Serum High-Density Lipoprotein: Effect of Change in Structure on Activity of Chicken Adipose Tissue Lipase

Abstract. *The high-density (1.063 to 1.21 g/ml) lipoprotein in human serum was analyzed as activator for a lipoprotein lipase isolated from chicken adipose tissue. The activating capacity was lost when the lipoprotein was extracted with a mixture of ethanol and ethyl ether (3:2 by volume) at — 10°C and it was restored upon incubation of the extracted protein with aqueous sols of either whole phospholipids or the lecithin fraction prepared from the high-density lipoprotein. Since phospholipid sols alone proved ineffective as substrate activators, the complex which forms upon incubation of the extracted lipoprotein with phospholipids appears to be a necessary requirement for lipoprotein lipase activity.*

The high-density lipoprotein (HDL) in normal human serum of density between 1.063 and 1.21 g/ml contains approximately 50 percent lipid and can be degraded to an essentially lipid-free apoprotein, which retains solubility in aqueous media (*1*, *2*) and avidity for lipids (*3*).

This apoprotein recombines in vitro with HDL phospholipids, forming a stable complex with a protein-phospholipid ratio similar to that of the parent lipoprotein (*4*). I now report results indicating that a lipid-protein complex, reassembled from inert constituents of HDL, is a necessary requirement for the lipolytic activity of a lipoprotein lipase (*5*) isolated from chicken adipose tissue.

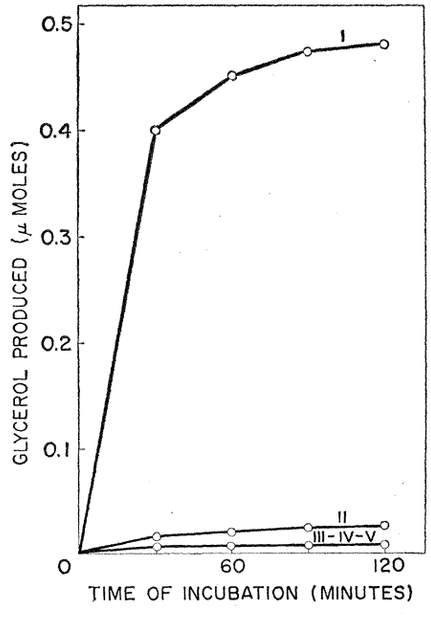

Fig. 1. Kinetics of glycerol release by lipoprotein lipase isolated from chicken fat. Nature of substrate activator as follows. *I*, apoprotein + phospholipid micelles; *II*, apoprotein + phospholipid micelles + 1*M* NaCl; *III*, apoprotein alone; *IV*, phospholipid micelles, alone; *V*, same as *I*, but with heated enzyme. Concentration of apoprotein in the system, 0.2 mg. Each point represents a mean of a duplicate determination.

Apoprotein and HDL were prepared from normal human serums (*1*). Whole phospholipids (lecithin, sphingomyelin, phosphatidylethanolamine, and lysolecithin) and the lecithin fraction were extracted from HDL with a mixture of ethanol and ethyl ether (3:1 by volume) and separated by silicic acid–column and thin-layer chromatography (*1*). The phospholipids were treated with high-frequency sound (20 kc/sec, 10 minutes, 4°C) and two parts (by weight) of the resulting aqueous sols were incubated with one part of apoprotein for 16 hours at 25°C. The mixture was used either directly or after purification by preparative ultracentrifugation or gel filtration. The techniques of purification and the physicochemical properties of the apoprotein phospholipid complex have been reported (*4*). Lipoprotein lipase was prepared from commercial frozen chicken fat (*6*), and its activity was tested against a coconut oil emulsion (*5*) by measuring glycerol production, which is known to parallel release of free fatty acids (*7*). The complete enzyme system contained: enzyme (20 mg/ml), 0.2 ml; Ediol (diluted to a concentration of 25 μmole of triglycerides per milliliter), 0.2 ml; HDL (2 mg of protein per milliliter), 0.1 ml; 1*M* $CaCl_2$, 0.02 ml; 10 percent bovine serum albumin, 0.2 ml; 1*M* NaCl, 0.2 ml; NH_4Cl-NH_3 buffer (*p*H 8.5, ionic strength 0.1) to a final volume of 1.2 ml. All components of the mixture were either dissolved or dialyzed against NH_4Cl-NH_3 buffer before use. The absolute amount of HDL protein was kept constant in all experiments. The final system was incubated at 37°C, and the glycerol released after 120 minutes of incubation was determined (*7*). Substrate (Ediol) and activator (HDL or apoprotein) in the given proportions were incubated at 37°C for 30 minutes before use. Determinations were made in duplicate and did not vary more than 10 percent. Values of glycerol produced were expressed as a difference between 120 minutes and zero time.

