LIPID BINDING PROTEIN (APOLIPOPROTEIN A-I) CONTAMINATION OF HIGH GRADE COMMERCIAL ALBUMINS

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1. Introduction

Human or bovine commercial albumins are frequently used in experiments involving lipoprotein metabolism, for maintaining oncotic pressure in organ perfusion systems in the study of synthesis and metabolism of lipoproteins [1–3] and as acceptors for fatty acids in studies of triglyceride catabolism by lipoprotein lipase (LPL, EC 3.1.1.34) [3–5] The albumin preparations are supposed to be pure and devoid of apolipoproteins, and besides their ability to bind fatty acids and lysolecithin, to be inert in lipid metabolism [6].

In the present report we provide evidence that some commercially available 'fatty acid poor' or 'fatty acid free' albumins both of human and bovine origin contain apolipoprotein A-I (apo A-I) in appreciable amounts. Apo A-I has been shown to bind avidly phospholipids and produce discoidal particles [7], to bind easily to studied lipoproteins [8] and to be the specific cofactor for one of the key enzymes in lipoprotein metabolism, lecithin : cholesterol acyl transferase (LCAT, EC 2 3 1.43) [9]. Therefore, the use of such albumin preparations in the study of lipoproteins may interfere with the interpretation of such experiments. We wish to caution against the use of commercial albumins in lipoprotein metabolism studies prior to quantifying apo A-I in them

* Since there may be differences in results obtained with various batches, we prefer not to identify the companies. Therefore, the various albumins are designated arbitrarily A to G and are identified by their lot no

2. Materials and methods

Albumins were purchased from Sigma Chemical Co. (St Louis, MO), Pentex, Miles Labs (Kankakee, IL) and Armour Pharmaceutical Co (Chicago, IL). Bovine albumins*. A, fatty acid free (lot no. 22), B, fatty acid poor (lot no. 28), C, fatty acid free (lot no. 7442), D, crystallized (lot no. 8020), E, fraction V (lot no. 47909). Human albumins F, fraction V (lot no. 134), G, fraction V (lot no. 8071)

Human apolipoprotein A-I was purified and monospecific antibodies to apo A-I were raised in rabbits as detailed in [10]. Bovine apo A-I was isolated from delipidated bovine high density lipoproteins (HDL) $d = 1.085–1\ 21$ g/ml, by gel permeation chromatography as in [11]. Its purity was checked by polyacrylamide gel electrophoresis, double immunodiffusion and immunoelectrophoresis [10]. Antibodies to bovine apo A-I were raised in rabbits according to the method described for human apo A-I [10]. These antibodies were shown to be monospecific, by double immunodiffusion and immunoelectrophoresis as detailed in [10].

Analytical methods protein was determined by the Lowry method [12] using bovine serum albumin as the standard. Human apo A-I was quantified by double antibody radioimmunoassay [10]. Bovine apo A-I was quantified by radial immunodiffusion in 1% agarose gel, 0.05 M barbital buffer (pH 8 2) containing 5% antibovine apo A-I serum, according to the method for human apo A-I [13]. The assay was shown to be specific, reacting only with bovine apo A-I and not with bovine albumin or globulins

The assay was accurate in quantifying apo A-I in bovine-HDL when compared to the results obtained by gel permeation chromatography [10,11]. The sensitivity of the assay was 20 μg/ml with a coefficient of variation of 8%. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) was by the method in [14] adapted from [15]. Phospholipid content was determined according to [16].

Various albumins, 300 mg, were incubated with 10 μM single lamellar vesicles of lecithin (prepared by Dr Y. Barenholz from egg yolk phosphatidylcholine (Makor Co., Jerusalem) by the method in [17]) in 6 ml 0.15 M NaCl containing 0.05 M Tris–HCl (pH 7.5) for 2 h at 23°C. At the end of the incubation the density was raised to 1.21 g/ml with solid KBr and centrifuged, using a 40.3 rotor in a Beckman L3-50 ultracentrifuge for 48 h 38 000 rev./min and 4°C. The tubes were sliced and the top 1.5 ml ($d < 1.21$ g/ml) was recentrifuged at the same density. The ultracentrifugally separated fractions were dialyzed for 48 h in 5 changes of 0.15 M NaCl containing 0.01% EDTA at 4°C. The incubation and ultracentrifugation procedures were repeated with the $d > 1.21$ g/ml fractions.

