Epitope mapping for the anti-rabbit cholesteryl ester transfer protein monoclonal antibody that selectively inhibits triglyceride transfer

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Abstract **Among the monoclonal antibodies (Mab) against rabbit plasma cholesteryl ester transfer protein (CETP), Mab 14-8F cross-reacted with human CETP and selectively inhibited triglyceride transfer but not cholesteryl ester transfer (**Ko, K. W. S., T. Ohnishi, and S. Yokoyama. **1994.** ***J. Biol. Chem.*** 269: **28206–28213). The epitope of this antibody was studied by using synthetic fragment peptides of rabbit and human CETP. Mab 14-8F reacted with the peptide R451-Q473 of human CETP near the carboxyl-terminal and not with the peptides representing any other regions, and inhibited the binding of human CETP to the goat antibody against its carboxyl-terminal peptide R451-S476. The experiments with a series of the fragment peptides in this region revealed that the epitope requires the segment 465-473 (EHLLVDFLQ) of human CETP or 485-493 (KHLLVDFLQ) of rabbit CETP (core epitope) though neither peptide by itself binds to the antibody. Both peptides needed extension at least by one residue beyond either amino- or carboxyl-end in order to show the reactivity to the antibody, but the effect was not highly residue-specific at least at the amino-end. Circular dichroism analysis demonstrated the increase of helical conformation by the extension of the "core epitope" peptides to either direction. Thus, the epitope is dependent on conformation of the core epitope induced by the presence of an additional residue(s) in either end. The core epitope occupies the central 64% of the reported linear epitope of Mab TP2, a widely used anti-human CETP monoclonal antibody that inhibits both cholesteryl ester and triglyceride transfer. Therefore, we conclude that the limited interaction of Mab with a common lipid interaction site causes selective inhibition of the transfer of triglyceride that has presumably lower priority than cholesteryl ester for the CETP reaction.**—Saito, K., K. Kobori, H. Hashimoto, S. Ito, M. Manabe, and S. Yokoyama. **Epitope mapping for the anti-rabbit cholesteryl ester transfer protein monoclonal antibody that selectively inhibits triglyceride transfer.** ***J. Lipid Res.*** **1999.** 40: **2013–2021.**

Supplementary key words cholesteryl ester transfer protein • cholesteryl ester • triglyceride • monoclonal antibody • epitope

Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein that catalyzes the non-directional transfer of neutral lipids, mainly cholesteryl ester (CE) and triglyceride (TG), between lipoproteins in plasma (1–3). CE in plasma lipoprotein is mainly generated by cholesterol esterification by lecithin:cholesterol acyltransferase (LCAT) in high density lipoprotein (HDL). TG in very low density lipoprotein (VLDL) or chylomicron is rapidly hydrolyzed by lipoprotein lipase and hepatic lipase and subsequently removed from plasma via the receptor-mediated hepatic uptake of the respective "remnant" particle (4). The main physiological role of CETP is to modify plasma lipoprotein metabolism by transferring CE from HDL and TG from VLDL/chylomicron by their heteroexchange (5, 6). As TG is kept hydrolyzed in lipoproteins other than low density lipoprotein (LDL), the size of HDL is kept small by CETP as CE is removed from and TG is supplied to HDL on an exchange basis. Consequently, HDL tends to be small in hypertriglyceridemic plasma (7, 8), and CE accumulates in HDL in the absence of CETP as LCAT continues to esterify cholesterol (9). Thus, the transfer of these two lipids by CETP is one of the keys for regulation of plasma lipoprotein profile.

A transfer-active site of human CETP for neutral lipid has been identified in the carboxyl terminal region by studying the epitope of an inhibitory monoclonal antibody (10, 11) and by using the mutant proteins (12–14). CE and TG compete for the same transfer-active site of the protein (5, 15), but CE is preferred over TG in the transfer reaction, being dependent to various extents on the structure of the lipid–substrate carrier lipoproteins (15–

Abbreviations: CETP, cholesteryl ester transfer protein; CE, cholesteryl ester; TG, triglyceride; LCAT, lecithin:cholesterol acyltransferase; HDL, LDL, and VLDL, high, low and very low density lipoproteins; Mab, monoclonal antibody; PBS, 10 mm phosphate buffer at pH 7.2 containing 150 mm NaCl; T-PBS, PBS containing 0.05% Tween-20.

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17). One of the monoclonal antibodies (Mab) we have raised against rabbit CETP, 14-8F (alternatively called 14-8H), inhibited the TG transfer but not CE transfer of rabbit and human CETP (6). There are also two other reports on such antibodies (18, 19). TG transfer by human and rabbit CETP is also selectively inhibited by the mercurial reagents to modify sulfhydryl groups (5, 20–22). These results are all consistent with the view that CE is given priority over TG in the interaction with a common interaction site of CETP (10, 15). Inhibition of TG transfer in plasma by Mab 14-8F resulted in prevention of the net CE transfer from HDL, providing direct evidence that CETP-mediated net lipid transfer is by an exchange mechanism at least in bulk plasma (6). Interestingly, an organomercurial cholesterol derivative, U-617, selectively inhibited the CE transfer which indicated the contribution of the cholesterol moiety in the interaction of CE with CETP (23).

