THE BINDING OF β_2-GLYCOPROTEIN-I TO HUMAN SERUM LIPOPROTEINS

Distribution among density fractions

Erika POLZ and Gerhard M. KOSTNER

Institute of Medical Biochemistry, University of Graz, Harrachgasse 21, A-8010 Graz, Austria

Received 9 April 1979

1. Introduction

Chylomicrons and VLDL were observed [1] to precipitate with SDS in the presence of a protein serum factor only. This factor has been characterized [2]. It is identical with β_2-glycoprotein-I, a human serum constituent discovered [3]. During our studies on the composition of the protein part of VLDL, we found that β_2GI is a regular constituent of apo-VLDL. This glycoprotein is ~4% by wt in VLDL [4].

Here we show that β_2GI is present in all major lipoprotein density fractions, especially in chylomicrons, VLDL and HDL. The highest amount however is found in the bottom fraction of the serum after preparative ultracentrifugation at a solution density (d) of 1.23 g/ml. Charge-shift electrophoresis as proposed [5] for serum lipoproteins clearly underlined the lipid binding property of β_2GI.

2. Material and methods

The serum of 2 male normolipemic volunteers aged 25 and 28 years was used. Neither had a family history of dyslipoproteinemia. Blood (150 ml) was drawn from each of them, after 12 h fasting and 5 h after a fatty meal, consisting of 1200 kcal lard and cream in addition to some carbohydrates and proteins. After clotting at room temperature (4 h) the serum was obtained by low speed centrifugation.

Chylomicrons were separated by high-speed centrifugation at 20 000 rev./min for 30 min. (Sorvall RC-5) and the top layer aspirated with a hypodermic syringe. Aliquots of the infranatant were adjusted to d = 1.070 g/ml by adding solid NaCl and to d = 1.23 g/ml by adding solid NaBr. The lipoprotein density classes were removed by preparative ultracentrifugation using the Ti50 rotor and a Beckman L3-50 ultracentrifuge. The following running conditions were chosen: VLDL, serum density, 18 h at 38 000 rev./min; VLDL + LDL, d = 1.070 g/ml, 24 h at 40 000 rev./min; VLDL + LDL + HDL, d = 1.23 g/ml, 48 h at 45 000 rev./min. The floating lipoproteins were removed by tube slicing and the infranatant was dialysed against 0.15 M NaCl (pH 7.5), containing 0.5 mg/ml NaN_3 and 0.5 mg/ml Na_2EDTA. After dialysis, each fraction was brought to the original volume by adding the appropriate amount of dialysate. In control experiments we isolated the main serum lipoproteins by sequential ultracentrifugation followed by two washes as outlined before [6].

Immunoquantitation of β_2GI was by the Mancini technique using M Partigen β_2-glycoprotein-I plates from Behring Werke (FRG). All plates had the batch no. 2007; the standard human serum (Behring Werke) had the batch no. 1003 DL. Immunodiffusion was performed only with the bottom fractions of the individual densities and calculation was done by difference from total serum. Since free and lipoprotein-bound β_2GI may exhibit different antigenic behaviour we delipidated all samples before immunoquantitation with 1,1,3,3-tetra-methyl urea by the method in [7].

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; β_2GI, β_2-glycoprotein-I; SDS, sodium dodecylsulfate

Address correspondence to: Professor Dr G. Kostner

Table 1

Lipid and β_2GI values and distribution of β_2GI in lipoprotein fractions of fasting and postprandial serum of two normal male subjects

	Serum			Serum[b] infranatant	Distribution of β_2GI							
	Glycerides (mg/100 ml)	Total cholesterol (mg/100 ml)	β_2GI (mg/100 ml)[a]	β_2GI (mg/100 ml)[a]	VLDL-C[d]	LDL-C[d] (mg/100 ml)	HDL-C[d]	Chylomicra	VLDL % total	LDL	HDL	bottom 1 21
Subject 1												
Fasting	55	165	17 0 ± 0 07	17 0 ± 0 06	11 0	91 3	62 7	0	9 2	1 3	18 2	71 3
Postprandial	128	175	18 6 ± 0 05	17 0 ± 0 04	25 6[c]	93 4	56 0	8 6	7 7	1 9	16 6	65 2
Subject 2												
Fasting	82	212	18 1 ± 0 06	18 1 ± 0 04	16 4	150 8	44 8	0	8 3	1 1	16 2	74 4
Postprandial	210	228	19 7 ± 0 04	18 1 ± 0 05	42 0[c]	145 7	40 3	8 2	7 1	1 5	14 4	68 8

[a] ± SEM

[b] Chylomicrons were removed at 20 000 rev /min for 30 min from serum and β_2GI was measured in the infranatant as described in section 2

[c] These values include the 'chylomicrons cholesterol' also

[d] VLDL-C, LDL-C, HDL-C = cholesterol content of the VLDL, LDL and HDL density fraction

This procedure did not affect the immunoquantitation as demonstrated in control experiments. Lipid analyses were carried out with conventional test combinations (Boehringer Mannheim) as in [8]. Charge-shift electrophoresis of β_2GI isolated from pooled human serum according to [9] was performed as suggested [10] in the presence of Triton X-100 alone and in combination with Cethyltrimethylammonium bromide and sodium deoxycholate, respectively. HDL cholesterol was determined as outlined in [8], VLDL cholesterol and LDL cholesterol values were calculated according to the Friedewald equation [11]:

$$\text{LDL cholesterol} = \text{serum cholesterol} - (\text{serum glycerides}/5 + \text{HDL cholesterol})$$

