THE EFFECT OF HUMAN ARGININE RICH APOPROTEIN ON RAT ADIPOSE LIPOPROTEIN LIPASE

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SUMMARY

Heterologous human arginine rich apoprotein purified by heparin affinity chromatography from very low density lipoproteins produces a pronounced inhibition of the activity of lipoprotein lipase obtained from rat adipose tissue when the apoprotein is added directly to the assay medium. If, on the other hand, only the triglyceride emulsion bound arginine rich apoprotein is presented to the enzyme, a two-fold increment in the activity of the enzyme is noted. The ratio of the substrate bound arginine rich apoprotein to the free apoprotein importantly influences the effect of this apoprotein on the lipoprotein lipase reaction. These findings suggest a potential receptor role for the protein in this enzyme-substrate interaction.

INTRODUCTION

The addition of arginine rich apoprotein, isolated from very low density lipoproteins, to assays of lipoprotein lipase has either been without effect (1) or resulted in an inhibition of the enzyme (2, 3). Despite the fact that the arginine rich apoprotein has a distinct affinity for heparin (4), which may be in close association with lipoprotein lipase (5), no role has been defined for it in triglyceride catabolism. The arginine rich apoprotein is a predominant constituent of triglyceride rich lipoproteins. In fact, one of the early metabolic events of the lymph chylomicron after reaching the plasma is the addition of the arginine rich apoprotein to the chylomicron (6, 7). Yet no function in triglyceride transport has been characterized for this apoprotein.

The present investigation describes an effect of the arginine rich apoprotein, isolated from human very low density lipoprotein (VLDL) by heparin affinity chromatography (4), on the activity of a crude lipoprotein lipase enzyme obtained from rat adipose tissue. On the basis of the influence of

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this apoprotein when bound to the triglyceride substrate as opposed to its effect in free form, a role for this apoprotein in the lipoprotein lipase reaction is proposed.

MATERIALS AND METHODS

Plasma was obtained from normal and hypertriglyceridemic (Type IV phenocopy) humans after a 14-hour fast and collected in EDTA (.1 mg/ml blood). The VLDL were isolated at 4° from plasma by conventional ultracentrifugal methods using a Beckman L5-65 centrifuge and 50 Ti rotor (1.8×10^8 g-min). They were repurified by an additional flotation through saline under the same conditions and dialyzed against 1,000 volumes of buffered 0.01% EDTA (pH 7.4) per volume of lipoprotein. After lyophilization, the lipoproteins were delipidated three times with 3:1 ethanol:ether (50 volumes per volume of lipoprotein) and dried under nitrogen.

The lipid free apoproteins were dissolved in a 2mM phosphate buffer (pH 7.4) containing 50 mM sodium chloride and 5 M urea. The urea soluble apoproteins (3 mg per ml buffer) were applied to a heparin affinity column which had been prepared by a method similar to that of Iverius (8). The apoproteins were eluted from the column using a linear salt gradient running from 50 mM to 500 mM sodium chloride. The arginine rich apoprotein fraction was isolated from all the other urea soluble apoproteins by virtue of its unique affinity for heparin (4). This arginine rich apoprotein was concentrated and repeatedly washed free of urea and phosphate on a PM 10 Amicon Filter system using distilled water, and then lyophilized in preparation for addition to the assay mixtures.

The epididymal fat pads of male Sprague Dawley rats were delipidated exactly as previously described (9), using repeated acetone/ether extractions. The resulting powder was extracted with a buffer (5 mg powder/ml buffer) containing 0.05 M NH_4OH/NH_4Cl pH 8.6 and briefly homogenized at 4° C, and centrifuged in a 2B Sorval for 30 min. at 800 g. The clear enzyme supernatant solution was decanted and used for the assays.

