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ISOLATION AND PURIFICATION OF AVIDIN FROM EGG WHITE BY IMMOBILIZED METAL AFFINITY CHROMATOGRAPHY

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ABSTRACT

Lysozyme and avidin were initially separated from egg white by cation exchange chromatography (Doulite C-464). Avidin was then isolated from lysozyme in pure form by immobilized metal affinity chromatography column (1.5 × 10cm) loaded with copper ions. The column was equilibrated with 0.02M phosphate buffer pH 7.7, containing 0.5M NaCl. Protein fraction obtained from Doulite column was applied on IMAC column, followed by washing with the starting buffer and eluting with pH gradient with 0.1M Acetic acid. Two peaks were obtained , the first peak represents the avidin, while , the second peak represents the lysozyme as tested by SDS-PAGA and HABA assay. The purity of avidin was increased to 75% by the IMAC process.

INTRODUCTION

Avidin was first detected as a factor in raw hen egg as albumen which caused an unusual dermatitis in rates when fed as their sole protein source (Boas, 1927). Subsequent studies established that the symptoms were due to deprivation of a vitamin, them called vitamin "H" but now known as biotin (Gyorgy et al.

1940). Avidin, it was found, bound biotin and made it unavailable to the organism.

Avidin is a protein exhibited high affinity for the vitamin biotin. In fact, the avidin-biotin interaction is characterized by one of the lowest dissociation constants known for a non-covalent ligand protein interaction (Green , 1963). This highly specific affinity is the basis for a heterogeneous group of biochemical techniques which utilized avidin. These include histochemical localization techniques for use with electron and light microscopy specific immunaoassays and even potential pharmacological application (Wilchek and Bayer, 1984).

Many steps are required for avidin purification. The earliest method of Dhyse (1954) consisted of six purification steps and the resultant protein product was about 50% active protein avidin (Green, 1975). Melamed and Green (1963) obtained high purity avidin with three successive purification steps on CMC and Amberltie CG-50 ion exchange resin, an effective but very time-consuming method. Two successful affinity methods for avidin have been described. Cautrecasas and Wilchek (1968) bound avidin to biocytin-Sepharose column and eluted with 6M Gu-Hcl at pH 1.5 . Avidin was completely denatured by this treatment but could be renatured by dilution, with a 90% yield. Honey and Orr, (1981) used iminobiotin which binds avidin with this approach yields of 95% avidin purity was reported.

Our previous paper (Al-Mashikhi et al. 1997) focused only on isolation of lysozyme from egg white by ion exchage. The purpose of this paper was to purify avidin as well as lysozyme from egg white by metal chelate interaction chromatography.

MATERIAL AND METHODS

Sepharose 6B , iminodiacetic and standard avidin and lysozyme protein were obtained from Sigma Chemical Co. (St. Louis, Mo) Butanediol diglycidyl ether (BGE) was obtained from Eastman Kodak Co. (Rochester, NY). Doulite C-464 cation exchange resin was obtained from Diamond Shamrock Co. (Cleveland, OH).

Isolation of lysozyme

Lysozyme was isolated from egg white as described by Al-Mashkhi et al. (1997). Homogenized egg white was applied to a column of Doulite C-464 which had been previously equilibrated with 0.1M Na-phosphate buffer. pH 7.7 at flow rate as to allow a contact time between the resin and egg white of 20-30 min. Immediately after egg white application, unadsorbed proteins were washed off the column with 5-10 column volumes of distilled water or starting buffer. Adsorbed proteins were eluted with buffer of increasing ionic strengh, using either step wise or gradient elution.

Purification of avidin

Sapharose 6B was activated and cross linked with BGE as described by Sunberg and Porath (1974) . IDA was bound to the activated Sepharose by the method of Parath and Olin (1983) . IDA-BGE Sepharose 6B was packed in to a column (1.5cm × 10.0cm) and the upper one-third to one-half was saturated with cupric ions as indicated by the blue color (2-3 ml of 0.05M $CuCl_2$) followed by washing first with distilled water and then with starting buffer consisting of 0.02M phosphate buffer pH 7.7 containing 0.5M NaCl .

Sample of protein (150 mg) peck obtained from Doutite C-464 column) was applied at a flow rate of 15-20ml/hr . Adsorbed proteins were eluted by a pH gradient generated by a two chamber device containing 400ml of starting buffer in the miximg chamber and 400ml of 0.1M acetic acid (pH 2.8). The eluted material was collected in 4.5 ml fractions. The fractions were monitored using A280 and pH measurements . Selected fractions were analysed for avidin content by the HABA assay.