With the enzyme system used (Table 1) Ediol alone proved an inert substrate. Activation was promoted by incubating the fat emulsion with HDL, either intact or after treatment with ethyl ether, a procedure which removes part of the neutral lipids and leaves a lipoprotein with its entire complement of phospholipids (*8*). The degree of lipolysis was dependent upon the amount of HDL protein in the system. In the range 0.1 to 0.4 mg the correlation was linear (glycerol produced in 120 minutes was 400 to 800 mμmole); above these values, lipolysis reached a plateau. Kinetics of glycerol release (Fig. 1) were similar to those reported by Korn (*6*).

Neither apoprotein nor phospholipid micelles alone activated the substrate. On the other hand, lipolysis was observed in the presence of reconstituted apoprotein phospholipid complex. The activity of the complex, which was dependent on the amount of apoprotein in the system, was lost after the lipoprotein was extracted with the ethanol-ether mixture (*1*) and was restored upon addition of phospholipid micelles. When lecithin was substituted for the phospholipid mixture in the recombination with apoprotein, the resulting complex was equally effective

Table 1. Role of substrate "activator" on the activity of lipoprotein lipase from chicken adipose tissue. Abbreviations: C, complete; αP, apoprotein; PM, phospholipid micelles.

Nature of the system	Glycerol produced* (mμmole)
C*	540
C with 1*M* NaCl	10†
C with heated enzyme 100°C, 5 minutes	10
C without Ediol	10
C without HDL	10
C, replace HDL with HDL (Et_2O)‡	500
C, replace HDL with αP	10
C, replace HDL with PM	10
C, replace HDL with αP and PM	480
C, replace HDL with αP and PM, but omit Ediol	10
C, replace HDL with αP and PM, add 1*M* NaCl	10
C, replace HDL with αP and PM, with heated enzyme	10

* For details see text. † 10 mμmole-lower limit of sensitivity of the analysis. ‡ HDL extracted with ethyl ether.

in the activation of the substrate. Sphingomyelin and phosphatidylethanolamine were not studied. None of the other serum nonlipoprotein components (albumin, α_1-glycoprotein, γ-globulin) or whole serum from which lipoproteins were removed by ultracentrifugal flotation at density greater than 1.21 (Spinco Model L, 114,000*g*, 24 hours, 16°C) were active either alone or after mixing with sols of HDL phospholipids. Modification of the substrate by ionic surfactants (sodium octyl to hexadecyl sulphate and benzyldimethyloctyl to nonadecyl ammonium chloride) in the presence or absence of apoprotein, was also ineffective in eliciting enzyme activity.

All of the enzyme preparations used produced comparable and reproducible results. As observed by Korn (*6*) they were inactivated by NaCl and showed a decline in activity upon prolonged storage at 4°C.

The results indicate that partial structural restoration of HDL apoprotein by addition of phospholipids is accompanied by restitution of function. This observation may prove useful in studies of the structure of HDL and in the analysis of its mode of formation. The mechanism of substrate activation by HDL must await isolation of lipoprotein lipase in pure form.