More recently, a new model for the CETP reaction mechanism has been proposed based on the crystal structure of human bactericidal/permeability-increasing protein, suggesting a role for a hydrophobic pocket in the amino-terminal domain for neutral lipid binding as interacting with a helical domain in the carboxyl-terminal (24, 25). The observation that selective inhibition of the TG transfer by the sulfhydryl modification disappeared when the amino-terminal cysteine was replaced in rabbit CETP (22) may also be consistent with the involvement of the amino-terminal in the active site with respect to the regulation of the lipid species specificity of the transfer reaction.

Thus, it is important to investigate the epitope for the Mabs that selectively inhibit the TG transfer in order to understand the active conformation and reaction mechanism of CETP, and accordingly the regulation mechanism of plasma lipoprotein profile by CETP. In this paper, we studied the epitope mapping for the monoclonal antibody against rabbit CETP that selectively inhibits the TG transfer (6). The epitope of this antibody was shown to be conformation-dependent and included the epitope of the Mab TP2 that inhibits both CE and TG transfer reactions (26, 27). The results therefore demonstrated that the limited interaction of the antibody with the same active site leads to the selective inhibition of the binding of CETP to a lipid having low affinity.

MATERIALS AND METHODS

Monoclonal and oligoclonal antibodies

To prepare anti-rabbit CETP Mab 3-11D (alternatively called 3-9F) and 14-8F, the hybridoma cells were injected into the peritoneal cavity of BALB/c mice and ascites cells were harvested approximately 3 weeks later (6). IgG was purified from ascites by affinity chromatography on protein A-Sepharose. The TG-selective inhibitory action was demonstrated with 14-8F and universal inhibition of the CE and TG transfer was shown with 3-11D, for both human and rabbit CETP (6). The antibody was also prepared by immunization of the goat with the peptide representing the last 26 amino acid residues of carboxyl-terminal of human CETP (R451-S476) (10) and was purified by DEAE-Toyopearl chromatography. The antibodies were conjugated with horseradish peroxidase when necessary (28).

Reactivity of the antibodies to synthetic peptides

All the fragment peptides of CETP used were synthesized by a solid phase method on a multichannel peptide synthesizer (29, 30) and purified by high performance liquid chromatography by Sawady Technology (Tokyo, Japan) on a commercial contract basis. Binding assay of the peptides to the antibodies was carried out by a solid-phase method (31, 32). Fifty microliters of 10 mm phosphate-buffered saline, pH 7.2 (PBS), containing 10 μg/mL of a synthetic peptide was added to the wells of a microplate and incubated for 2 h at room temperature. The plate was washed three times with 0.05% Tween-20 in PBS (T-PBS) and allowed to stand with 300 μL T-PBS for 1 h at room temperature. After the T-PBS was discarded, the monoclonal antibodies, 0.5 μg of the purified IgG in 50 μl T-PBS, were added to the wells to incubate for 2 h at room temperature. The plate was rinsed with T-PBS three times, 50 μl of peroxidase-conjugated anti-mouse IgG of goat was added, and the microplate was incubated for 1 h at room temperature. After washing three times, 50 μl of the substrate solution containing 0.3% *o*-phenylenediamine-^{2}HCl and 0.003% H_2O_2 was added and incubated for 15 min at room temperature. Thereafter, 50 μl of 1.5 N H_2SO_4 was added. The color development was read with a microplate reader (Toso MPR-A4i, Tokyo, Japan) by measuring optic absorbance at 492 nm. The absorbance at 620 nm was subtracted as a background. Alternatively, the reactivity of the peptides to CETP was examined by a competitive binding assay against the Mab 14-8F. Fifty micrograms of the human CETP isolated from the plasma (33) in PBS was incubated in the wells for 2 h. After blocking with 25% Block Ace, the peroxidase-conjugated Mab 14-8F, 4 μg/mL, and 400 μg/ml of each peptide, both in the T-PBS, were mixed in 1:1 (v/v) and added to the CETP wells. The bound antibody was measured by the same colorimetric technique as mentioned above. The insoluble peptides in T-PBS were dissolved in 10% NH_3 and the pH was brought back to neutrality by 1.5 N H_2SO_4, excepting a few peptides insoluble even by this treatment. The values were expressed as the percentage of the control without peptide (100% binding). Correlation of the competitive assay (Y) to the direct solid phase binding assay (X) was best described as $Y = 86.09 \times \exp(-0.582\ X)$ with $r = 0.766$ for the 23 peptides eventually dissolved in the buffer (**Fig. 1**).

