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An Indirect Lectin Affinity Bioassay for Uromodulin

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Uromodulin is a monomeric glycoprotein excreted in large amounts in urine. Most of the assay methods for quantifying uromodulin rely on the availability of antibodies to this glycoprotein. This study reports a new bioassay for uromodulin without requiring the presence of antibodies. The bioassay is based on the lectin affinity of the glycoprotein and the known interaction between uromodulin and the tumor necrosis factor. The measuring range of the bioassay described in this paper is between 0.13 and 1.25 μg/ml, which makes it a potential method for clinical use.

Key Words: glycoprotein, methods, biological assay

Abbreviations used. THG, Tamm-Horsfall glycoprotein; TNF, tumor necrosis factor; Con A, concanavalin A.

Introduction

Uromodulin, also known as Tamm-Horsfall glycoprotein (THG), derived from human pregnancy urine, is a monomeric glycoprotein of 85 kDa[1,2]. It is produced in the thick ascending limb (TAL) and early distal convoluted tubule (DCT) and is excreted in large amounts in urine. The immunosuppressive property of Uromodulin is based on its glycosylation pattern that is different THG obtained from males and non-pregnant women[3]. Although the exact physiological functions of uromodulin are yet to be clarified, a number of researchers have reported its possible involvement in the regulation of ion transport, the urine-diluting mechanism of nephrons, the pathogenesis of stone formation and in some forms of acute renal failure[4]. Uromodulin has also been reported to have a high binding affinity for tumor necrosis factor (TNF)[5,6].

Several assay methods have been developed to quantify uromodulin. Among these, radioimmunoassay (RIA)[7] and enzyme-linked immunosorbent assay (ELISA)[8] are widely used. Although immunoassays are selective and sensitive, these methods rely on the availability of specific polyclonal or monoclonal antibodies. This study reports a sensitive and specific bioassay for uromodulin without the presence of antibodies. The bioassay is based on the lectin affinity of the glycoprotein[9] and the known interaction between uromodulin and TNF[5,6]. The results are monitored by using the mortality index of a mouse fibroblast L929 cell line.

Experimental

1. Purification of Uromodulin: Uromodulin was isolated from the pooled 24 h urine of pregnant donors by repeated salt precipitation in 0.58 M NaCl[1,10]). Samples of salt-precipitated material were further purified by running over a cyanogen bromide-activated concanavalin A (Con A) sepharose (Sigma Chemical Co. St Louis, MO) column with a total bed volume of 100 ml. Bound material was eluted with two bed volumes of 250 mM methyl-mannoside in phosphate-buffered saline (PBS), pH 7.2. Following dialysis against three changes of distilled water for 48 h at 4°C, the sample was lyophilized and resuspended in PBS containing 0.1% SDS and chromatographed on a 2.5 by 90 cm column packed with Bio Gel A 1.5 m (200-400 mesh size) (Bio-Rad Inc. Richmond CA). The absorbance of fractions was monitored at 280 nm and the major elution peak fractions were pooled.

2. Protein Determination: The protein concentration was determined as described[11].

3. Assay Protocol for Uromodulin: The bioassay was carried out in 96-well immunolon microtiter plates (Dynatech Lab, Chantilly, VA). The wells were coated with 200 μl solution of Con A (5 μg/μl) in 0.1 M Na_2CO_3, pH 9.0, for 16 h at 4°C. The wells were then emptied and washed three times with PBS containing 0.1% Tween-20, followed by the addition of 0.5% (w/v) BSA for 1 h to saturate the plastic surface. The wells were washed twice with a solution containing 9 g/L NaCl, 0.2 g/L Tween-20, and 0.5 g/L NaN_3. Different concentrations of isolated uromodulin, from 0.13 to 1.50 μg/ml, in 100 μl PBS were added into wells in triplicate and incubated for 30 min with continuous shaking. Next, a constant amount of TNF (100 μl of 0.1 μg/μl stock, a total of $3x10^3$units) in Dulbecco's modified eagle medium (DMEM) was added and incubated for another 30 min. All incubations were at room temperature. The amount of free TNF was monitored using L929 cells. All reagents were of analytical grade.

