.In conclusion the information derived may be useful in the classification of *Trichophyton* species.

Key words: Protein fraction; Trichophyton mentagrophytes Var.erinacei ;Gel filtration method

Introduction

The prevalence of dermatophyte infections varies greatly, at least 10-15% of the world populations. Trichophyton mentagrophytes, a Zoophilic dermatophyte causes a superficial infection of skin by invading and parasitizing only the non-living keratinized layers [1]. However, many of limgus-host interactions are dependent on specific moieties and enzymes produced by dermatophytes and yeasts [2-5]. For example, protein molecules represent one of the major components of the fungal cell, through, it occurs as mannoprotein polymers or enzymes that play a vital role in host invasion [6].

Despite the different techniques which have been used for the separation and the purification of proteins, the use of gel filtration chromatography permits a rapid and effective separation when the protein has high molecular weight [7]. However, there is no available data on the Chemical definition of the constituents of *Trichophyton mentagrophyres* var. *erinacei,* except some papers reported on this strain and other strains [8-13]. In lraq, data represent cornerstone in this region of the world about dermatophytes and yeasts infection [14-l7].The aim of this investigation was to extract, separate and determine proteins molecular weight from

dermatophyte isolate, *Trichophyton mentagrophyres* var. *erinacei* using a gel filtration chromatography.

Materials And Methods

Test organism:

Conventional methods of isolation, identification were used, then the residual mycelia were stored at -20°C until proceeded for extraction and elution chromatography [18].

A strain of *Trichophyton mentagrophyres* var. *erinacei* was kindly supplied by Dr. A.H. Aubaid were cultured for four weeks on sabouraud's 2% glucose Agar (SGA,Oxide); pH 5-6 at 27°C.

Protein extraction:

The crude aqueous extract from mycelia (2,62gm) was mixed with 50ml of 70% ammonium sulphate and put in refrigerator for 24h with magnetic stirrer. The mixture was centrifuged for l2 min. at 12,000 rpm using ultracentrifuge. The sediment was taken and put in dialysis tube (Mwt. cut-off: 6-8xl0^{10} dalton) for dialysis process for 48h in refrigerator with magnetic stirrer against distilled water (distilled water was replaced every 6h). The dialysate was dried using freezdrying machine (Lyophilizer,Virtis comp. U.S.A). The recovery weight was 0.05gm (protein ratio=1.88%).[19].

Column Chromatography of Protein of *Trichophyton mentagrophytes* Var. *erinacei*:

A column of sephadex G-100 (26x0.8 cm) was employed after being buffered and washed with 0.5 M Tris-HCl buffer (pH 7.5).The column was eluted with the same buffer and at a flow rate of 2.5 ml per 45 min .The column was calibrated with blue dextran 2000 to estimate the column void volume, then with standard molecular weight proteins (Thyroglobulin,669,000;Ferritin,440,000;Aldolase,158,000; Bovine serum albumin 67,000; Ovalbumin, 43,000 ;Chemotrypsinogen,25,000 and Ribonuclease A ,13,000)Dalton.A molecular weight calibrated curve (Fig.1) defines the relationship between the elution volumes of a set of standard proteins and the logarithm of their respective mol.wt.. K_{av} values for each protein were estimated using the following equation [20]:

$$Kav = \frac{Ve - Vo}{Vt - Vo}$$

Where:

K_{av} = a distribution coefficient

V_e = elution volume for the protein

V_o =column void volume : elution volume for blue dextran 2000

V_t = total bed volume

Dried dialyzed protein was dissolved in a starting buffer solution and volume of 1,0 ml was applied to the column. Step-wise elution was made with three kinds of buffer systems of varying ionic strength: 0.5M Tris-HCI buffer (pH 7.5); IM Tris-HCI buffer (pH 7.5) and IM Tris-HCI buffer containing IM NaCl (pH 7.5). Each 2.5m| of the eluent was collected by means of fraction collector (LKB: Sweden). An ultra-violet absorption measurements of each fraction at 280nrn using LKB spectrophotometer was made values of absorption were plotted against the elution volume (Fig. 2), then the molecular weight of the separated fractions was calculated.

Amino acid analyses of separated protein fractions were done by their digestion with 6N HCI. The hydrolysate was subjected to one dimensional thin layer chromatography with the developing solvents, n-butanol: acetic acid: water (4: l:2). Amino acids were detected by ninhydrin reaction. Cystine was identified by the nitroprusside reaction [7].