Antibody competition assay

The goat antibody to the human CETP carboxyl-terminal was competed by Mab 14-8F for the binding to human CETP. Mab 3-11D, 1 μg in 50 μL PBS, was added to wells of the microplate and incubated overnight at 4°C for immobilization. The microplate was washed three times with T-PBS and 350 μl of T-PBS containing 0.2% bovine serum albumin was added to the wells for another incubation for 2 h at room temperature. After rinsing with T-PBS, 50 μl of the 20–320 time-diluted human serum sample with T-PBS was added to each well and incubated for 2 h at room temperature. After another wash with T-PBS, 50 μl of the peroxidase-conjugated goat anti-CETP carboxyl-terminal antibody in T-PBS was added to the wells with or without 1 μg/mL of Mab 14-8F and incubated for 1 h at room temperature. The binding of the goat antibody was measured as mentioned above.

pH dependency of the reactivity of the monoclonal antibodies

Mab 3-11D was used as the capturing antibody immobilized in the wells, and the peroxidase-conjugated 14-8F was used as the detection antibody. For the pH dependency, 25 mm phosphate-buffered saline was used for the pH range of 5.5 to 8.0 and 25 mm citrate-buffered saline containing 0.05% Tween 20 was used for the pH range of 4.0 to 6.0. To investigate 3-11D, the dilution of human serum for the first CETP-capturing reaction was in the

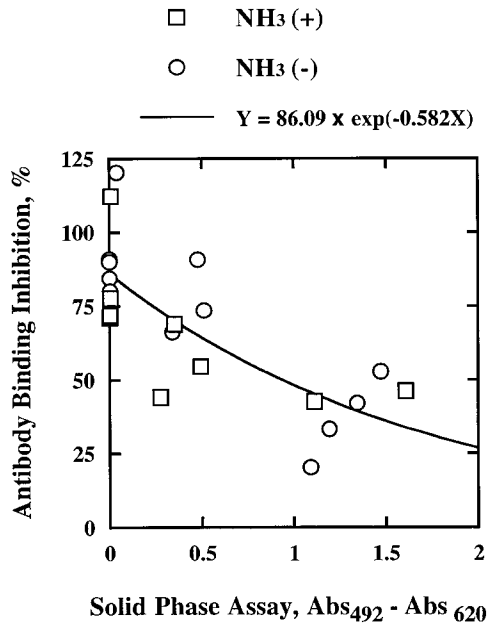

Fig. 1. Relationship of the binding competition assay to the solid phase binding assay for the binding of the fragment peptides of human and rabbit CETP. The methods are described in the text. The results were compared for the 23 measurable peptides including the 18 peptides shown in the figures and the 5 shorter peptides that do not bind to CETP in the solid phase assay. Circles indicate the peptides dissolved without using NH_3 and squares indicate those that required 10% NH_3 treatment for solubilization. The solid line curve indicates the exponential function obtained by least squares regression as the optimum fit, $Y = 86.09 \times \exp(-0.582X)$ ($r = 0.766$).

pH 4.0 to 8.0 range, and the second reaction with the peroxidase-conjugated 14-8F was performed in T-PBS. To investigate 14-8F, T-PBS was used for the dilution of human serum, and the second reaction with the peroxidase-conjugated 14-8F was done in the pH 4.0 to 7.5 range.

Circular dichroism

The peptides were dissolved in 1% NH_3 water solution to make 1 mg/mL and the solution was diluted by 5 times with 50% trifluoroethanol (TFE) in PBS to make the final peptide concentration 250 μg/mL. Circular dichroism (CD) spectra were measured in a JASCO J-500A at 24°C between 200 and 250 nm with the pathlength of 1 mm. Four cumulative scannings were integrated into each spectrum. Conformation estimate was given by the analysis of the CD spectrum profile (34).

RESULTS

A series of the peptides was tested for the reactivity to the Mab 3-11D and 14-8F to cover the amino- to carboxyl-terminals of human CETP (35). **Table 1** shows that Mab 3-11D bound none of these peptides, perhaps being consistent with the original implication that this antibody is against a highly conformational epitope rather than the epitope located in a certain region of the sequence (6). In contrast, Mab 14-8F reacted only with the peptide R451-Q473 that represents the region near the carboxyl-terminal of CETP. The goat antibody against the 26 carboxyl-terminal residues peptide of human CETP (R451-S476) also reacted only with this peptide. In addition, Mab 14-8F inhibited the binding of the CETP captured by 3-11D to the goat antibody (**Fig. 2**). Thus, Mab 14-8F recognizes the epitope of human CETP between the residues R451 and Q473.

TABLE 1. Reactivity of the anti-CETP monoclonal and oligoclonal antibodies to the human CETP fragment peptides

Human CETP Fragment Peptide	Antibody		
	3-11D	14-8F	Anti-C-Terminal Antibody
C1-A19	0.002	0.001	0.005
N24-D42	0.001	0.001	0.000
T44-Q63	0.003	0.001	0.000
S68-Q87	0.003	0.001	0.007
D110-L129	0.004	0.001	0.000
D132-C143	0.002	0.002	0.000
L150-W162	0.002	0.002	0.000
K239-R259	0.001	0.001	0.000
D290-E306	0.004	0.000	0.000
E364-L382	0.004	0.000	0.000
R451-Q473	0.005	0.531	1.398
D460-L468	ND	0.002	0.000
E465-S476	ND	0.275	0.271
D460-D470	ND	0.002	0.168
D460-Q473	ND	0.589	1.104

Mab 3-11D and 14-8F as well as the goat antibody against the 26 residue peptide representing the C-terminal of human CETP were tested for the binding to the human CETP fragment peptides that represent the regions covering from the N- to C-terminals of the protein. The values represent the difference of the optic absorbance at 492 nm from that at 620 nm.