Results and Discussion

1. Purification of Uromodulin The urine samples were collected from pregnant women as the immunosuppressive properties of uromodulin make the pregnant women rich in this glycoprotein[1]. Figure 1 shows the chromatographic elution profile during the purification of the salt-precipitated sample. The first peak, containing blue dextran as indicator, was followed by the uromodulin peak, which comprised approximately three-fourths of the applied material (Fig. 1, fractions between 40-80). The following fractions (Fig. 1, fractions between 80-100) consisted of the peptides differing from uromodulin in molecular weight as well as lectin affinity since they did not bind to Con A-coated immunolon plates. Analysis on a 7.5% (w/v) SDS-PAGE showed that eluting the material from a Con A sepharose column eliminated heterogenous fractions of salt-precipitated sample, as a single band of 85 kDa protein was detected on the Coomassie brilliant blue-stained gel (data not shown).

2. Lectin Affinity Assay: The quantitative method for uromodulin presented in this paper originated from previous reports on the lectin affinity of glycoproteins[9], lectin-like interaction between uromodulin and TNF[5], and the decreased cytolytic activity of TNF on monolayers of L929 cells in the presence of uromodulin[12]. Binding specificity makes lectins highly useful in the purification and characterization of glycoproteins. A lectin affinity bioassay reported by Electricwala employed immobilized lectins to quantify tissue plasminogen activator using its chromogenic substrate[9]. Among the lectins the author used, Con A showed relatively higher binding affinity for the glycoprotein and binding was proportional to the amount

of sample. Binding of uromodulin to TNF was first reported by Sherblom *et al.*[5]. The authors reported a lectin-like interaction of uromodulin with TNF via its mannose residues. On the other hand, Lambert *et al.* have identified an inhibitory effect of uromodulin on the cytolytic activity of TNF for monolayers of L929 cells when the cells were pre-treated with the glycoprotein and washed before the addition of the cytokine[12]. The rationale of the bioassay in this study is based on these three separate reports. When a constant amount of TNF is added to Con A-coated, Uromodulin-added plates, the cytolytic activity of TNF in L929 cells is limited to the fraction of free-TNF, unbound to uromodulin. Therefore, when a "Con A/uromodulin/TNF sandwich" is applied to a L929 cell culture known to respond to TNF, the mortality of the cells as a percentage can be converted into the amount of uromodulin. The optimal concentrations of sandwich components, Con A and TNF, were as described in the previous reports[9,12]. The blocked fraction of TNF did not exert its cytolytic activity and no interaction of TNF with either Con A or BSA was detected.

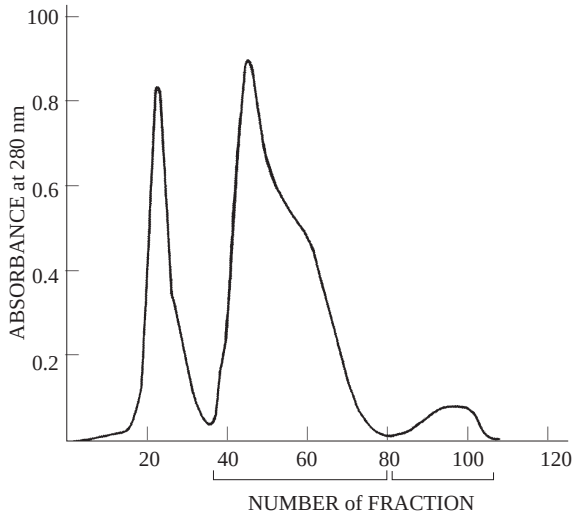

Figure 1. Chromatographic elution profile during the purification of the salt-precipitated sample of Uromodulin from a Con A sepharose column. Fractions underlined were pooled separately and lyophilised. The flow rate and volume of each fraction were 6 ml/h and 3.4 ml, respectively. See Experimental section for the details of purification.

Figure 2 shows a representative plot of dose response in the form of percent mortality (solid line) and percent viability (broker line) of L929 cells versus uromodulin concentration (μg/ml). The results are expressed as the average of the three parallel measurements. A proportional decrease in L929 cell mortality is detected with the increased concentrations of uromodulin (Fig. 2, solid line). Dose-dependent change in L929 survival also supports the previous reports on the individual interactions of the assay components[5,9,12]. The measuring range of the method is between 0.13 and 1.25 μg/ml of uromodulin. Therefore, this bioassay has the potential to be used in clinical laboratory studies.

