Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 817 (2005) 145–151
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Isolation of plasma albumin by ethanol extraction is inappropriate for isotope ratio measurements during the acute phase response

Rita Jacobs[a,1], Hans Demmelmair[b,1], Peter Rittler[a], Josef Kellermann[c], Berthold Koletzko[b], Maximiliane Krick[a], Karl-Walter Jauch[a], Wolfgang H. Hartl[a,*]

[a] *Department of Surgery, Klinikum Grosshadern, Ludwig-Maximilian University, Marchioninistr. 15, D-81377 Munich, Germany*
[b] *Department of Pediatrics, Dr.v. Haunersches, Kinderspital, Ludwig-Maximilian University, Munich, Germany*
[c] *Analytical Protein Chemistry Group, Max-Planck Institute of Biochemistry, Martinsried, Germany*

Received 12 August 2004; accepted 29 November 2004
Available online 15 December 2004

Abstract

Isolation of high-purity albumin from plasma is essential to study albumin kinetics in vivo with tracer techniques. Because of its simplicity ethanol extraction has been repeatedly used for albumin purification. However, it cannot be excluded that this single-step procedure completely prohibits contamination by other proteins, especially those known to be produced at an accelerated rate during the acute phase response. In the present study, we wanted to examine the reliability of ethanol extraction in different clinical conditions and to study the effects of potential impurities on albumin enrichment during stable isotope tracer studies. SDS-PAGE revealed a contaminating protein band at about 25,000 Da in healthy subjects and postoperative patients during the acute phase response, but not in critically ill patients. According to densitometry about 8% of proteins after ethanol extraction were contaminants. To examine potential contaminant effects on tracer enrichment 1-[^{13}C]-leucine was given to healthy subjects and postoperative patients. Blood samples were taken after various amounts of time, and albumin enrichments (tracer/tracee ratios) were determined from isotope ratios obtained by mass spectrometry. Irrespective of the magnitude of tracer enrichment, postoperative tracer/tracee ratios were significantly higher (on average +10%) in samples exclusively analysed by ethanol extraction than in samples which had undergone additional electrophoretic purification. No significant effect of the contaminant was seen in healthy subjects. N-terminal protein sequencing revealed contaminants to mainly consist of apolipoprotein A-1. Its physiology and pathophysiology may sufficiently explain its variable effects of albumin enrichment. Our findings suggest that exclusive ethanol extraction is inappropriate for albumin isolation in tracer studies performed during the acute phase response. Ethanol extraction may also not be advisable in all other situations known to be associated with a rise in apolipoprotein A-1 turnover.

Keywords: Albumin; Ethanol extraction; Mass spectrometry; Acute phase response

1. Introduction

Isolation of highly pure albumin from plasma is a prerequisite for tracer studies designed to examine albumin turnover in vivo. For scientific purposes, several methods for albumin isolation have been examined in the past. Traditional methods like cold ethanol purification according to Cohn [1] work via fractionation but cannot produce sufficiently pure albumin [2,3]. Alternatively, single-step chromatographic methods have been employed [2,4], but these also cannot guarantee the desired high degree of purity [2,3]. Other procedures such as multi-step chromatography [3,5] or automated FPLC [6] will produce the desired purity, but are either laborious and time costly [3,5], or technically demanding [6].

Because of its simplicity dissolution of albumin in acidified ethanol [7] has become popular for albumin isolation in biological tracer experiments. This method has repeat-

* Corresponding author. Tel.: +49 89 7095 3510; fax: +49 89 700 4418.
E-mail address: whartl@gch.med.uni-muenchen.de (W.H. Hartl).
[1] Both authors contributed equally.

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.11.056

edly been claimed to yield sufficiently pure albumin [7–13]. However, because of the nature of this single step procedure this view has been questioned in the past [6,14,15]. Furthermore, if impurity exists indeed after ethanol extraction, no one has so far studied the relevance of this phenomenon for the accuracy of enrichment measurements. It was the aim of the present study to examine purity of albumin after ethanol extraction from samples obtained in different clinical conditions and to analyse interfering effects of potential contaminants on albumin enrichment during tracer application.