Avidin assay

Avidin activity was measured by the 2(4-hydroxy azo benzene)- benzoic acid (HABA) spectrophotometric method of Green (1965) with minor modification. To 2ml sample solution 1 ml of 0.2M sodium phosphate buffer, pH7.2 was added , followed by 0.1 ml of 2 mM HABA . The absorbance of the avidin-HABA complex was read at 500 nm. Then 0.1 ml of 0.4 mM biotin solution was added to displace HABA and A500 was read again. The concentration of avidin was calculatd according to the following equation :

$$\text{Avidin}(g/l) = \frac{\text{Volume assay} \times \text{MW avidin} \times \Delta A_{500}}{\text{Volume sample} \times E \times 4}$$

Where MW : molecular weight of avidin (68000)

4 : number of biotin binding sites per avidin molecular ;

E : 34000 The extinction coefficient of the avidin - HABA complex.

Protein assay

Protein concentration of samples were determined form their absorbance at 280nm. Protein content was calculated based on a $E^{1\%}$ values of 26.4 for lysozyme and 15.4 for avidin (Anon. 1981, Green 1975). Since lysozyme is the main contaminant of the avidin containing fractions, avidin purity can accurately be estimated according to the following equation :

$$\text{Avidin Purity}(\%) = \frac{\text{A.s. a.}}{\text{A.s. a.} + \frac{A280 - (\text{A.s. a.} \times 1.54)}{2.64}}$$

Where A. s. a. : Avidin specific activity (mg / l) ;

A_{280} : Absorbance of fraction at 280nm ;

1.54 : $E^{0.1\%}$ avidin at 280nm ;

2.64 : $E^{0.1\%}$ lysozyme at 280nm.

Electrophoresis

Sodium dodecyl sulfate-polyacylamide gel electrophoresis (SDS-PAGE) was done in a vertical slab unit by the method of Laemmli (1970) as outlined by Al-Mashikhi and Nakai (1987).

RESULTS AND DISCUSSION

Isolation of avidin

Avidin and lysozme are basic proteins and can be isolated together from egg white with the use of a Doulite C-464 Cation exchange column in neutral or slightly alkaline pH, Lysozyme was successfully isolated by Doulite C-464 column (Al-Mashikhi et

al 1997). THe lysozyme fraction obtained from our previous paper was applied on metal chelate interaction chromatography column. Figure 1 shows the elution profile of adsorbed proteins on IDA-BGE-Sepharose 6B loaded with copper ions. Almost all of the proteins in the sample applied were bound to the column as indicated the low absorbance at 280 nm of the eluant following sample application. Upon elution with the pH gradient, two peaks were obtained by observing the A_{280} profile. The first peak was rich in avidin activity as confirmed by the HABA spectrophotometric assay.

Figure 2 shows the SDS-PAGE profile of the fraction obtained from egg white by Doulite C-464 chromatography and metal chelate affinity chromatography . The avidin and lysozyme bands were identified by comparison to standard proteins . The protein fraction from the doulite C-464 process contains lysozyme as the major protein fraction (Lane 4 Figure 2) ; the avidin band is faintly visible, being only about 14% of the protein in this fraction. Considerable separation and consequent purification of the two proteins, avidin and lysozyme were achieved by IMAC process. The first peak eluted from IMAC column contains primarily avidin as the major component (Lane 3). Based on the avidin content from HABA assay and the total protein content by A_{280} measurement, the first peak contains approximately 75% avidin, on a protein basis. The second peak eluted from IMAC column contains primarily lysozyme (Lane 2) ; no avidin was detected in this fraction by either SDS-PAGE or HABA assay.

These preliminary experiments demonstrate the high capacity of IMAC column for avidin and lysozyme , and feasibility to use IMAC as a second column procedure to achieve purification of

the avidin fraction from Doulite chromatography. It is possible that better resolution of the avidin and lysozyme peaks from IMAC column can be achieved by changing the elution conditions.