Binding to human serum CETP of Mab 3-11D and 14-8F was examined in various pH. Mab 3-11D showed no significant pH-dependent change of the binding in the pH range of 5 to 7.5. In contrast, the binding of 14-8F to CETP captured by 3-11D showed substantial pH dependency with the maximum reactivity at a weakly acidic con-

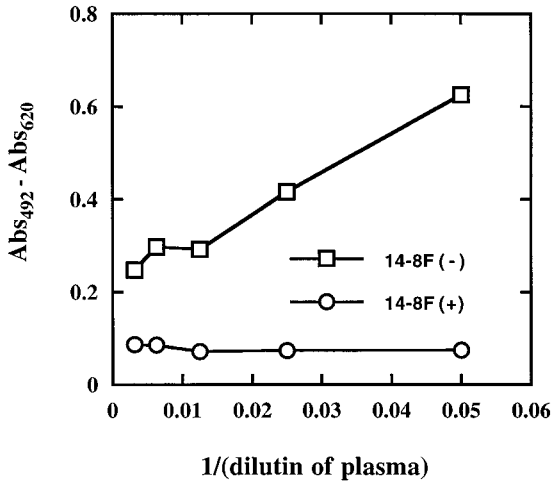

Fig. 2. Inhibition by Mab 14-8F of the binding of the goat antibody against the carboxyl terminal hexacosapeptide of human CETP to human plasma CETP. CETP was captured by immobilized Mab 3-11D by incubating with human plasma and then the binding of the anti-peptide goat antibody was measured in the absence and presence of Mab 14-8F. The experimental detail is described in Methods.

dition of pH 5 to 5.5. Thus, a pH-sensitive factor(s) may contribute to the Mab 14-8F binding, such as the epitope conformation.

In order to find the more precise location of the epitope, more fragment peptides were synthesized for the region of the 26 residues of the carboxyl-terminal of human CETP R451-S476 to study the binding of Mab 14-8F. The antibody reacted with the peptides D460-Q473 and F463-L475 but failed to bind the peptides R451-P464 and D460-L468. As well, the goat anti-CETP carboxyl-terminal antibody bound to peptide D460-Q473 but not D460-L468. Therefore, further analysis was concentrated to the region of F463-S476.

This region corresponds to the segment F483-S496 of rabbit CETP with matching amino acid sequence except for substitution of human E465 by K485 in rabbit (36, 37). This substitution was also introduced to study the interaction of the peptides with Mab 14-8F. **Figure 3** demonstrates the reactivity of the series of the peptides by two independent methods to detect the region critical for the binding to the antibody. The competitive binding assay was incomplete and less sensitive because of the insolubility of the many peptides and because of the nature of the assay method in itself. Nevertheless, the results by both methods were largely consistent for the measurable peptides. The peptides F463-Q473, E465-S474, and E465-S476 all showed strong binding to the antibody and the binding of the peptide P464-Q473 was also positive, while the peptide 465-473 (EHLLVDFLQ) did not bind to the antibody at all. In the competitive assay, the peptide 465-473 (EHLLVDFLQ) competed against the 14-8F rather than the peptide P464-Q473. Thus, though the peptides by themselves do not react to Mab 14-8F in the solid phase assay, their extension beyond either end resulted in the positive binding to the Mab. The effect of the amino-end extension was canceled by shortening the peptide at the carboxyl end by one residue (F463-L472) in the solid phase assay (Fig. 3). Also, monodecapeptide (HLLVDFLQSLS) that represents the consensus sequence in the carboxyl-terminal of human and rabbit CETP was negative, showing that the extension to the carboxyl-terminal does not recover the loss of the reactivity of the peptide by omitting 465E for human or 485K for rabbit.

Similar results were obtained with a series of the peptides following the rabbit CETP sequence in either assay system (Fig. 3). The peptides F483-Q493, P484-Q493, and K485-S496 all demonstrated clear positive binding but the peptide 485-493 (KHLLVDFLQ) was negative. Thus, the segments 465-473 (EHLLVDFLQ) of human CETP and 485-493 (KHLLVDFLQ) of rabbit CETP are absolute requirements for the epitope against Mab 14-8F (the core epitopes), but they are not adequate by themselves for in-