Results and discussion :

The elution curve of protein fractions during three kinds of buffer systems of varying ionic strength at 280nrn is shown in Fig.2, With 0.5M Tris -HCl buffer as eluent, two small peaks appeared in the curve at absorbance value (0.23) and (0.194), respectively. While during the elution with 1M Tris-HCI buffer, also one peak appeared, with high absorbency (0.83). When we used IM Tris-HCI buffer containing lM NaCl as eluent; one large peack appeared with absorbance value (0.867). The fractions corresponding to these peaks were named as F_I. F_{II}, F_{III}, and F_{IV} respectively (Fig. 2).

The molecular weight of the isolated fractions was calculated depending on K_{av} values. The molecular weights of these protein fractions ranged from 102,000,220,000,425,000 and 610.000 Daltons after compared with standard proteins (table 1).On the other hand. the amino acid analyses of the isolated protein revealed the abundance of the following amino acids: Glutamic acid, Glycine,Proline, Cystine, Valnein and Methionine after comparison with the R_f values of standard amino acids (table 2).

The dermatophytes, as well as other fungi, actively secrete products including digestive enzymes such as proteinase and saccharidases into the growth medium. Some of these enzymes may be distinctive and can be distinguished by analytical methods [21]. The first purpose of using protein separation technique in this study was to determine type pattern for *Trichophyton mentagrophyres* var. *erinacei* cultured on sabouraud's 2% glucose broth. This pattern comprising four protein fractions with M.wt. ranging 102,000,220,000,425,000 and 610.000 Daltons. However, the gel filtration chromatography seems to be an adequate method for protein separation because it separates molecules according to size: the large molecules which cannot enter the pores of the gel, are excluded and emerge in the void volume, and the small molecules which can enter, the smaller molecules are retarded. So, this analytical method may be useful method to distinguish in the classification of *Trichophyton* species. This fact was found by other investigators [22] which stated that some taxonomic problems in the genus Trichophyton, for example, controversy exists about the relationship of *T. meginni* to *T. kuryargei*.

