

An enzymatic method for the detection of human serum albumin

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Abbreviations: DB, dextran blue; HSA, human serum albumin; LDH, lactate dehydrogenase; CSF, cerebrospinal fluid

Abstract

Albumin is the most abundant protein in human serum. A dye-binding method is commonly used in clinical laboratories for its estimation using different types of dyes. However, all these dye methods were interfered by a variety of compounds. Here we present a method for the detection of albumin in human serum and other biological fluids. The principle is based on the fact that lactate dehydrogenase isoenzyme-5 (LDH-5) binds specifically to Dextran-Blue (DB). Albumin inhibits the binding of LDH-5 with DB. Absence of LDH activity in DB fraction after gel filtration indicates the presence of albumin in sample and vice versa.

Keywords: albumin, human, LDH-5, serum

Introduction

Human serum albumin (HSA) is synthesized in liver and is the most abundant protein in human serum, representing 40-60% of the total protein (Lindsey, 1996). The earliest method for its determination involved the salting-out of the globulins with sodium sulfate, leaving the albumin in solution (Silverman and Christenson, 1994). The albumin was then determined by the Kjeldahl method and, later, by the Biuret color development (Gomes *et al.*, 1998). The method commonly used today is one involving dye binding and shift in color when a dye is bound by albumin (Silverman and Christenson, 1994; Lindsey, 1996; Gomes *et al.*, 1998). A variety of dyes have been used, including methyl orange, 2-(4'-hydroxyazobenzene) benzoic acid (HABA), bromocresol

green (BCG), and bromocresol purple (BCP) (Thakkar *et al.*, 1997; Kessler *et al.*, 1997a). Methyl orange is non-specific for albumin; β -lipoproteins and some α 1- and α 2-globulins also bind to albumin and have a low sensitivity (Kessler *et al.*, 1997a). In addition, several compounds such as salicylates, penicillin, conjugated bilirubin, and sulfonamides, interfere with the binding of albumin to the dye. Interfering substances such as bilirubin and salicylates do not affect BCG; however, hemoglobin can inhibit the binding of albumin to dye (Kessler *et al.*, 1997b). Even though BCG is the dye most commonly used today, there are repeated reports that the measurement of albumin by BCG overestimates albumin levels (Karon *et al.*, 1997). Several formats of electrophoresis, radioimmunoassays (RIA) and immunoassays are available for albumin determination (Karon *et al.*, 1997; Kessler *et al.*, 1997b), which are more appropriate for body fluids in which albumin concentration is normally low (i.e., urine and CSF). The RIA and immunoassays are relatively expensive and unlikely to be applied except for special diagnostic purposes. Recently, the albumin-blue (AB) series of fluorescent albumin dyes have been described (Kessler *et al.*, 1997a). The limited solubility of the reagent solution so far prevented its application in routine analysis. A new derivative of AB, AB580, with improved solubility has become available recently and is used in autoanalyser for albumin determination in serum and urine (Karon *et al.*, 1997). Except for blood serum, albumin concentrations in body fluids such as urine are usually low and, therefore, require sensitive and selective method for their detection and estimation. Enzymes have been extensively used nowadays in the analytical laboratories due to their high specificity and sensitivity (Whitaker, 1991; Rej, 1998). In this study we present a simple method for the detection of human albumin in biological sample using LDH-5 and DB.

Materials and Methods

Chemicals

NADH, sodium pyruvate, Dextran Blue (DB), human serum albumin (HSA), bovine serum albumin (BSA) and potassium phosphate were from Sigma, St. Louis, U.S.A. Sepharose-CL-6B gel was from Pharmacia-LKB Biotechnology, Uppsala, Sweden. The LDH-5 was purified from rat liver as described elsewhere (Javed *et al.*, 1995). All biological fluids were obtained from the clinical laboratory of the Aga Khan University Hospital

(AKUH) and assayed after centrifugation within 3 days. This study followed the protocol of the Human Subject Protection Committee of AKUH and all volunteers were informed prior to participation.

Principle of the assay

This assay is based on high affinity binding of DB with LDH-5 (Leatherbarrow and Dean, 1980; Javed and Naru, 1998; Javed *et al.*, 1999). This binding of LDH-5 with DB was specifically inhibited by HSA (Javed and Naru, 1998; Javed *et al.*, 1999). The presence of the LDH activity in DB fraction after gel filtration indicates the absence of albumin while the absence of LDH activity indicates the presence of albumin.