2. Experimental

2.1. Subjects

Studies were performed in six healthy volunteers, 10 postoperative patients and five critically ill patients. Postoperative patients had had limited colorectal cancer and had undergone curative, elective abdominal surgery. These patients were studied during the acute phase response after surgery, had an uneventful postoperative course and were free from signs of organ malfunction and local or systemic infection. No patient (before surgery) had a history of previous weight loss or clinical and laboratory signs of malnutrition or metabolic diseases. Critically ill patients were severely septic and had been in the intensive care unit since more than 10 days. Except in critically ill patients, informed consent was obtained from all subjects after the experimental protocol had been explained in detail. In critically ill patients an informed consent was not required since neither blood samples were taken for the purpose of the study, nor did we perform experimental measures of any kind. Blood analysis was exclusively done in left-overs from blood samples drawn for routine blood gas analysis. The study was approved by the local institutional review board (protocol #134/97).

2.2. Experimental protocol

After 10 p.m., all subjects/volunteers remained postabsorptive, except for consumption of mineral water. On the next day, heparinized venous or arterial blood was obtained from each subject. Then, all volunteers consumed an oral bolus (4 mg/kg) of 1-[^{13}C]-leucine (Tracer Technologies, Sommerville, Massachusetts, USA, 99.3 at.% enrichment) at 7 a.m. Subsequent blood samples were taken at 2, 4, 5, 6 and 8 h after tracer application. In seven postoperative patients a primed-constant intravenous infusion of 1-[^{13}C]-leucine was started at 7 a.m. The isotope infusion rate was 0.16 μmol/kg min (prime 9.6 μmol/kg) and lasted 3, 6 or 10 h. Arterialized blood samples were taken at the end of isotope infusion. In all subjects who received 1-[^{13}C]-leucine, an additional blood sample was collected before isotope administration to determine the background enrichment of protein-bound leucine.

2.3. Procedure

2.3.1. Ethanol extraction

Ethanol extraction was performed according to Korner [7] and Slater and Preston [13]. Five hundred microliters of 10% trichloroacetic acid (TCA) at 4 °C were added to 500 μl serum. The protein fraction was separated by precipitation. Samples were centrifuged at 11,000 rpm for 10 min (Hettich Mikroliter, Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was removed and the pellet was re suspended in 500 μl absolute ethanol. The ethanolic albumin solution was centrifuged again at 11,000 rpm for 10 min. The ethanolic supernatant and 5 ml of distilled water were transferred to an ultrafiltration device (50,000 molecular mass cut-off Centriplus ultrafiltration cone; Centriplus YM-50 Millipore Corporation Bedford, Massachusetts, USA) placed above an universal bottle. Samples were centrifuged at 5000 rpm for 30 min or until the fluid had been completely cleared by the filter. The filtrate was discarded. Sediments attached to the filter were resuspended by adding 1 ml of distilled water to the inverted back side of the filter and by spinning the samples at 1500 rpm for 15 min (Hettich Roto Silenta/RPm, Hettich Zentrifugen, Tuttlingen, Germany). Samples were than divided into several aliquots for further analyses (analytical electrophoresis only, hydrolysation/leucine derivatization/mass spectrometry only, preparative electrophoresis with subsequent hydrolysation/leucine derivatization/mass spectrometry).

2.3.2. Analytical electrophoresis

An aliquot of the supernatant was used to monitor albumin purity or identification, using a 10% sodium dodecyl sulphate polyacrylamide vertical slab gel electrophoresis (SDS-PAGE) and visualization by Coomassie staining. Electrophoretically obtained bands of samples were compared with bands of commercially obtained human albumin and molecular weight markers (rainbow molecular weight marker RPN 800 and 756, respectively, Amersham Pharmacia Biotech, Uppsala, Sweden). Electrophoresis was performed using a vertical slab gel apparatus (PerfectBlue^{TM} double gel system Twin S, PEQLAB Biotechnologie GmbH, Erlangen, Germany). The 12.06 ml resolving gel was prepared using 4.4 ml of solution containing 30% acrylamide, 0.8% bisacrylamide and 69.2% H_2O. To this solution we added 120 μl of 10% sodium dodecyl sulfate (SDS), 3 ml of 1.5 M Tris/HCl buffer at pH 8.8, 4.4 ml H_2O, freshly prepared 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 80 μl) and freshly prepared 10% ammonium peroxodisulfate (60 μl). The stacking gel was prepared using 1.26 ml of the above acrylamide solution, 75 μl of 10% SDS, 1.95 ml of 0.5 M Tris/HCl buffer at pH 6.8, 2.55 ml H_2O, freshly prepared 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 108 μl) and freshly prepared 10% ammonium peroxodisulfate (54 μl). The electrode buffer contained 192 mM glycin, 25 mM Tris and 10 g SDS in 1 l H_2O, and the sample buffer 20 μg bromophenolic blue, 0.1 M Tris/HCl at pH 6.5, 20 g glycerol and 4 g SDS in 100 ml