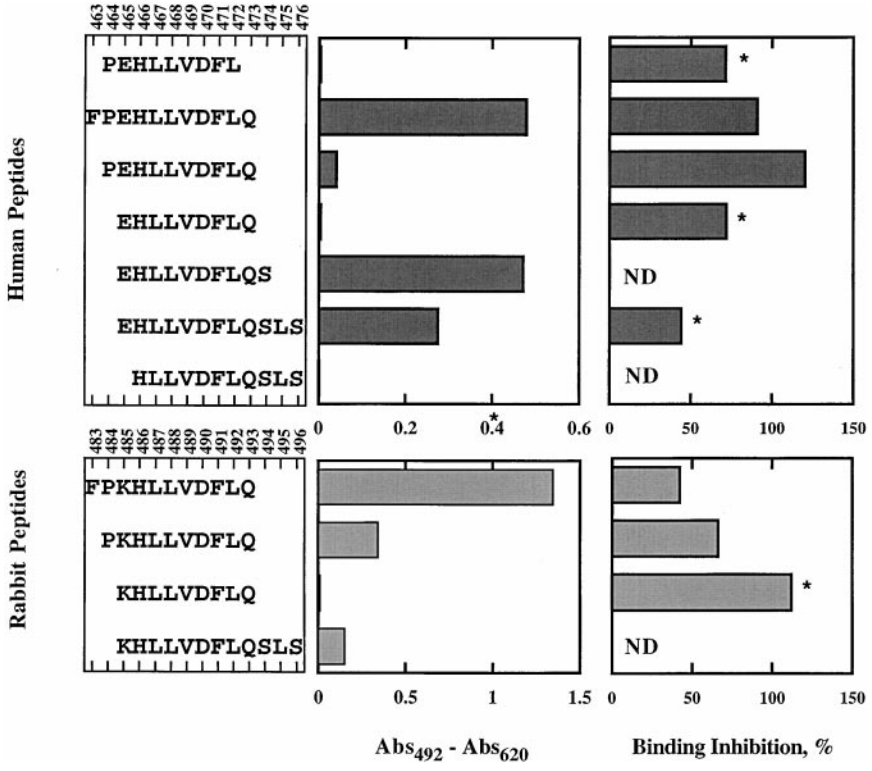

Fig. 3. Binding to Mab 14-8F of the peptides representing the regions near the carboxyl terminal of human and rabbit CETPs. The number indicates the position corresponding to the residual number of respective protein. Binding is expressed as the absorbance at 492 nm minus that at 620 nm as a background for the solid phase assay, and as the percentage to the control in the competitive inhibition assay. ND indicates not determined due to the insolubility of the peptide. Asterisk indicates that the peptide required the NH_3 treatment for solubilization.

teraction with the antibody. The extension by at least one residue beyond the either carboxyl- or amino-end of the segments is required for the interaction.

The role of the carboxyl-end extension in the reactivity is shown in **Fig. 4**. The results are again in good agreement between both assay systems in that the reactivity seems decreased as the carboxyl-end is extended further. **Figure 5** shows the role of the amino-end extension. The reactivity was substantially higher with the two-residue extension than with the one-residue, especially in human CETP, being consistent for both assay systems. However, the extension by more than two residues does not cause a further increase of the binding in either CETP. Interestingly, substitution of P (464 for human and 484 for rabbit) by C did not influence the effect of extension, as shown in **Fig. 6**. Also, substitution of the alternative E or K (465 for human or 485 for rabbit) by D did not abolish the binding.

Figure 7 shows the CD spectra of the peptides. As the "core epitope" peptides were extended beyond the amino-end, the negative peak at 204 nm became deeper indicating the increase of the helical conformation though not showing the typical alpha-helix spectrum. The extension beyond the carboxyl-end resulted in more prominent induction of alpha-helical conformation from the CD spectrum point of view. **Table 2** represents the conformation estimates obtained from the analysis of the CD spectra. The increase of alpha and beta conformations was demonstrated by the extension of the peptides to either end.

DISCUSSION

Mab 14-8F against rabbit CETP cross-reacted with human CETP and selectively inhibited the transfer of TG over CE (6). The epitope of this antibody was probed by using synthetic peptides representing various segments of rabbit and human CETP. The experimental results revealed that the epitope resides in the segment 465-473 (EHLLVDFLQ) of human CETP (35) or 485-493 (KHLLVDFLQ) of rabbit CETP (36, 37) though neither of these peptides by itself interacts with the antibody. Both peptides needed to be extended at least by one residue beyond either the amino- or carboxyl-end in order to gain the reactivity to the antibody. Also, the effect of the extension was not residue-specific, especially at the amino-end. Thus, the epitope is dependent on the conformation of this "core epitope" that is induced by an additional residue(s) to either end of the segment. To support this view, conformational change was observed in the CD spectrum by the extension of the "core epitope" peptides. Because 465E in human and 485K in rabbit seemed exchangeable, this position may not be included in the "core"