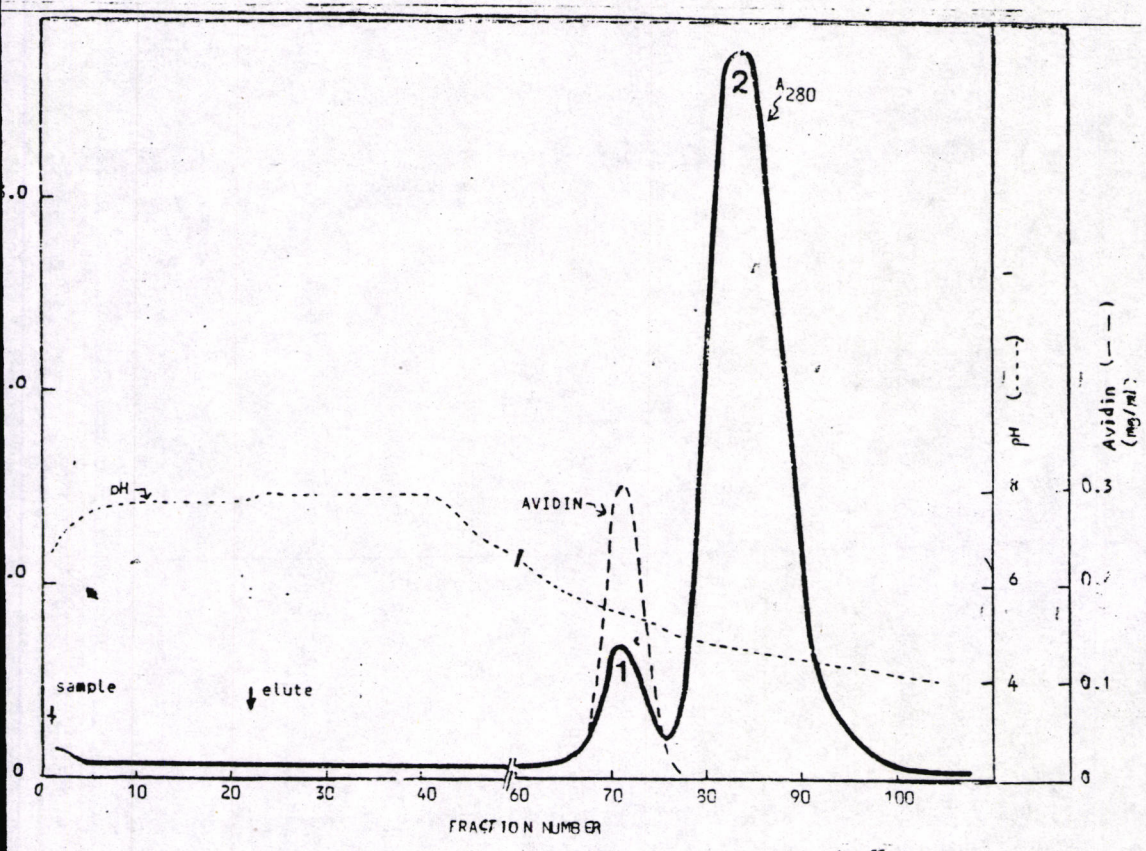

Figure 1. Elution, profile of adsorbed proteins from immobilized metal affinity chromatogrphy (IMAC). Lysozyme rich fraction sample from doulite C-464 column was applied to IMAC column and eluted with pH gradient of 0.02M phosphate buffer (pH 7.7) containing 0.5M NaCl and 0.1M acetic acid (pH 2.8). Each fraction contained 4.5 ml.

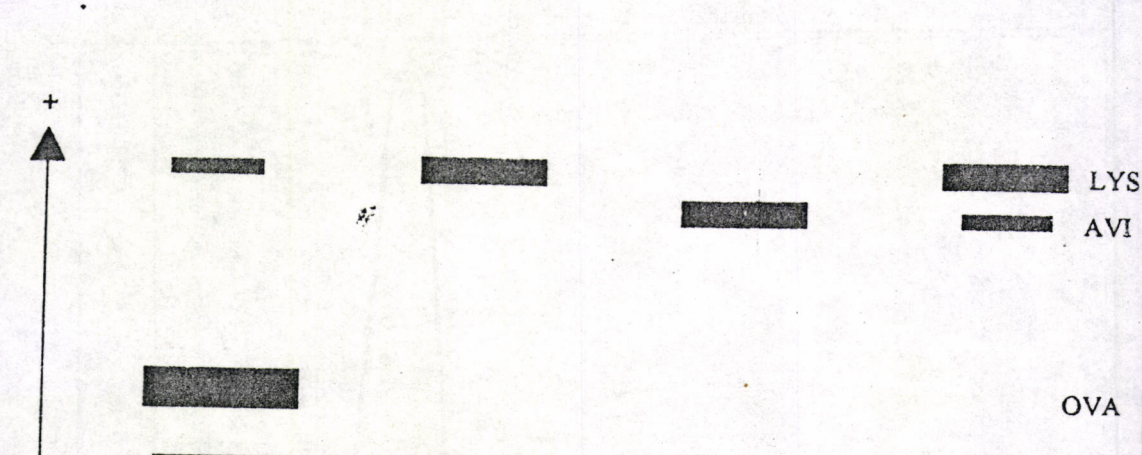

Figure 2 SDS-PAGE analysis of fraction separated from egg white by doulite C-464 column and immobilized metal affinity chromatography. Lane 1. Egg white proteins, Lane 2 peak 2 from IMAC , Lane 3 peak1 from IMAC, Lane 4 lysozyme fraction from Doulite column. LYS, Lysozyme; AVI,Avidin ; OVA, Ovalbumin; OVT, Ovotransferrin.