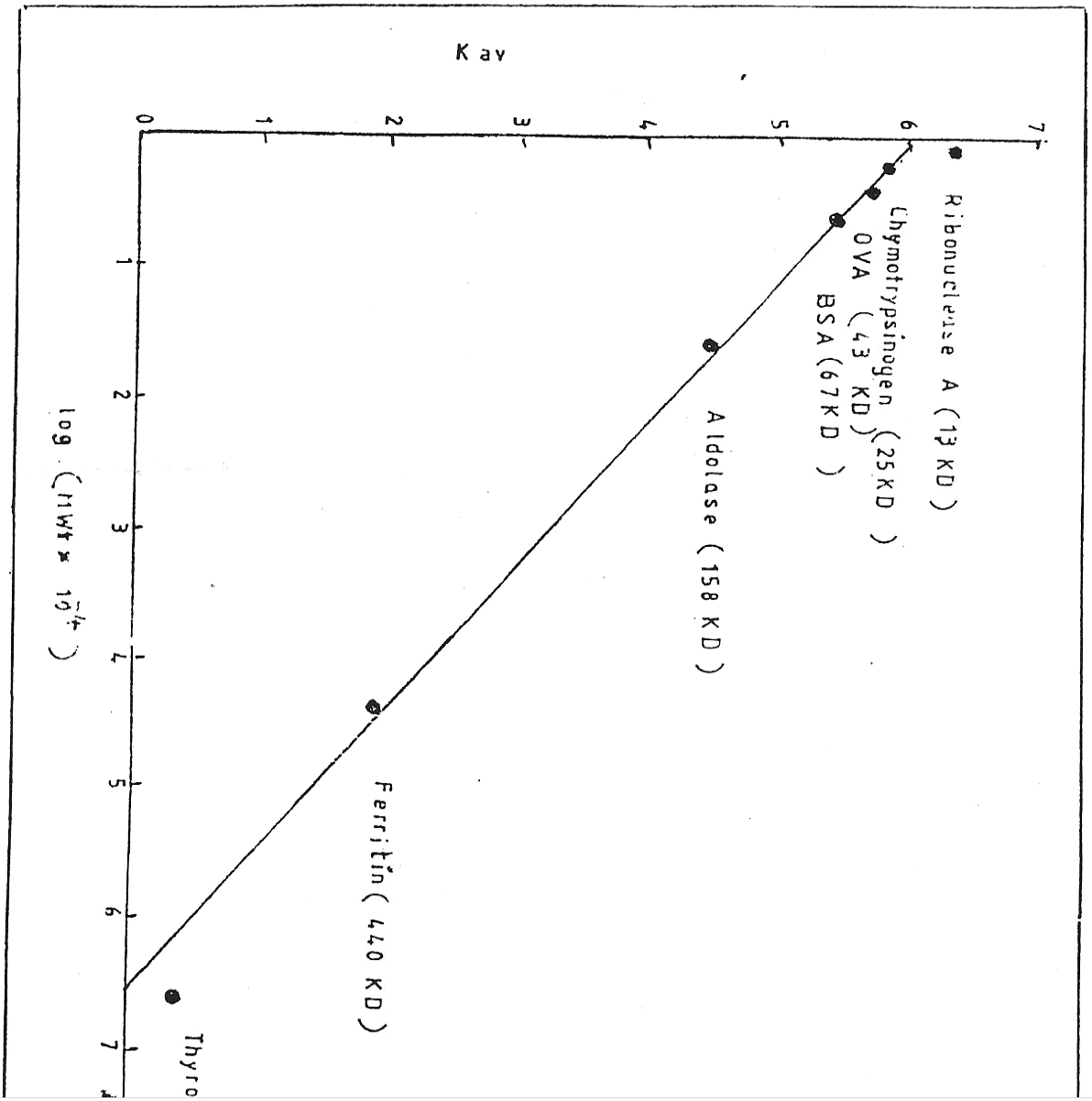

Fig. 1: A standard curve to estimate molecular weight protein fractions using correlation between the K_{av} of reference protein vs log of molecular weight.

