# β-Peptides as Inhibitors of Small-Intestinal Cholesterol and Fat Absorption

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Dedicated to our friend and colleague Prof. *J.-M. Lehn* on the occasion of his 60th birthday

Selective lipid transport through the brush-border membrane in the small intestine of mammals is mediated by membrane-bound proteins, the so-called scavenger receptors of class B, type I or II (SR-BI or -BII). These, in turn, are inhibited by certain proteins and synthetic α-peptides that have an amphipathic helix as the binding motif (*Fig. 1*). In whole cells (test with human colonic carcinoma cells, CaCo-2), on the other hand, the inhibitors are subject to proteolysis. We have now tested six β-peptides (hexa-, hepta-, and nonamers **1** – **6**), each carrying one to seven water-solubilizing side chains of either Ser or Lys, with a brush-border-membrane (BBM) vesicle model system (rate and $IC_{50}$ values in *Figs. 2* and *3*) and with a tightly packed monolayer of CaCo-2 cells (rate in *Fig. 4*), to find that the rate of transport of cholesterol can be reduced to what may be considered the passive diffusion ('background') level. There is a correlation between the ability of the β-peptides to form an amphipathic-type $3_{14}$-helical secondary structure in MeOH and their inhibitory effect (*Table 1* and *Fig. 5*). Although the inhibitory activity of the β-peptides is in only the mM range (*Table 2*), it is to be compared with no activity at all of previously tested α-peptides and proteins (built of L-amino acids) in CaCo-2 cells. Furthermore, these active β-peptides (**1**, **5**, and **6**) contain only seven or nine residues and must be considered simple, first-generation models capable of mimicking the biological activity of amphipathic α-peptide helices in living whole cells.

**Introduction and Goals.** – Recently, scavenger receptors of class B (SR-B) type I and II were shown to be integral proteins of the small-intestinal brush-border membrane (BBM), where they facilitate the uptake of cholesterol and other dietary lipids from either mixed bile-salt micelles or small unilamellar phospholipid vesicles [1]. These lipid particles function as donors or carriers of water-insoluble dietary lipids in the lumen of the small intestine [2]. The protein SR-BI appears to be of pivotal importance in cholesterol metabolism and homeostasis [3a]: in liver and steroidogenic tissues, high-density lipoprotein (HDL) is the physiological ligand of this receptor [3b]. Apparently, SR-BI also plays a key role in the transport of free and esterified cholesterol from HDL to cells, a process referred to as *selective lipid transport* [4][2]), as well as the reverse process termed *reverse cholesterol transport* [5]. Scavenger receptors

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[1]) Part of the Ph.D. Theses of *M. W.* (Dissertation ETH No. 13049) and *S.A.* (Dissertation ETH No. 13203), Zürich, 1999.

[2]) In *selective transport*, constituent lipid molecules of the HDL particle move selectively *via* SR-BI receptor to the acceptor membrane and eventually to the cytosol of the cell. This process is distinct from *endocytosis* where the whole HDL particle, including the proteins, is taken up by the cell.

of class B are multiligand receptors [6] characterized by a lack of lipid specificity: they function rather as a port or docking receptor [1]. When lipoproteins and other lipid particles dock at SR-B, the receptor mediates the bidirectional flux of lipid molecules between the donor particle and the acceptor membrane. The lack of lipid specificity of SR-BI is demonstrated by the observation that SR-BI catalyzes the transport of lipid molecules as structurally diverse as free and esterified cholesterol, plant sterols, phospholipids, triacylglycerols, and products of hydrolysis resulting from these compounds [1]. Furthermore, one of our laboratories reported recently that binding of HDL and apolipoproteins to BBM-resident SR-B induces the inhibition of cholesterol and fat absorption at the level of the BBM [1][7]. Amphipathic $\alpha$-helices in the inhibiting proteins have been identified as the structural motif underlying this inhibitory effect [7] (*Fig. 1*).

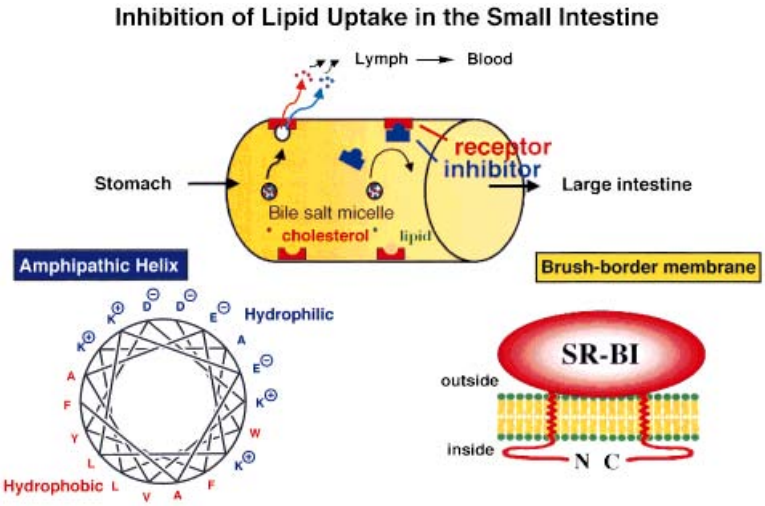


Fig. 1. *Cartoon representation of cholesterol and lipid transport (and its inhibition) through the intestinal BBM by the SR-BI protein (scavenger receptor of class B, type I)*. An amphipathic helix has been shown to be involved in the binding of high-density lipoproteins and apolipoproteins (inhibitors) to the SR-BI protein. As shown in this cartoon, SR-BI is associated with the BBM by two putative anchoring domains. The octadecapeptide Ac-$(Xaa)_{18}$-$NH_2$ used for the demonstration [1][7] of this effect (with rabbit BBMV) is presented as a helical wheel, with the one-letter abbreviation for the 18 amino acids. With CaCo-2 cells, there is no inhibition by apolipoprotein or by the octadecamer due to proteolysis!