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References and Notes

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Immunoglobulin M Allotypes of the Rabbit: Identification of a Second Specificity

Abstract. *Rabbit antiserums, originally prepared to react specifically with rabbit immunoglobulin allotypes Ab5 and Ab6, also react with some normal rabbit serums that clearly do not contain Ab5 or Ab6 allotypes. This reaction is due to antigenic specificity present on rabbit immunoglobulin M (IgM) and not on rabbit immunoglobulin G. Serums that contain this IgM allotype do not react with an antiserum that reacts with the known rabbit IgM allotype, Ms1. This specificity may therefore be identified as a second rabbit IgM allotype, Ms2.*

Rabbit immunoglobulin allotypes are genetically controlled antigenic specificities which may differ from one individual to another. Immunization of a rabbit not having a given allotypic specificity with the serum (or immunoglobulin) of another rabbit bearing the given allotypic specificity results in the formation of antibodies in the serum of the immunized rabbit which react with the given allotypic specificity (*1*). To date, six different allotypic antigenic specificities have been well defined. Allotypes Aa1, Aa2, and Aa3 are controlled by one chromosomal locus "a," and are located on the H-chain of rabbit immunoglobulin G (IgG); allotypes Ab4, Ab5, and Ab6 are controlled separately by a second chromosomal locus "b" and are generally thought to be located on the L-chain of rabbit IgG (*2*). The determinants controlled by both loci are present on serum IgG and immunglobulin M (IgM) and on immunoglobulin A (IgA) and IgG in colostrum (*3*).

An allotypic determinant present on rabbit IgM and not on rabbit IgG has been identified and has been named Ms1 (*4*). The hypothetical allele or alleles for the IgM allotype Ms1 controlled by the gene locus Ms, or any other allotypic determinant of IgM, has not previously been reported. I now report preliminary identification of a second allotypic antigenic determinant on rabbit IgM.

The allotypic specificities of the immunoglobulins of a given rabbit are determined in this laboratory by "double-diffusion-in-agar" precipitin reactions between the appropriate specific antiserums to an allotype and the serum obtained from the rabbit being tested. During the course of allotyping the serums of rabbits with antiserums to the six well-characterized immunoglobulin allotypes Aa1, Aa2, Aa3, Ab4, Ab5, and Ab6 (*5*), faint precipitin bands with antiserums directed against Ab5 and Ab6 appeared 2 to 3 days after the major precipitin bands (Fig. 1) appeared.

With one Ab4,5 serum two lines were observed with the antiserum to Ab5. One was the result of a reaction with the Ab5 antigenic determinant, whereas the second faint line was probably the result of a different group of molecules as the line passed through the precipitin line formed by the reaction of antiserum to Ab5 with Ab5 (Fig. 1). Thus an intraspecies antigenic difference present on a serum protein other than IgG was tentatively identified. According to current terminology this antigenic determinant could be called an allotype.

The next step was to ascertain on which serum protein this allotypic determinant was located. The late appearance of the precipitin band, the location close to the antigen well, and the tendency of the lateral margins of the band to curve toward the antigen well (Fig. 1) indicated a protein in low concentration with a diffusion constant less than that of the reacting antibody. All of these observations seemed most consistent with serum IgM. Accordingly individual serums containing the antigen were separated on Sephadex G-200. The antigenic determinant of interest was located only in the first peak, that is, the high-molecular-weight area containing IgM (Fig. 2). Treatment of individual serums containing this antigenic specificity with 2-mercaptoethanol destroyed the capacity of these serums to form a precipitin band when they were reacted with the appropriate antiserums, but the a and b loci reactivities were not affected. Similarly mercaptoethanol also destroyed the reactivity of Msl-containing serums when they were reacted with antiserums to Msl. To define the macroglobulin on which the determinant was located immunoelectrophoresis was performed (Fig. 3). These analyses indicated that the allotype identified was indeed an allotypic determinant present on rabbit IgM.

To determine whether this IgM allotype represented a previously unreported determinant or was only a rediscovery of the previously reported IgM allotype Ms1, normal serums that were not Ab6 or Ab5 but contained the IgM allotype under investigation and