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SOME KINETIC PROPERTIES OF PLASMA LECITHIN-CHOLESTEROL ACYLTRANSFERASE IN HYPER-α-LIPOPROTEINEMIA IN MAN

A. A. Nikiforova, E. M. Ivanova, and E. G. Alksnis

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Lecithin-cholesterol acyltransferase (LCAT; E.C. 2.3.1.43) catalyzes the transacylation reaction, namely transfer of a fatty acid radical from the β-position of lecithin to the hydroxyl group of cholesterol (CH) with the formation of a cholesterol ester (CHE) and lysolecithin [7, 8]. Both substrates (lecithin and cholesterol), and also apoproteins A-I and C-I which are activators of this reaction, are components of high-density lipoproteins (HDL), with which LCAT forms a substrate-enzyme complex in the blood [4]. Recently the protective role of HDL in the development of atherosclerosis and one of the complications of this disease, namely ischemic heart disease, has been demonstrated [2, 3, 12, 13, 15]. The biochemical mechanisms which lie at the basis of elevation of the blood HDL level and also the role of LCAT in this process remain unexplained. Albers [5] showed by analytical isofocusing that five LCAT isozymes are present in human blood plasma. These findings have not yet been confirmed. The physiological role of the individual isozymes likewise awaits elucidation.

The aim of this investigation was to study some kinetic properties of LCAT in the blood plasma of patients with hyper-α-lipoproteinemia, enabling the presence of LCAT isozymes in the blood to be detected.

EXPERIMENTAL METHOD

Blood was taken from healthy men aged 25-35 years with persistent hyper-α-lipoproteinemia (their HDL cholesterol, or α-CH level in all cases exceeded 80 mg/dl, corresponding to an HDL concentration of more than 500 mg/dl according to the results of analytical ultracentrifugation [1]). The total HDL fraction isolated from the subjects' blood plasma within the density range of 1.063-1.21 g/ml was used as substrate for the LCAT reaction. The source of enzyme was lipoprotein-free plasma obtained from the subjects of the investigation by removal of all classes of lipoproteins (LP) from it. The method of taking blood, obtaining plasma, isolating HDL, and obtaining 4-^{14}C-cholesterol-labeled HDL and lipoprotein-free

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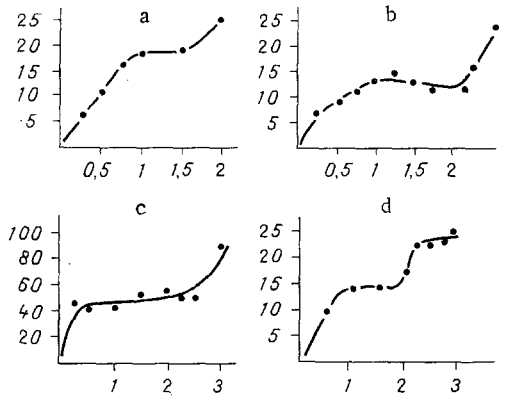

Fig. 1. Velocity of LCAT reaction in infranatant from subjects with hyper-α-lipoproteinemia depending on substrate concentration. Abscissa, concentration of nonesterified HDL cholesterol ($\times 10^{-3}$ M); ordinate, quantity (in nanomoles) of nonesterified HDL cholesterol undergoing esterification during incubation for 1 h, calculated per milliliter in infranatant. a-d) α-CH concentrations 95, 89, 130, and 90 mg/dl respectively.

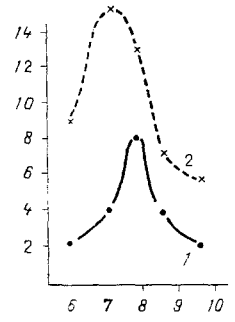

Fig. 2

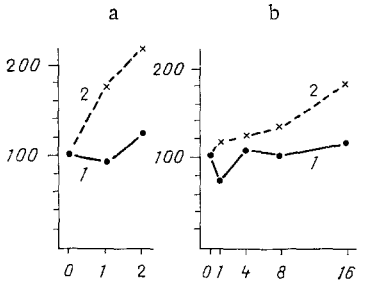

Fig. 3

Fig. 2. Dependence of velocity of LCAT reaction on pH of medium with two substrate concentrations: 1 mM (1) and 3 mM (2) of HDL cholesterol. Abscissa, pH values; ordinate, quantity (in nanomoles) of nonesterified HDL cholesterol undergoing esterification during incubation for 1 h, calculated per milliliter of infranatant.

Fig. 3. Effect of different apo-E concentrations on velocity of LCAT reaction with two substrate concentrations: 1 mM (1) and 3 mM (2) (velocity of LCAT reaction in samples without apo-E taken as 100%). Abscissa, apo-E concentration in sample (in μg); ordinate, change in velocity of esterification of CH in presence of apo-E (in %). a, b) α-CH concentrations 130 and 90 mg/dl respectively.

plasma (the infranatant), as well as the methods, of determining α-CH, and nonesterified HDL cholesterol and protein in the infranatant, were described previously [14]. The velocity of the LCAT reaction was judged by determining labeled CHE formed from ^{14}C-nonesterified CH and lecithin of HDL on incubation of the latter with the enzyme (infranatant). The incubation mixture contained 0.03 ml of infranatant (protein content 2 mg), 0.04 or 0.12 ml of labeled HDL (the concentration of nonesterified HDL cholesterol was 1 and 3 mM respectively), and 0.13 or 0.05 ml of 0.01 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl, 1 mM EDTA, and 10 mM mercaptoethanol, the total volume of the sample being 0.2 ml. When studying the dependence of velocity of the LCAT reaction on substrate concentration, the latter was

varied between 0.5 and 3 mM of nonesterified HDL cholesterol. Instead of the infranatant, buffer was added to the control samples. The conditions of incubation, stopping the reaction, fractionating the lipids by thin-layer chromatography, and counting radioactivity were described previously [14]. The velocity of the LCAT reaction was expressed in nanomoles nonesterified CH undergoing esterification during incubation for 1 h, calculated per milliliter of infranatant, Apoprotein E (apo-E) was generously provided by A. D. Denisenko.