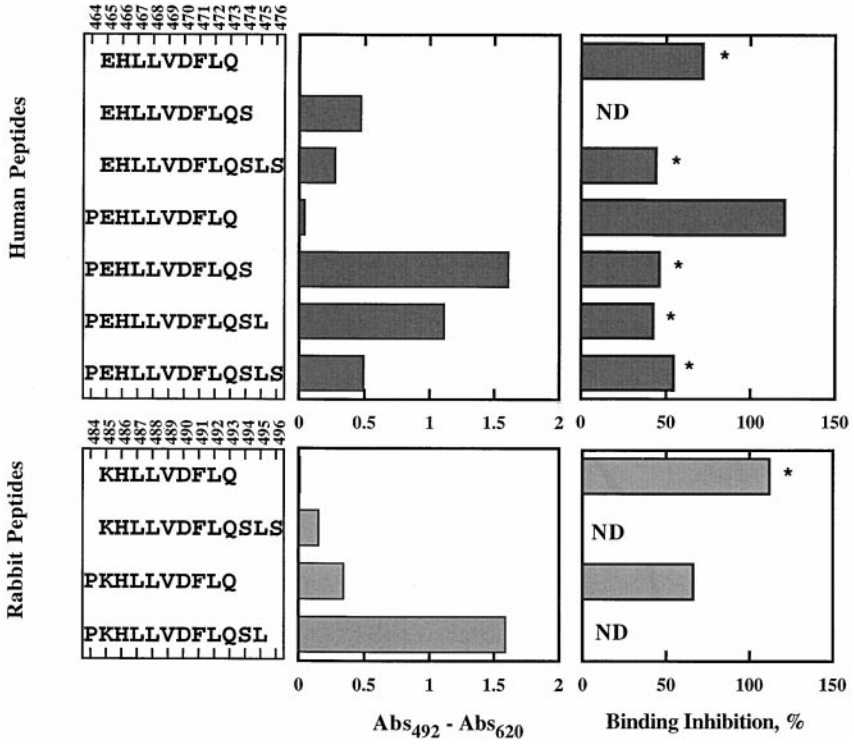

Fig. 4. Effect of the carboxyl extension of the "core epitope" peptide on the binding of the peptides to Mab 14-8F. Common peptide represents the consensus sequence in the amino terminal of human and rabbit CETP that includes 8 of 9 residues of the "core epitope" by eliminating the amino end residue, plus three residues extension in the carboxyl end. Binding is expressed as the absorbance at 492 nm minus that at 620 nm as a background for the solid phase assay, and as the percentage to the control in the competitive inhibition assay. ND indicates not determined due to the insolubility of the peptide. Asterisk indicates that the peptide required the NH_3 treatment for solubilization.

A site responsible for the lipid binding and transfer activity of human CETP has been searched by identifying the epitope of the inhibitory monoclonal antibody TP2 (10, 11) and by studying the transfer activity of the recombinant CETPs having a series of the carboxyl-terminal truncation (12, 14) or point mutations (13). The results led to the conclusion that it is located in the carboxyl-terminal region. The transfer activities for TG and CE are attenuated simultaneously in these experiments so it was rational to assume a common binding site for the neutral lipids for the transfer activity of CETP (10, 14). This view was consistent with the experimental data that CE and TG compete for the transfer reaction (5, 15).

The competitive transfer experiments demonstrated that CETP prefers CE over TG for the interaction and subsequently for the transfer reaction (5, 15), and the extent of this selectivity depends on the structure of the lipid-carrying lipoprotein particles (15–17). As the net transfer of CE from HDL to other lipoproteins in bulk plasma largely depends on the hetero-exchange of CE and TG (6), the selectivity between CE and TG in the transfer reaction would contribute significantly to the regulation of plasma lipoprotein profile by CETP. Indeed, the selective inhibition of the TG transfer by Mab 14-8F inhibited the net removal of CE from HDL in spite of the active CE transfer in bulk plasma in vitro (6). Thus, a differential mechanism for CE and TG transfer is of physiological importance to understand the regulation of plasma lipoprotein profile.

Differentiation of the CE and TG transfer by CETP has been demonstrated in two different ways. First, at least three Mabs, LT-J1 raised against human CETP (21), 14-8F that we have raised against rabbit CETP (6), and CMTP2, against cynomolgus monkey CETP (19), demonstrated the selective inhibition of the TG transfer. Fab fragment of the universal inhibitory antibody TP2 also showed only partial inhibition of the CE transfer while the complete inhibition of the TG transfer was retained (10). Second, TG transfer by human and rabbit CETP is selectively inhibited by the mercurial reagents that modify the sulfhydryl group (5, 20–22). These results are all consistent with the view that CE has priority over TG for the interaction with a common site of CETP (15). Mab 14-8F also inhibited the transfer of pyrene-labeled CE (15), and this is consistent with the speculation that the weak affinity of these lipids is caused by the bulkiness of the molecules that do not ideally fit the interaction site of CETP (6, 10).

Results of the present study demonstrated that Mab 14-8F has an interaction site within the carboxyl-terminal region of CETP that has already been identified as an active site (10–14). However, it is not a simple linear epitope but appears to require a certain conformation for the binding to the antibody. This "core epitope" occupies the central