REFERENCES

1- Al-Mashikhi, S. A. , Al-Baldawi, A. M. and Torahi, F. A. 1997. Isolation of lysozyme from egg white by cation exchange chromatography. (Accepted in Iraqi J.Sci).

2- Al-Mashikhi , S. A. and Nakai, S. 1987 . Separation of ovotransferrin from egg white by immobilized metal affinity chromotagaphy. Agric. Biol. Chem. 51:2881-2887.

3- Anonymous, 1981. Lysozyme, product No. L-6876. Sigma Chemical Company. St. Louis, Mo.

4- Boas, M. 1927. Effect of desiccation upon nutritive properties of egg white. Biochem. 21 : 712 .

5- Cautrecasas, P. and M. Wilchek 1968. Single step purification of avidin from egg white by affinity chromatography on biocytin-Sepharose. Biophys. Res. Commun. 33 : 235.

6- Dhyse, F. G. 1954. A practical laboratory preparation of avidin concentrate for biological investigation. Proc. Soc. Exp. Biol. Med. 85 : 515 .

7- Green, N. M. 1963. Avidin : The use of [14C] biotin for kinetic studies and for assay. Biochem. J. 85 : 585 .

8- Green, N. M. 1965. A spectrophotometric assay and biotin based on the binding of dyes by avidin. Biochem . J . 94 : 23 C.

9- Green, N. M. 1975. Avidin Adv. Protein Chem. 29 : 85.

10- Gyorgy, P. , C. S. Rose, K. Hofinann, D. B. Melville, and V. du Vigneaud. 1940. A further note on the identity of vitamin H. with biotin. Science 29 : 609 .

11- Heney, G., and G. A. Orr. 1981. The purification of avidin and its drivatives on 2-iminobiotin -6- aminohexyl Sepharose 4B, Anal. Biochem. 114 : 92 .

12- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 : 680.

13- Melamed, M. D. and N. M. Green 1963. Avidin 2 . Purification and composition. Biochem. J. 89 : 591 .

14- Porath, J. and B. Olin 1983. Immobilized metal chromatography of biomaterials. Serum protein affinty for gel immobilized iron and nickel ions. Biochem. 22 : 1621 .

15- Sunberg, L. and J. Porath 1994. Preparation of adsorbents for biospecific affinity chromatography. I. attachment of group containing ligands to insoluble polymers by means of bifunctional oxiranes. J. Chromatogr . 90 : 87.

16- Wilcheck, E. A. and M. Bayer 1984. The avidin-biotin complex in immunology. Immunology Today 5: 39 .

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فصل وتنقية الافيدين من بياض البيض بواسطة كروماتوكرافي الالفة المعدنية

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الخلاصة

تم اولا فصل اللايسوزايم و الافيدين من بياض البيض المجنس والمخفف بنسبة 1 : 1 بواسطة عمود التبادل الايوني الموجب (Doulite $C_4 6_4$) . ثم تنقية وفصل الافيدين عن اللايسوزايم بواسطة عمود كروماتوكرافي الالفة المعدنية (1.5 × 10 سم) والمحمل بأيونات النحاس . وبعد اجراء الموازنة للعمود بواسطة منظم الفوسفات (0.02 مولار) وأس هيدروجيني مقدار 7.7 والحاوي على 0.5 مولر كلوريد الصوديوم. تم امرار خليط الافيدين واللايسوزايم على العمود وأجريت عملية فصل البروتينات المرتبطة بواسطة الاس الهيدروجيني المتدرج . تم الحصول على قمتين احدهما تمثل الافيدين والاخرى تمثل اللايسوزايم عندما اجري الفحص بواسطة الهجرة الكهربائية وكذلك اختبار فعالية الافيدين لوحظ إن نقاوة الافيدين قد ارتفعت الى 75 ٪ عند استخدام عمود كروماتوكرافي الالفة المعدنية.