Procedure

The Sepharose-CL-6B gel was packed in column (17 cm × 0.5 cm) and equilibrated with 10 mM potassium phosphate buffer containing 0.02% sodium azide, pH 7.5. One mg DB was added to 1 ml sample (\pm HSA) and applied on top of the column. The microfractionator (Gilson, Wisconsin, USA) collected one ml fractions and the flow rate was controlled to about 40 ml/h by Microperpex peristaltic pump (LKB, Uppsala, Sweden). To measure LDH activity, we added 465 μ l of DB fraction to 1ml cuvette. Twenty five μ l substrate buffer (12 mM sodium pyruvate and 1 M potassium phosphate pH 7.5) were mixed and kept at 30°C for about 10 min. The reaction was then started by adding 10 μ l of 0.9 mM NADH and the rate was monitored at 340 nm in spectrophotometer (Beckman DU70, USA) for 2-3 min (Javed *et al.*, 1995; Javed and Naru 1998).

Results

In the presence of DB in the samples, LDH-5 co-eluted with DB in void volume of sepharose column (Figure 1). However, in the presence of HSA in samples along with DB, LDH-5 activity was absent from DB fractions and eluted in later fractions of column chromatography (Figure 1). All human body fluids that contained albumin (serum, albuminuric urine, plural fluid, ascites, peritoneal fluids, etc) showed no LDH-5 activity in DB fractions after gel filtration and LDH-5 activity was observed in the later fractions. The minimum volume of normal human serum needed to completely block the LDH-5 binding with DB was found to be 2 μ l, that equals to be about 0.08 mg of albumin. We tried to estimate the albumin by calibrating a standard curve but we could not reproduce the results. By adding serum to DB-LDH samples from animals (*i.e.*, animal albumin) like sheep, rabbit, mouse, rat, hamster and pigeon, the DB-LDH complex appeared in void volume. There was no effect on binding of DB with LDH-5 even up to 40 mg/ml of animal albumin.

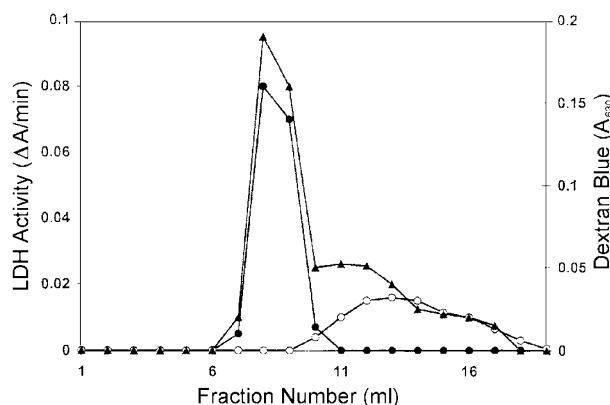


Figure 1. Gel filtration of DB-LDH-complex in the presence (○-○) and absence (●-●) of human serum albumin. The method is described in the text. DB peak is shown as (▲-▲) which is eluted in the void volume. The pattern of the Fig was like (●-●) when we added the serum in the samples from various animal species or BSA as mentioned in the results.

Similarly there was no effect of BSA on the binding of DB with LDH-5.

Discussion

Various methods have been used for the detection of albumin in different biological samples (Karon *et al.*, 1997; Kessler *et al.*, 1997a; Kessler *et al.*, 1997b; Thakkar *et al.*, 1997). Each method has some merits and demerits and the major drawback is the interference by other proteins and metabolites (Kessler *et al.*, 1997a). Here, we described a new method for the detection of HSA. We believe that the gel filtration method is more specific than the dye binding methods (Karon *et al.*, 1997; Kessler *et al.*, 1997a; Kessler *et al.*, 1997b; Thakkar *et al.*, 1997) because the DB specifically binds to LDH-5 and it does not have any affinity even for LDH-1. Similarly, the HSA specifically compete with the binding of LDH-5 with DB and albumin from other animals and other serum proteins from human serum do not compete with binding of DB with LDH-5 (Leatherbarrow and Dean, 1980). We have also observed that the binding of LDH-5 with DB is inhibited only by HSA, as the serum from animals, like sheep, rabbit, mouse, rat, hamster and pigeon (which contained animal albumin and not human albumin) were unable to block the binding of LDH-5 with DB and LDH activity appeared in the void volume in the presence of even up to 40 mg/ml of animal albumin (speculating that these animal serum also have about 4 gm/dl of albumin with total serum protein as about 6-8 gm/dl). This method is more simple and sensitive to detect micro quantity of albumin. The same gel has been used for many months and even can be used for many years in the presence of 0.02% sodium azide. Furthermore, LDH-5 can easily be purified in large quantities from rat liver (Javed *et al.*, 1995)

and was found to be stable for years at 4°C.

In conclusion, the detection of HSA in biological fluids by this method is more specific relative to dye binding methods and can be applied in many pathological conditions. The dye binding and electrophoresis are standard but need special equipments, which may not be available in ordinary clinical laboratories, particularly in developing countries. Furthermore this new assay for HSA detection is a useful addition to the analytical sciences.

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