VITAMIN E BINDING PROTEINS IN HUMAN SERUM

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Summary The distribution of vitamin E in human serum lipoprotein fraction was investigated by measuring the amount of vitamin E in various protein fractions prepared by ultracentrifugation, gel filtration and electrophoresis. The larger part of the total serum vitamin E (up to 44%) was present in the α_1 -lipoproteins, while the β -lipoproteins contained smaller concentrations (up to 26%).

It is well known that lipids and fat-soluble vitamins in serum are bound to proteins and therefore are soluble in water. Thus it can be considered that vitamin E, one of the fat-soluble vitamins, is also bound to proteins. Many investigations have shown that the β -lipoprotein fraction is the major fraction for the transport of vitamin E (1-6). On the other hand, results by LEWIS *et al.* (7) indicate that a significantly larger part of the vitamin E (54%) is carried in the α_1 -lipoprotein fraction than in the β -lipoprotein fraction (20%).

The purpose of present study is to determine, whether vitamin E is bound mainly to β -lipoproteins as generally assumed or to α_1 -lipoproteins.

MATERIALS AND METHODS

Human serum. Serum samples from 50 to 100 healthy persons were pooled and stored at 4°C and analyzed within 1 week after sampling. Hemolytic, chylaceous sera and Au antigen positive sera were omitted.

Antisera. Anti- α_1 -lipoprotein serum and anti- β -lipoprotein serum were supplied by the Behring Institut. The anti-albumin serum was prepared by immunizing rabbits with a purified albumin (8, 9).

Standard proteins. The β -lipoprotein standard and the albumin standard were provided by Hoechst Japan Co., Sendai. The α_1 -lipoprotein standard was prepared from human serum by a ultracentrifugal flotation procedure and preparative polyacrylamide gel electrophoresis (shaded area in Fig. 7 represents combined

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fractions utilized for α_1 -lipoprotein standard).

Serum proteins were fractionated by ultracentrifugation according to OKAJIMA (11) and by the Sepharose 4B gel filtration procedure modified by QUARFORDT *et al.* (10). Each fraction was analyzed for its vitamin E and lipoprotein concentration. Vitamin E was assayed by Thompson's fluorometry procedure modified by ABE (12). Identification of each lipoprotein was analyzed Ouchterlony's double immunodiffusion method (13), while for the quantitative analysis Mancini's single radial immunodiffusion method and partially rocket immunoelectrophoresis (14, 20), was employed.

RESULTS

Each serum was fractionated into chylomicrons, VLD-lipoproteins,³ β -lipoproteins, α_1 -lipoproteins and VHD-lipoproteins³ by ultracentrifugation at a density of 1.006, 1.063 and 1.21 g/ml. The amounts of vitamin E and lipoprotein found in each fraction are shown in Figs. 1, 2 and 3 and the corresponding distribution in percent is summarized in Table 1.

Ultracentrifugal supernatants were analyzed by means of electrophoretic methods (15, 16). The result of this immunoelectrophoresis is shown in Fig. 4. The photograph shows that 1 ml of the top layer of the supernatant having a density of 1.063 g/ml contains VLD-lipoproteins and β -lipoproteins. A 3.75% polyacrylamide gel electrophoresis indicated that this supernatant also contained chylomicrons. The top layer of supernatant with a density of 1.21 g/ml contained

Fig. 1. Vitamin E distribution pattern obtained by ultracentrifugal flotation a density of 1.006 g/ml.

³ VLD-lipoproteins: very low density lipoproteins.

VHD-lipoproteins: very high density lipoproteins.

Fig. 2. Vitamin E distribution pattern obtained by ultracentrifugal flotation at a density of 1.063 g/ml.

Fig. 3. Vitamin E distribution pattern obtained by ultracentrifugal flotation at a density of 1.21 g/ml.

Table 1. Distribution of serum vitamin E in human lipoprotein fractions.