The assay of lipoprotein lipase was performed in a manner similar to the procedure of LaRosa, et al. (10). The total assay mixture had the same glyceryl $1^{14}C$ trioleate concentration, 139 nmoles per 6 ml, and specific activity (3960 dpm/nmole) as previously described, but used a 0.1 M Tris buffer pH 8.6 and the addition of 0.2 ml fasting normal human serum per 6 ml assay mix. The basic assay mixture, containing the solvent free glyceryl $1^{14}C$ trioleate and 0.1 ml 1% triton, was prepared in 6 ml of 0.1 M Tris and sonified For the control incubations, one-half of this mixture (3 ml) was layered under buffer and centrifuged at 30,000 rpm at 8° using a 40.3 Beckman rotor and an L5-65 Beckman ultracentrifuge. The substrate was retrieved in the top 3 ml after centrifugation (spun substrate). The other half of the substrate (3 ml) was assayed without ultracentrifugation (unspun substrate). The arginine rich apoprotein was added to the basic substrate mixture (25 - 100 µg/ml) in the experimental vials, and 3 ml of the substrate was ultracentrifuged prior to assay, and the other 3 ml assayed without centrifugation. The assays were begun by adding 50 µl of heparin (100 U/ml), Sigma Chem., 0.1 ml of 1% human albumin, 0.1 ml fasting human serum and either 0.5 ml of enzyme preparation or 0.5 ml of buffer to 3 ml of the basic mixture. The assays were run for one hour at 27°, and the hydrolyzed free fatty acids isolated by a conventional method (10) and assayed for radioactivity.

TABLE I

The Effect of Direct Additions of Differing Human Arginine Rich Apoprotein Concentrations on Rat Adipose Lipoprotein Lipase

Concentration of Added ARP[a] µg/ml	Lipase Activity nmol FFA[a]/hr/ml
0	0.29 (8)[b]
25	0.27 (6)
50	0.22 (4)
75	0.16 (6)

[a] ARP = arginine rich apoprotein; FFA = free fatty acid

[b] Results are the means of triplicate determinations. The figure within the parenthesis indicates the fractional standard deviations expressed as percent.

RESULTS

When arginine rich apoprotein was directly added to the assay mixture in concentrations from 25 - 75 µg of protein per ml, a suppression in the activity of the crude lipoprotein lipase from the rat adipose tissue (Table I) was observed. The degree of suppression was dependent upon the amount of the apoprotein added to the mixture. A greater inhibition was noted for the assays with the larger contents of the added apoprotein.

If the triolein substrate were mixed with 100 µg/ml of the arginine rich apoprotein, a greater degree of inhibition was noted (Table II) than for the lower concentrations of this apoprotein. This concentration of apoprotein produced an approximately 75% inhibition of the enzyme activity. When the triglyceride triton emulsion was centrifuged away from the added arginine rich apoprotein before the assay, an appreciable amount (10 - 20%) by amino acid analysis) of the arginine rich apoprotein floated up with the substrate

TABLE II

The Effect of Triglyceride Bound and Unbound

Arginine Rich Apoprotein on Rat Lipoprotein Lipase

Experiment	Lipase Activity[a] nmol/hr/ml	
	Uncentrifuged Substrate	Centrifuged Substrate
Control (No ARP[b])	0.31	0.34
ARP 33 (100 µg/ml)	0.09	0.59
ARP 426 (100 µg/ml)	0.06	0.48
ARP 518 (100 µg/ml)	0.09	0.54
ARP 21 (100 µg/ml)	0.07	0.61

[a] The activity figures represent the means of duplicate determinations of lipoprotein lipase assayed as described in the text.

[b] ARP = arginine rich apoprotein.

(Figure 1). The bulk of the apoprotein (>80%) remained in the infranate unassociated with glyceride radioactivity. These triglyceride emulsions which had been exposed to the arginine rich apoprotein appeared to take up the apoprotein as does the native lymph chylomicron when it enters the plasma (6). When this centrifuged substrate, containing arginine rich protein, was incubated with the enzyme a reproducible increment in enzyme activity was observed (Table II). The centrifuged substrate demonstrated almost twice the activity of the control centrifuged substrate and was almost eight times more active than the uncentrifuged substrate which had the bulk of the arginine rich apoprotein in the substrate unbound form.