3. Results and discussion

Table 1 shows the serum lipid values of the 2 male volunteers before and after the test meal. HDL cholesterol values were measured, LDL cholesterol and VLDL cholesterol values were calculated according to [11]. This should give only a rough estimate of the individual serum lipoprotein concentrations. Five hours after the fatty meal, the triglyceride values of the serum increased 2–3-fold; there was a slight increase of the serum cholesterol values only. The HDL cholesterol values in both cases were in the fasting state 10% lower than in the postprandial. A concomitant raise of the VLDL cholesterol was observed 5 h after the meal. As demonstrated in table 1 the fasting β_2GI values were 17.0 and 18.1 mg/100 ml, which is well within the limits found in normals (Behring reports a normal range of 15–20 mg/100 ml). After the fatty meal, the β_2GI values increased by 8.6% and 8.2%. This increase was completely due to the portion of β_2GI transported in the chylomicron fraction, suggesting the β_2GI might be newly formed and/or secreted during chylomicron synthesis. Another possibility could be that 'nascent' chylomicrons pick up some β_2GI from the lymph. After removal of all lipoproteins from the serum at $d = 1.23$ g/ml we found ~70% of the β_2GI in the bottom fraction. 16–18% were transported with HDL and 7–9% with VLDL. The LDL fraction (d = 1.006–1.063 g/ml) contained in all cases < 2% total β_2-GI. Since postprandial serum contained more β_2GI in LDL it is possible that β_2GI in that density class is transported by the intermediate density lipoproteins. Relatively little difference was observed between fasting and postprandial serum concerning the amount of β_2GI transported in the individual density classes except for the chylomicron fraction.

Since β_2GI with M_r 40 000 sediments only slowly during removal of lipoproteins by ultracentrifugation, there remained the possibility that the presence of this glycoprotein in lipoprotein density fractions was due to contaminations. We therefore isolated VLDL, LDL and HDL in the conventional way by sequential ultracentrifugation followed by 2 washes. Although accurate quantitation in each fraction was not possible we observed roughly the same proportion of β_2GI in VLDL, LDL and HDL as found in the above procedure. To prove further the lipid binding property of β_2GI, charge-shift electrophoresis was carried out with β_2GI purified according to [9]. Figure 1 shows the electrophoretic mobility of β_2GI in presence of positively as well as negatively charged detergents.

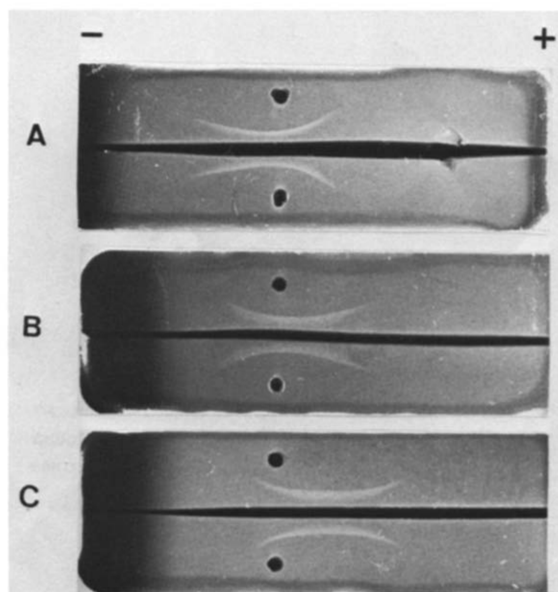

Fig.1. Charge-shift electrophoresis of β_2GI in the presence of Triton X-100 + Cethyltrimethylammonium bromide (plate A), Triton X-100 (plate B) and Triton X-100 + sodium deoxycholate (place C). Experiments were carried out as in [10]. β_2GI applied to the holes was 0.7–1.5 g/ml. Anti-β_2GI was purchased from Behring Werke.

Since the migration rate and direction changes in the presence of charged detergents, it can be assumed according to [10] that β_2GI in fact is an amphipathic protein which binds detergents by hydrophobic interaction Further experiments are currently in progress indicating that β_2GI preferentially binds to triglyceride-rich lipid emulsions (in preparation)

Acknowledgements

This work was supported by the Osterreichische Forschungsrat, project 3734 The technical assistance of H Grillhofer is appreciated

References

[1] Burstein, M and Scholnik, H R (1972) Life Sci 11, 177–184
[2] Burstein, M and Legman, P (1977) Protides of the Biological Fluids (Peeters, H ed) pp 407–410, Pergamon Press, Oxford
[3] Schultze, H E , Heide, K and Haupt, H (1961) Naturwiss 23, 719
[4] Polz, E , Kostner, G and Holasek, A (1979) submitted
[5] Utermann, G and Beisiegel, R (1979) FEBS Lett 97, 245–248
[6] Kostner, G M (1976) in Handbuch der Inneren Medizin, VII/4 Fettstoffwechsel, pp 125–171, Springer, Heidelberg
[7] Kane, P J , Sata, T , Hamilton, R L and Havel, R J (1975) J Clin Invest 56, 1622–1634
[8] Kostner, G M (1976) Clin Chem 22, 695
[9] Finnlayson, J S and Muskinski, J F (1967) Biochim Biophys Acta 147, 413–420
[10] Helenius, A and Simons, K (1977) Proc Natl Acad Sci USA 74, 529–532
[11] Friedewald, W T , Levy, R I and Fredrickson, D S (1972) Clin Chem 18, 499–508