Fraction	Density (g/ml)	Vitamin E content (%)
Chylomicron and VLD-lipoproteins	<1.006	10
β-Lipoproteins	1.006-1.063	26
α ₁ -Lipoproteins ₂ (HDL ₂)	1.063-1.12	28
α ₁ -Lipoproteins ₃ (HDL ₃)	1.12-1.21	16
VHD lipoproteins and other serum proteins	>1.21	20
Total		100

Fig. 4. Immunoelectrophoretic analysis of ultracentrifugal supernatant. Antigen: a, 1 ml of top layer of the supernatant at a density of 1.006 g/ml; b, 1 ml of top layer of the supernatant at a density of 1.063 g/ml; c, 1 ml of top layer of the supernatant at a density of 1.21 g/ml. Antibody: anti-whole human serum (AWHS) prepared by immunizing rabbits in our laboratory. Anti- α_1 -lipoprotein serum (A α_1 -L) was purchased from the Behring Institut. Staining of lipoproteins was carried out with Sudan Black B in 60% ethanol as described by SAKAGISHI (21) and staining of proteins was carried out with Ponceau 3R.

α_1 -lipoproteins and albumin in addition to the various components of the supernatant also found at a density of 1.063 g/ml. These lipoprotein fractions contained over 80% of the total serum vitamin E. Vitamin E was found at least in the following three kinds of lipoprotein fractions; 1) the chylomicrons and/or the VLD-lipoprotein fractions, 2) the β -lipoprotein fraction, and 3) the α_1 -lipoprotein fraction. The α_1 -lipoprotein fraction contained a major part of the total serum vitamin E, while the β -lipoprotein fraction contained the remainder.

The vitamin E content of each lipoprotein fractionated by the gel filtration procedure is shown in Fig. 5. The first protein peak contained β -lipoproteins and the second peak contained α_1 -lipoproteins and other components. As a result of the gel filtration and the ultracentrifugation experiments, it was established that β -lipoproteins contained *ca.* 20 to 25% of the serum vitamin E. Since the second peak, which is a low molecular weight peak, contained α_1 -lipoproteins and most of the other serum proteins, it could not be determined how much vitamin E was bound specifically to the α_1 -lipoproteins alone. Therefore, an attempt was made to completely separate the various other components from the α_1 -lipoproteins by ultracentrifugation method at a density of 1.21 g/ml. The sera (of the top 1 ml layer) were pooled and analyzed by Sepharose 4B gel filtration. The vitamin E distribution pattern thus obtained is shown in Fig. 6, and indicates that the concentration of the α_1 -lipoproteins was reduced to one-half. The concentration of vitamin E also decreased to a lower level than that of the whole serum shown in Fig. 5, but the α_1 -lipoproteins peak coincided with the highest peak of vitamin E. From these results, it was concluded that α_1 -lipoproteins was the main carrier of

vitamin E in a human serum and was able to bind about 45% vitamin E. Although the α_1 -lipoproteins and the β -lipoproteins could not be separated from each other by means of gel filtration, such a separation could be done by polyacrylamide gel electrophoresis. Lipoproteins were purified by the ultracentri-

Fig. 5. Vitamin E distribution pattern of the whole human serum obtained by gel filtration on Sepharose 4B.

Fig. 6. Vitamin E distribution pattern of lipoprotein fractions prepared by gel filtration on Sepharose 4B.

Fig. 7. Polyacrylamide elution profile of human lipoprotein fractions obtained by ultracentrifugal flotation.

Table 2. The ratio of the vitamin E concentration in α_1 -lipoproteins and in β -lipoproteins.