DISCUSSION

A number of observations have suggested that the arginine rich apoprotein has a function in the plasma transport of triglycerides. It has been noted

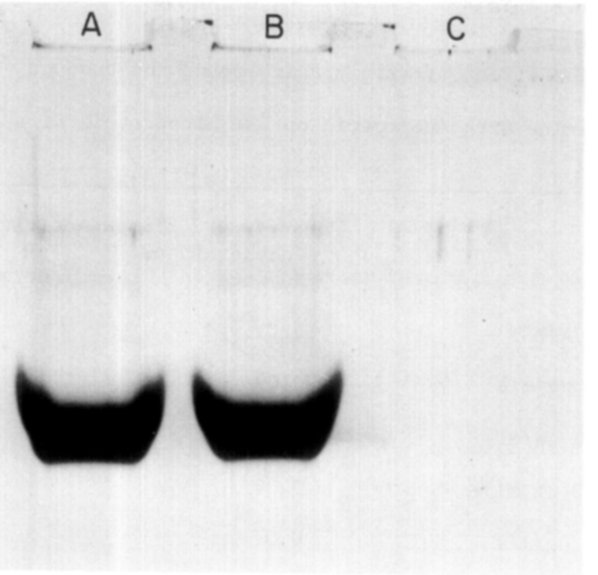

Figure 1. The sodium dodecyl sulfate - 10% polyacrylamide electrophoretic pattern of: A) the pure arginine rich apoproteins added to the incubations; B) the ultracentrifuged triglyceride emulsions which had been exposed to the arginine rich apoprotein; C) the ultracentrifuged control triglyceride emulsions.

that in humans (11) the arginine rich apoprotein is predominantly on the triglyceride rich lipoproteins with very little on any other lipoprotein group. It has also been observed (4) that the arginine rich apoprotein is the only apoprotein of the relatively soluble group of proteins on triglyceride rich lipoprotein to have an appreciable affinity for heparin. Thirdly, it has been recognized (6) that one of the early metabolic events of a chylomicron upon entering the plasma is the addition of the arginine rich apoprotein to it. All these observations suggest some role for this apoprotein in the metabolism of plasma triglyceride.

Despite these suggestive data indicating a role for the arginine rich apoprotein in triglyceride transport, assays of this apoprotein have either revealed no effect of the apoprotein on the lipoprotein lipase (1) or a suppression of activity from the apoprotein (2, 3). From the data presented here, it is apparent why a differing assay result might be anticipated depen-

ding upon the conditions chosen for the assay: in particular, the amount of the arginine rich apoprotein added. The data described strongly indicate an effect of this heterologous apoprotein on rat adipose tissue lipoprotein lipase activity. The effect on the assay appears dependent on the relative amount of apoprotein bound to the triglyceride substrate and the amount of apoprotein unbound. When the substrate is made rich in the bound species by ultracentrifugally elminating the unbound protein, there is an approximate two-fold stimulation in the activity of the enzyme. When the lipoprotein lipase triglyceride reaction occurs in a milieu of predominantly free arginine rich apoprotein as seen with direct addition to the incubation system, there is an inhibitory phenomenon observed similar to that noted previously (2, 3).

These data are compatible with a receptor role for the arginine rich apoprotein associated with triglyceride rich lipoproteins in the lipoprotein lipase reaction. When the protein is bound to substrate, a positive influence on the hydrolysis of substrate is noticed even in bulk solution; whereas when the protein is substrate-free, the anticipated inhibitory effect is then observed. The affinity of the arginine rich apoprotein for heparin (4) most likely is the property that determines its proposed receptor role for lipoprotein lipase. Korn (5) has suggested an intimate association between tissue lipoprotein lipase and heparin although such an association under physiologic conditions has not been proven. However, the strong affinity of the enzyme for heparin (12) makes it reasonable to propose heparin as the mediator of an arginine rich apoprotein-lipoprotein lipase interaction. The inhibitory effect of the substrate free arginine rich apoprotein observed here could be on the basis of tying up the enzyme heparin complex with a "false substrate" or by competing with the enzyme for heparin binding sites. The much greater affinity of the enzyme for heparin (12) makes the second explanation somewhat unlikely.

The relative amounts of the arginine rich apoprotein that are bound to the appropriate triglyceride rich substrate as opposed to that amount of apoprotein unbound, or bound to non-triglyceride substrate, may importantly

govern the activity of the lipolytic system *in vivo*. The *in vivo* relationship of substrate and enzyme is quite different from these *in vitro* bulk solution conditions. Most of the enzyme appears to be fixed in tissue, probably associated with acidic glycosaminoglycans (5), but the substrate circulates in plasma. A receptor protein on the substrate would potentially be even more critical for the association of enzyme and substrate *in vivo*.

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