3. Results and discussion

Using the sensitive radioimmunoassay technique (10 ng/ml) we were able to detect and quantify human apo A-I directly in human albumins (table 1). With the radial immunodiffusion method for bovine apo A-I we could detect apo A-I in only one bovine albumin preparation (B). In order to overcome this low sensitivity we have concentrated apo A-I, taking advantage of the known

Table 1
Quantification of apo A-I in commercially available albumins (bovine and human)

Albumins	Apo A-I (μg/g albumin)[a]			Protein (mg/g albumin)
	In commercial preparation[b]	In $d < 1.21$ g/ml[c]	In $d > 1.21$ g/ml[d]	In $d < 1.21$ g/ml[c]
Bovine				
A	0[f]	15.2 ± 2.9	0	2.9 ± 0.3
B	197.0 ± 25.3	172.8 ± 13.5 (16.7 ± 2.3)[e]	0	5.1 ± 0.4
C	0	0	0	0.18 ± 0.02
D	0	0	0	0.21 ± 0.03
E	0	0	0	0.25 ± 0.04
Human				
F	3.2 ± 0.5	2.7 ± 0.5 (0.3 ± 0.05)[e]	0	0.16 ± 0.02
G	1.8 ± 0.25	1.6 ± 0.3 (0.15 ± 0.04)[e]	0	0.78 ± 0.05

[a] Bovine apo A-I content in bovine albumins (A–E) and human apo A-I in human albumins (F,G)
[b] Assayed directly on solubilized original albumins (20% w/v)
[c] Quantification of apo A-I or total protein on $d < 1.21$ g/ml fraction following incubation with liposomes. The results are expressed per 1 g albumin subjected to incubation
[d] Apo A-I in $d > 1.21$ g/ml fraction following two incubations with lecithin liposomes
[e] Results in parentheses express the additional apo A-I recovered in lecithin vesicles following a second incubation and centrifugation
[f] Less than 100 μg bovine apo A-I per 1 g bovine albumin and < 50 ng human apo A-I per 1 g human albumin

Results are mean ± SD of 3 experiments

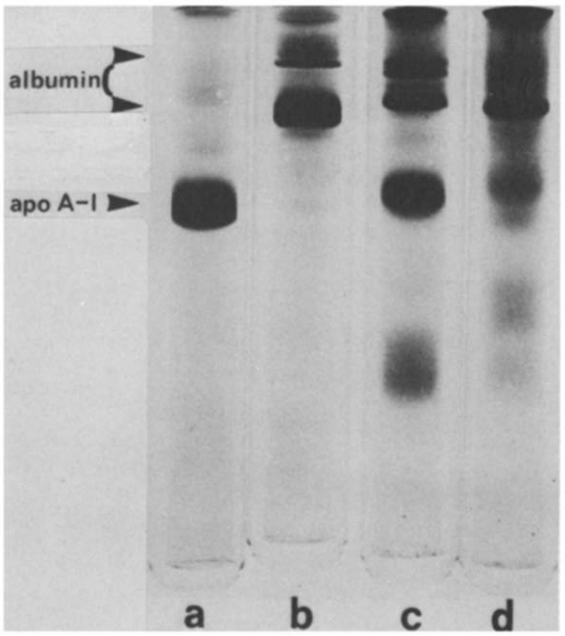

Fig.1. SDS–PAGE of. (a) bovine apo A-I (50 μg); (b) bovine albumin B (50 μg); (c) liposomes incubated with bovine albumin B ($d < 1.21$ g/ml) (50 μg protein), (d) liposomes obtained following a second incubation with albumin B (25 μg). The gels were stained in 0.2% Coomassie blue Note the presence of a protein band in gels (c) and (d) with similar electrophoretic mobility as bovine apo A-I (a).

affinity of apo A-I for phospholipid liposomes [7]. Following incubation of the albumin preparations with egg yolk phosphatidylcholine we have isolated the liposomes by floatation at $d = 1.21$ g/ml (table 1). Prior to the incubation with albumins, the liposomes were nonreactive in both immunoassays.

SDS–PAGE of these incubated liposomes ($d < 1.21$ g/ml) revealed the presence of a protein with identical mobility (molecular weight) as apo A-I of the corresponding species (fig.1). The same pattern as shown in fig.1 for bovine albumin B was also observed with albumin A. Similar results were also obtained with human albumins (F and G) (not shown in fig.1). Immunoassay of these liposomes enabled quantification of apo A-I in an additional commercial bovine albumin (A) (table 1). Repeating the incubation and floatation procedures enabled us to remove all the immunoassayable apo A-I from the human albumins (table 1).

Apo A-I is a metabolically active apolipoprotein and participates in several steps in lipoprotein metabolism. It binds phospholipids [7], removes cholesterol from cells in tissue culture [18] and is a specific activator for LCAT [9]. Therefore, its introduction into in vitro systems in lipoprotein studies may interfere with the metabolic processes under investigation. Several available commercial albumins both of human and bovine origin, even in the 'fatty acid free' forms, contain considerable amounts of this important apoprotein. Thus, we would like to caution against the use of these albumin preparations (especially in large quantities) without a prior check for the presence of apo A-I. In preliminary experiments it seems that one can remove this apoprotein by absorption on phosphatidylcholine vesicles.

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