EXPERIMENTAL RESULTS

Dependence of the velocity of the LCAT reaction on concentration of substrate (nonesterified HDL cholesterol) in four subjects with hyper-α-lipoproteinemia is shown in Fig. 1. It will be clear from Fig. 1 that kinetic curves of complex character, with one (a, b, c) or two (d) plateaux, and with a second maximum of reaction velocity observable at high substrate concentrations (2.5-3 mM nonesterified HDL cholesterol), were obtained in all cases. Discovery of the complex character of the kinetic curves for LCAT against substrate led to the decision to study the effect of pH of the medium on reaction velocity with two substrate concentrations (1 and 3 mM of nonesterified HDL cholesterol), corresponding to the two peaks on the kinetic curve. As Fig. 2 shows, if the concentration of nonesterified HDL cholesterol was 1 mM the optimum of reaction velocity was observed at pH 8.0, whereas with a substrate concentration corresponding to the second peak (3 mM) the pH optimum was shifted toward the acid side, at 7.4. A difference was thus found in the pH optimum for LCAT in different substrate concentrations. The results obtained with low substrate concentrations in these experiments agree well with the data of Jahani et al. [9], who used only low concentrations of nonesterified HDL cholesterol or liposomal CH and, under those conditions, found that the pH optimum for LCAT is 8.0.

In the next series of experiments the effect of apo-E on velocity of the LCAT reaction was studied. The effect of apoproteins such as A-I, A-II, C-I, C-II, C-III, and D on the kinetics of the LCAT reaction has in fact been well studied [5]. However, there are no data in the literature on the effect of apo-E on LCAT. The possibility of complex formation between apo-E and CH was nevertheless demonstrated in [10, 11]. These investigations, together with our own data [14], showing positive correlation ($r = +0.89$) between the velocity of the LCAT reaction and the apo-E concentration in a group of subjects with hyper-α-lipoproteinemia, led us to investigate the effect of this apoprotein of LCAT with two substrate concentrations. It will be clear from Fig. 3 that apo-E had no appreciable effect on the velocity of the LCAT reaction with low substrate concentrations (1 mM of nonesterified HDL cholesterol). Meanwhile with high substrate concentrations (3 mM) apo-E activated the reaction velocity; its activating effect, moreover, increased with an increase in apoprotein concentration in the medium. The complex character of the curves of reaction velocity versus substrate concentration, with the presence of one or even two plateaux, together with the difference in pH optimum and the action of apo-E, suggest that the blood plasma of individuals with hyper-α-lipoproteinemia contains at least two LCAT isozymes. These data agree with results in the literature [5]. The complex character of the curve of velocity of CH esterification versus substrate concentration can evidently be explained by the presence of isozymes in the subjects' plasma, one of which corresponds to the hyperbolic, the other to the S-shaped character of the curves in Fig. 1. The S-shaped character of the curve of reaction velocity versus substrate concentration for one isozyme is evidence that this form is active mainly in high substrate concentrations. Incidentally, it is under those conditions that the activating effect of apo-E on LCAT is observed. It is not yet possible to conjecture what the mechanism of the activating action of apo-E is. It can be tentatively suggested that apo-E increases affinity of the substrate for the LCAT isozyme with an S-shaped reaction velocity curve. This isozyme evidently exhibits its action only when the blood HDL concentration rises. At the same time, the possibility cannot be ruled out that the presence of this LCAT isozyme in the plasma may be a regulating factor when the blood HDL concentration rises.

The results of this investigation shed light on some facts discovered by the writers previously. For instance, we found no correlation between velocity of the LCAT reaction and the HDL level in a group of subjects including individuals with hypo-, normo-, and hyper-α-lipoproteinemia [14]. The same conclusion was drawn by Albers, who found no correlation between the enzyme concentration, determined by radioimmunoassay, and the blood HDL level in a population group of subjects [6]. Meanwhile, comparison of LCAT activity in two groups of subjects — with high and normal blood HDL levels — revealed a statistically significant

decrease in the activity of this enzyme in subjects with hyper-α-lipoproteinemia [14]. It can be tentatively suggested that the low LCAT activity in this case is due to a decrease in activity of its isozyme which has a hyperbolic type of reaction velocity versus substrate curve, for the experimental conditions were optimal for determination of the activity of precisely this isozyme (concentration of nonesterified HDL cholesterol 1.5-1.75 mM, pH 8.0). However, considering the fact that the total velocity of the CH esterification reaction in subjects with a high HDL level is unchanged [14], it can be postulated that a decrease in the activity of one isozyme must be accompanied by increased activity of another. Consequently, in individuals with hyper-α-lipoproteinemia, activity of the isozyme with a hyperbolic type of curve is depressed, but at the same time, activity of the isozyme with an S-shaped reaction velocity curve, with maximal activity at high substrate concentrations, is increased. On the whole, it can be concluded from these results that when the physiological role of LCAT is studied, besides determining the total velocity of CH esterification or the plasma enzyme concentration, it is also essential to study activity of individual isoforms of this enzyme.

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