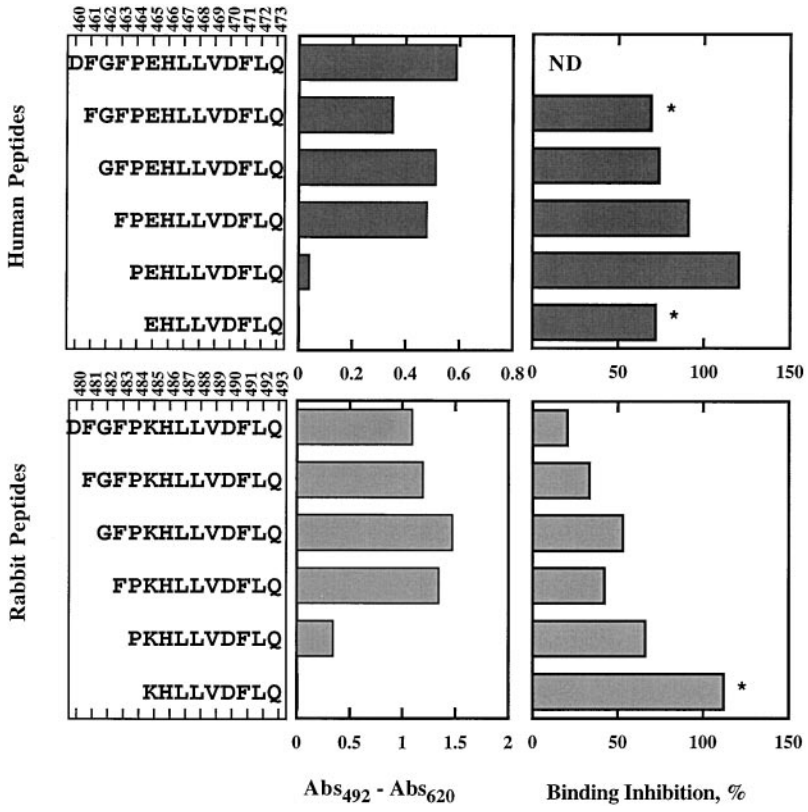

Fig. 5. Effect of the amino end extension on the binding of the peptides to Mab 14-8F. Binding is expressed as the absorbance at 492 nm minus that at 620 nm as a background for the solid phase assay, and as the percentage to the control in the competitive inhibition assay. ND indicates not determined due to the insolubility of the peptide. Asterisk indicates that the peptide required the NH_3 treatment for solubilization.

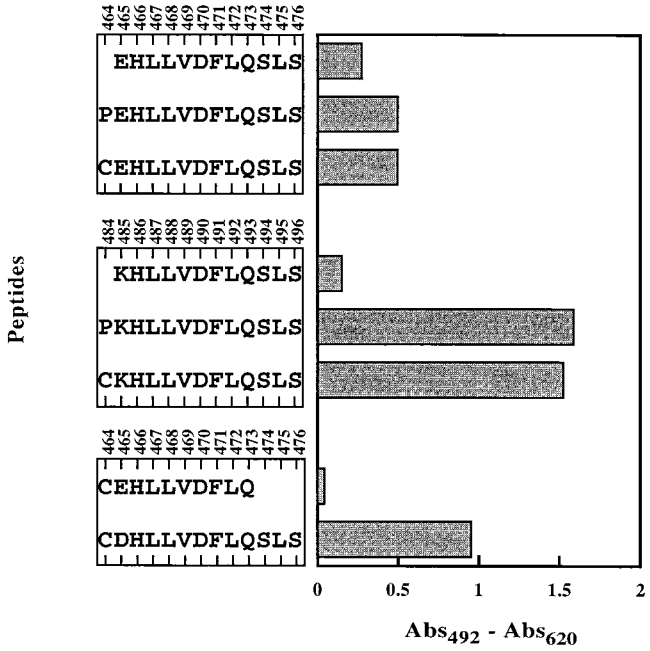

Fig. 6. Effect of the substitution of the residues on the binding of the peptides to Mab 14-F. The next residue to the amino end of the "core epitope" sequence was substituted by cysteine to phenylalanine. Also, the alternative site for human and rabbit (465E and 485K) of the "core epitope" peptide was substituted by aspartic acid.

TABLE 2. Conformational analysis of the peptides

	α-Helix	β-Sheet	Random Structure
Human CETP peptides			
1. EHLLVDFLQ	17.3	4.1	78.6
2. FPEHLLVDFLQ	19.9	9.3	70.8
3. EHLLVDFLQSLS	37.9	12.5	49.6
4. PEHLLVDFLQSLS	39.3	15.6	45.1
Rabbit CETP peptides			
5. KHLLVDFLQ	15.0	5.2	79.8
6. FPKHLLVDFLQ	20.6	2.1	77.3
7. KHLLVDFLQSLS	31.7	10.6	57.7
8. PKHLLVDFLQSL	34.2	15.9	49.9

Percent contents of α-helix, β-sheet, and random structure were calculated from the CD spectra in 50% TFE shown in Fig. 7 (34).

64% of the reported epitope of Mab TP2 (residues D460 and F463 to L475 of human CETP) (12, 13), the widely used anti-human CETP Mab that reportedly inhibits both CE and TG transfer (26, 27).

Tall (1), Swenson et al. (10), and Wang et al. (13) proposed that there are two amphiphilic alpha-helices separated by a proline in the last 26 carboxyl-terminal amino acids of human CETP and these helical regions are directly responsible for the interaction of CETP with the hydrophobic faces of the neutral lipid molecules. They demonstrated that Mab TP2 recognizes the epitope including one of these helical segments of CETP (10) and speculated that the binding of the antibody to the hydrophilic face of the helix interferes with its interaction with neutral lipid (13). The "core epitope" of Mab 14-8F corresponds to a part of the same helical segment, 9 of the 14 residues of the TP2 epitope (10, 13), that makes a hydrophobic face of three leucines and one phenylalanine (L467, L468, F471 and L472). Thus, Mabs 14-8F and TP2 recognize a similar region of CETP, but the former Mab binds in a more restricted manner. It is not enough to interfere with the binding of CETP to CE but enough to inhibit the TG binding (6, 10, 15).