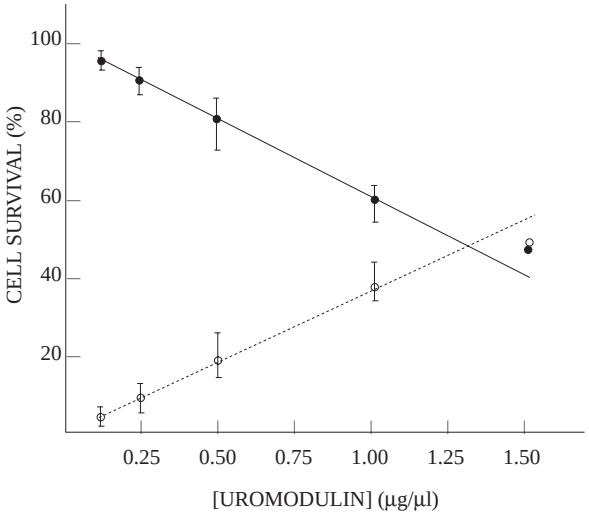

Figure 2. A representative dose response curve in L929 cell line. Results are expressed as the average of three parallel measurements of percent mortality (—-), and percent viability (- - -), versus uromodulin concentration (μg/ml). Error bars are shown. See the text for the details of the experiment.

The other assay procedures, such as RIA[7] and ELISA[8], involve the antibodies to estimate uromodulin. However, measurements using polyclonal antibodies may accommodate a population of molecules that recognizes an antigenically dominant epitope on the glycoprotein. This may result in competition for the same region of the molecule, which leads to a relatively low level of specific binding. The utilization of site-specific or monoclonal antibodies may improve the sensitivity of measurements; however, uromodulin exhibits a considerable polymorphism[13]. Thus, monitoring uromodulin with monoclonal antibodies might lead to lower registrations of the actual amount. The bioassay presented in this study does not have a prerequisite for antibodies. On the other hand, methods based on the spectrophotometric protein determination of salt-precipitated material are not useful, as urine samples of pregnant women contain a number of immunosuppressive glycopeptides[14]. Some of these compounds may be the degradative products of uromodulin and their presence can prevent the reliable estimation of the true magnitude.

Taken together, this is the first report on the use of lectins in a quantitative assay for Uromodulin by employing TNF and a cell culture. However, it requires further improvements and simplifications to be used in the clinical analysis of other biological fluids, i.e., serum and urine samples from men and non-pregnant women. The applicability of this method may show variations as the alteration of the sugar moiety of uromodulin by pathological conditions[15] can influence the affinity of the glycoprotein to the lectins. Considering the measuring range of the method presented in this paper, a higher dilution may be required as the concentration of Uromodulin reaches up to 4 mg/l in urine samples with different pathology[8].

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References

1. I. Tamm and F. L. Horsfall, **J. Exp. Med. 95,** 71-97 (1952).
2. A. V. Muchmore and J. M Decker, **Science 229,** 479-81 (1985).
3. R. L. Easton, M. S. Patankar, G. F. Clark, H. R. Morris and A. Dell, **J. Biol. Chem. 275,** 21928-38 (2000).
4. F. Kokot and J. Dulawa, **Nephron 85,** 97-102 (2000).
5. A. P. Sherblom, J. M. Decker and A. V. Muchmore, **J. Biol. Chem. 262,** 5418-25 (1988).
6. P. J. McLaughlin, A. Aaikawa, H. M. Davis, R. G. Ward, A. Barkan, R. A. Sells and P. M. Johnson, **Clin. Sci. 84**, 243-6 (1993).
7. F. A. Duffy, R. D. Marshall and C. Wrong, **Clin Nephrol 28,** 39-47 (1987).
8. A. Glauser, W. Hochreiter, P. Jaeger and B. Hess, **Nephrol. Dial. Transplant. 15,** 1580-87 (2000).
9. A. Electricwala, **Biochim. Biophys. Acta. 990,** 53-58. (1989).
10. R. H. Schwartz, J. D. Van-Ess, A. G. May, E. A. Schanck and R. B. Freeman, **Transplantation 16,** 83-87. (1973).
11. M. M. Bradford, **Anal. Chem. 72,** 248-54.(1976)
12. C. Lambert, R. Brealey, J. Steele and G. A. Rook, **Immunology 79,** 203-10 (1993).
13. T. Nesselhut, W. Rath, E. Grunow, G. Kaufholz, U. Ostermai, N. Cillien and W. Kuhn, **Eur. J. Obstet. Gynecol. Reprod. Biol. 48,** 23-31. (1993).
14. A. V. Muchmore, J. M. Decker, R. M. Blaese and B. Nilsson, **J. Exp. Med. 180,** 1672-1685. (1984)
15. T. Olczak, M. Olczak, A. Kubicz, J. Dulawa and F. Kokot, **Int. J. Clin. Lab. Res. 29,** 68-74 (1999).