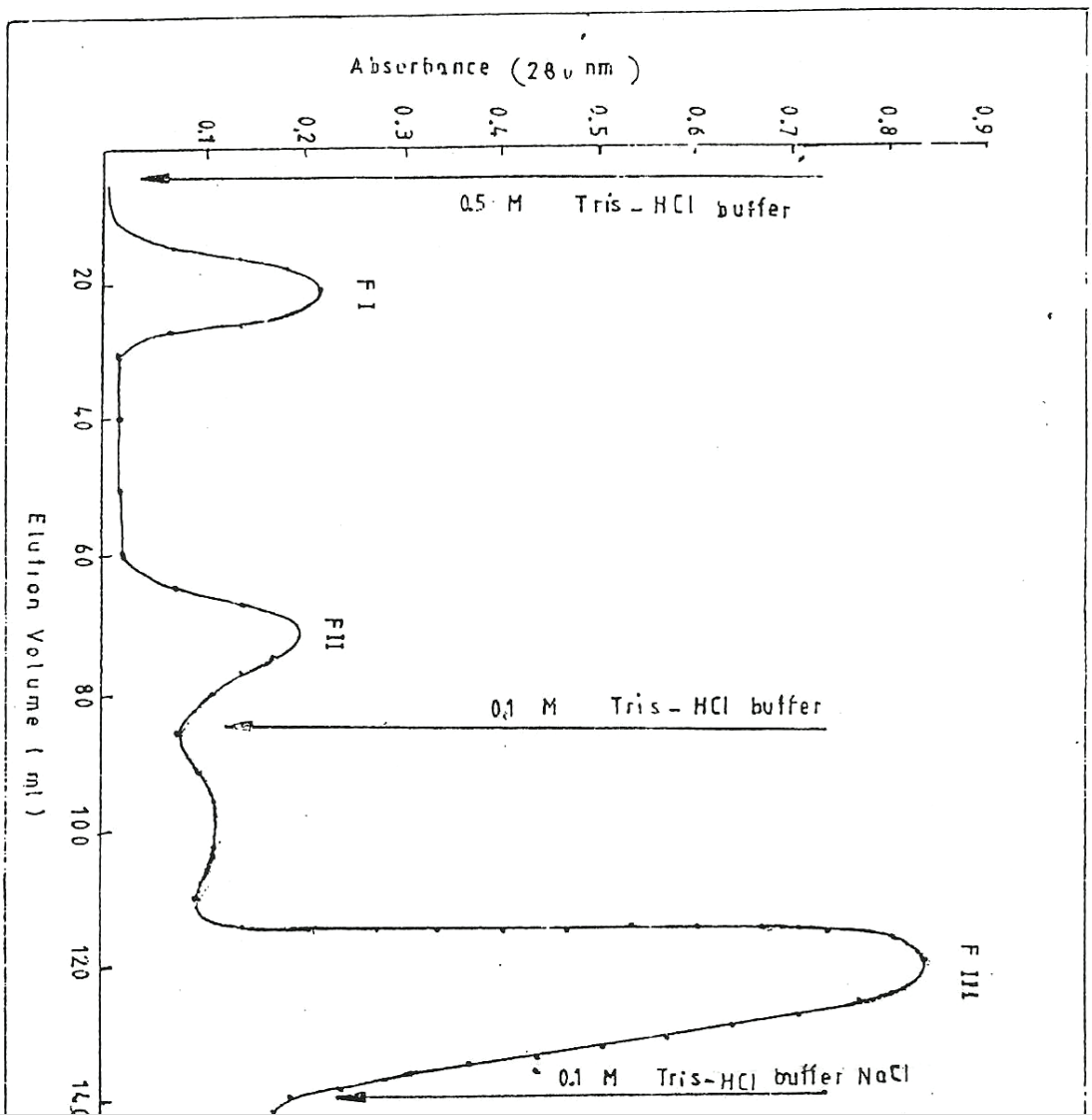

Fig. 2: Sephadex G-100 gel filteration of protein fractions absorbance at 280nm.

Table 1 : *K*av values and molecular weight of fractions isolated by gel filteration chromatography.

Fraction No.	K_{av} value	Molecular weight (Dalton)
F_I.	0-363	610,000
F_{II}	2.04	425,000
F_{III}	4.00	220,000
F_{IV}	5.09	102,000

Table 2 : Amino Acid contents in the protein isolated from *Trichophyton mentagrophytes* Var. *erinacei* and their R_f with it's R_f values of standard amino acid

Amino Acid	Spot size as a relative amount (cm)
Glycine	1.8
Threonine	1.5
Serine	1.6
Glutamic acid	1.8
Valine	1.7
Cystine	1.7
Histidine	1.5
Aspartic acid	1.5
Alanine	1.1
Phenylalanine	1.0
Isoleucine	1.0
Methionine	1.7
Proline	1.7
Lysine	0.9

The potential of gel filtration chromatography to resolve complex mixture of proteins has not been broadly applied to the mycotic pathogens The major application thus for has been in characterization of antigenic extracts from yeast-form cell wall [23] or culture broth filtrate [24]. At present, disc electrophoresis in polyacrylamide gels and analytical ultracentrifugation applied to several genera of dermatophyte provided distinctive patterns of proteins from culture filtrate which differentiated the genera *Epidermophyton*, Microsporum and *Trichophyton* [25-26].

Amino acids analyses of the total protein showed an agreement with a previous study [17] reported about the antigenic constituents of the same isolate under the present study, with exception, the isolated number or kind of amino acid was less when compared with the previous study. This may be due to the differences or manner of procedure used in both studies.

ln conclusion, this study is considered as part of series studies [14-17] complished on the isolate *Trichophyton mentagrophyres* var. *erinacei* which represent the common agent of dermatophytoses in this region of the world and the gained data of the present study added an interest informations for further studies in differentiation between dermatophytes species.

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عزل وتشخيص البروتين المفصول من مستخلص فطريات Tricophyton mentagrophytes var erinacei وباستخدام تقنية كروموتوغرافيا الترشيح الهلامي

افراح زاير رسن

قسم الكيمياء–كلية العلوم–جامعة البصرة

الخلاصة:

تم عزل وتشخيص البروتين المفصول من مستخلص فطريات Tricophyton mentagrophytes var erinacei وباستخدام السيفاديكس تدريج 100 في تقنية كروموتوغرافيا الترشيح الهلامي ,وقيس الامتصاص الضوئي للأجزاء البروتينية المفصولة عند الطول الموجي (280) نانوميتر وقيست الاوزان الجزيئية لهذه الأجزاء البروتينية ووجد أنها تتراوح بين 102,000و 220,00و425,000و610,000 دالتون, كما حددت نوعية الاحماض الامينية التي اظهرت وجود غلبة الاحماض الامينية التالية حامض الكلوتامك, الكلايسين,البرولين,الفالين,المثيونين واخيرا السستين على بقية الاحماض الامينية الاخرى .

وقد قدمت النتائج الحالية بان التقنية المستخدمة مفيدة للتمييز بين أنواع فطريات Trichophyton