The "core epitope" requires the extension beyond either

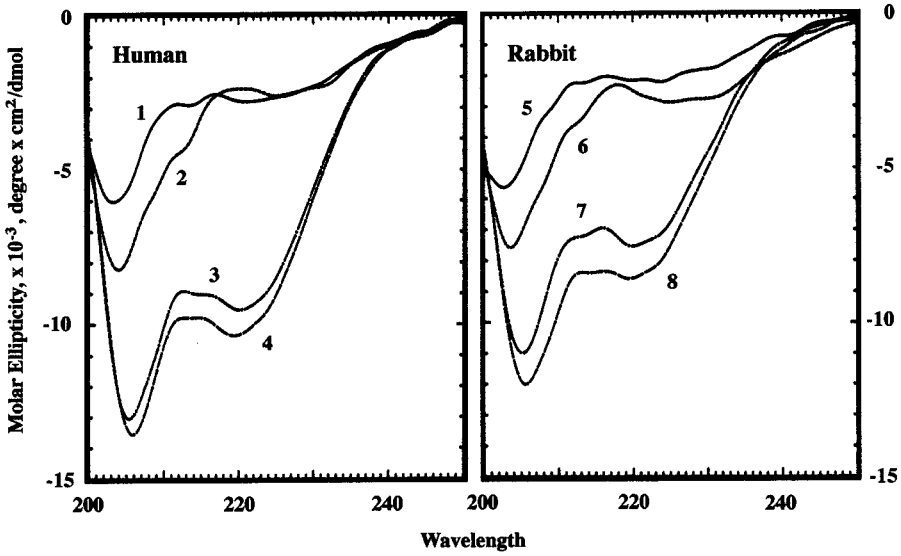

Fig. 7. The CD spectrum of the peptides in 50% TFE. The experimental detail is described in Methods. The right panel shows the data of the human CETP fragment peptides, and the left panel shows the results of the rabbit CETP fragment peptides. The curve 1, the human "core epitope" peptide EHLLVDFLQ; 2, FPEHLLVDFLQ; 3, EHLLVDFLQSLS; 4, PEHLLVDFLQSLS; 5, the rabbit "core epitope" peptide KHLLVDFLQ; 6, FPKHLLVDFLQ; 7, KHLLVDFLQSLS; 8, PKHLLVDFLQSL.

end by at least one residue for the recognition by the antibody, indicating that the epitope is dependent on the conformation induced by these extensions. As the TP2 epitope includes three more residues in the amino-end and two more residues in the carboxyl-end, this epitope peptide by itself seems long enough to generate the conformation recognized by the antibody. This view may also be supported by the greater pH dependency of the binding of Mab 14-8F, which can still be interpreted alternatively as the conformational change of the antibody.

Previous CD studies demonstrated that the conformations of rabbit and human CETPs are similar and both contain more beta sheet than alpha helix (38, 39). The CD spectra of the peptides taken in 50% TFE were rather alpha helix-predominant showing that the carboxyl terminal region of CETP is indeed helix-rich (34). The results also demonstrated that the higher helical conformation is induced as the "core epitopes" are extended to either amino or carboxyl end. The data were thus consistent with the results from the antibody binding studies.

Selective inhibition of TG transfer by the sulfhydryl modification disappeared when the amino-terminal cysteine in rabbit CETP was removed (22). This result suggests that this cysteine residue is not involved in disulfide linkage and the amino-terminal interacts with the carboxyl-terminal as a part of the active site structure. However, the epitope mapping study showed no indication of the interaction of Mab 14-8F with this region. Crystal structure of human bactericidal/permeability-increasing protein revealed two hydrophobic pockets for lipid binding (24). As this protein has a high similarity to CETP both in structural and functional aspects, the structure–function model for CETP may also be modified based on this result (25) that the hydrophobic pocket in the amino-terminal domain rather than that in the carboxyl-terminal is responsible for neutral lipid binding and the carboxyl-terminal helix participates in the functioning of this pocket. If this model is valid, the amino-terminal domain is directly responsible for neutral lipid transfer activity and the helical segments in the carboxyl-terminal rather play the role of a co-factor. Additional investigation is needed to clarify these points.

We conclude that the carboxyl-terminal segment of CETP, E465–S476 of human and K485–S496 of rabbit that contributes to forming an amphiphilic helix is responsible for both TG and CE transfer. The antibodies that recognize this region have a potential to inhibit the transfer activity of CETP. The limited interaction of the antibody results in selective inhibition of the transfer of TG, which may not fit so well as CE in the CETP "lipid interacting pocket" due to its molecular size, or may have lower "affinity" for the interaction than CE.

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