Sample No.	Applied sample	Ratio
1	Whole serum	2.5
2	Whole serum	3.2
3	Lipoprotein fraction	2.9
4	Lipoprotein fraction	2.5

$$\text{Ratio} = \frac{\text{vitamin E concentration bound to } \alpha_1\text{-lipoproteins}}{\text{vitamin E concentration bound to } \beta\text{-lipoproteins}}$$

fugation at a density of 1.21 g/ml, pooled, and subsequently separated by 3.75% preparative polyacrylamide gel electrophoresis (17) (Fig. 7). In this treatment, β -lipoproteins remained in the gel and did not appear in the eluted fluid. As shown in Fig. 7, the vitamin E peak coincided with the α_1 -lipoproteins peak. The amount of vitamin E, which was found in each lipoprotein, was calculated from the results of the Sepharose 4B gel filtration. The ratio of vitamin E concentration in α_1 -lipoproteins and in β -lipoproteins is shown in Table 2. The concentration of vitamin E bound to α_1 -lipoproteins was about three times larger than that in β -lipoproteins.

It is generally considered that α_1 -lipoproteins are heterogeneous substances (18) but it has not been established whether vitamin E is bound equally to the various subclasses of the α_1 -lipoproteins. The result of gel filtration on Sephadex G-200

Fig. 8. Vitamin E distribution pattern of whole human serum obtained by gel filtration on Sephadex G-200.

Fig. 9. Vitamin E distribution pattern obtained by ultracentrifugal flotation at a density of 1.12 g/ml.

is shown in Fig. 8. Two distinct peaks of vitamin E appeared, whereby one corresponds to the position of the β -lipoprotein peak and the other is situated between the two peaks of β - and α_1 -lipoproteins. In order to clarify which lipoprotein was the real carrier of the vitamin E belonging to the second peak, the serum was adjusted to a density of 1.12 g/ml and centrifuged. The result of this centrifugation is shown in Fig. 9. Infranatant fractions contained about 64% of the serum vitamin E. Furthermore, about 28% of the vitamin E was found to be bound to HDL₂ (high-density lipoproteins₂) (Table 1).

DISCUSSION

SHITARA *et al.* (22) found a good correlation between the serum vitamin E level and the serum cholesterol level in an investigation of the rural residents of Yamagata Prefecture, Japan. It is generally thought that a major part of the serum cholesterol is presented in the β -lipoproteins and VLD-lipoproteins. Therefore, it was considered initially that the major part of the serum vitamin E is bound to the β -lipoproteins. However, the present results show that a larger part of the total serum vitamin E (up to 44%) is present in the α_1 -lipoproteins while only a lesser amount is found in the β -lipoproteins (up to 26%). It is suggested that a correlation between the serum vitamin E and the serum cholesterol level may be explained by a correlation between the serum lipoproteins and the serum lipids.

AFTERGOOD *et al.* (23) has shown that the administration of oral contraceptive drugs to rats or humans decreased the vitamin E and the α_1 -lipoproteins level while increasing the cholesterol and β -lipoproteins in the serum. This result seems to prove that α_1 -lipoproteins are the major fraction for the transport of vitamin E.

As mentioned above, vitamin E is bound to several lipoproteins which transport vitamin E to the various tissues. It is however still unclear whether all the serum vitamin E binding proteins transport vitamin E to the various tissues, or whether specific vitamin E transporting proteins are required. RAJARAM (19) reported that serum VLD-lipoproteins are involved in carrying vitamin E to the various tissues in addition to their important role in the transfer of the vitamin absorbed across the intestine. But the results of the present investigation show in contradiction to his idea, that in the stationary state, less than 10% serum vitamin E is bound to VLD-lipoproteins while the greater part of the vitamin is bound to α_1 -lipoproteins, β -lipoproteins and other serum proteins (Fig. 1). According to studies of KAYDEN's *et al.* (5), the exchange rate which is obtained by incubating vitamin E-deficient erythrocytes with several lipoprotein fractions, indicates that both the LDL and HDL fractions release vitamin E to the erythrocytes to a larger extent than VLD lipoproteins. We suppose that α_1 -lipoproteins and/or β -lipoproteins are involved in the transport of the vitamin to the various tissue, and further studies are in progress to elucidate what sort of proteins participate in the transportation of serum vitamin E to the intracellular organelles.

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