As has been shown by one of our groups, short-chain $\beta$-peptides (oligomers of $\beta$-amino acids) containing as few as six residues have a high propensity to fold into well-defined secondary structures (helices, pleated sheets, turns) both in solution and in the solid state [8a]. This was shown by circular dichroism, NMR, and X-ray diffraction for $\beta$-peptides containing as substituents in the 2- and/or 3-position of the $\beta$-amino acid the side chains of familiar L-$\alpha$-amino acids, *e.g.*, Ala, Val, Leu, Phe, Lys *etc.*[3])[4]). $\beta$-Peptides

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[3]) For reviews, see [8a]. For full papers describing synthesis and structure of $\beta$-peptides with proteinogenic side chains, see [8b].

[4]) The $\beta$-peptides used in the present study have been described in [9a], or mentioned in a preliminary communication [9b]; see also the Ph.D. thesis of *S. A.*[1]).

composed of more than five $\beta^2$-, $\beta^3$-, and $\beta^{2,3}$-amino acids (derived from the corresponding L-$\alpha$-amino acids) fold preferentially into left-handed $3_{14}$ helices [8][9]. In addition to the propensity to form helical structures, this class of compounds is characterized by another important feature: $\beta$-peptides are resistant to all kinds of mammalian proteases and peptidases, including the most aggressive ones such as pronase and proteinase K [10]. These properties warrant closer scrutiny of this class of compounds as peptidomimetics in medicinal chemistry [11].

Here we address the question of whether $\beta$-peptides capable of forming helical structures can mimic apolipoproteins and natural $\alpha$-peptides in their inhibitory effect on sterol and fat uptake at the level of the small-intestinal BBM. It was of special interest to learn whether $\beta$-peptides would be active in the whole-cell (CaCo-2 cell) test where $\alpha$-peptide inhibitors are cleaved by proteases and peptidases [1].

**Results.** – We employed the previously described [9] $\beta$-peptides H-($\beta^3$-HPhe-$\beta^3$-HAla-$\beta^3$-HLys)$_2$-$\beta^3$-HPhe-OH (**1**), H($\beta^3$-HVal-(*R*)-$\beta^3$-HAla-$\beta^3$-HLys-(*R*)-$\beta^3$-HPhe-$\beta^3$-HVal-(*R*)-$\beta^3$-HAla-$\beta^3$-HLys-OH (**2**), H-(2*R*)-($\alpha$Me)-$\beta^3$-HAla-(2*R*)-($\alpha$Me)-$\beta^3$-HVal-$\beta^2$-HVal-$\beta^3$-HLys-(2*R*)-($\alpha$Me)-$\beta^3$-HAla-(2*R*)-($\alpha$Me)-$\beta^3$-HLeu-OH (**3**), H-($\beta^3$-HLys)$_7$-OH (**4**), H-$\beta^3$-HPhe-$\beta^3$-HAla-$\beta^3$-HSer-$\beta^3$-HPhe-$\beta^3$-HAla-$\beta^3$-HSer-$\beta^3$-HPhe-OH (**5**), and H-($\beta^3$-HAla-$\beta^3$-HLys-$\beta^3$-HPhe)$_3$-OH (**6**), each carrying at least one water-solubilizing proteinogenic side chain (of Lys or Ser). They were used to follow the transport of lipids and cholesterol or cholesteryl esters, either from unilamellar lipid vesicles into the membrane of BBM vesicles, or through the CaCo-2 cells.

The kinetics of cholesteryl oleoyl ester[5]) uptake into the membrane of brush-border membrane vesicles (BBMV) from small unilamellar lipid vesicles as the donor in the presence and absence of $\beta$-heptapeptide **1** are depicted in *Fig. 2*. The kinetic curve is biphasic and could be adequately fitted by the sum of two exponential functions. The pseudo-first-order rate constants (half-life $t_{1/2}$) derived from curve fitting were $k_1 = 0.39 \pm 0.04$ min$^{-1}$ ($t_{1/2} = 1.8$ min) for the initial fast phase and $k_2 = (9.33 \pm 2.2)$ $10^{-3}$ min$^{-1}$ ($t_{1/2} = 74$ min) for the ensuing slow phase. In the presence of $\beta$-peptide **1** (1 mg/ml), the uptake kinetics were markedly slowed (*Fig. 2*). The kinetic curve observed in the presence of $\beta$-peptide **1** was still biphasic, however, and the values of the pseudo-first-order rate constants derived from curve fitting were significantly smaller than those measured in the absence of the $\beta$-peptide and the half lives correspondingly larger: $t_{1/2} = 10 \pm 2$ min and $t_{1/2} = 13 \pm 3$ h for the initial fast phase and the second slow phase, respectively.

The concentration dependence of the inhibitory effect of $\beta$-peptide **1** is shown as dose-response curve in *Fig. 3*. The $IC_{50}$ value, *i.e.*, the concentration of the inhibitor required to produce an inhibition of 50%, was derived from curve fitting (with a modified *Hill* equation), yielding values of $0.85 \pm 0.08$ mg/ml (*Table 1*) and $n = 1.0 \pm 0.1$.

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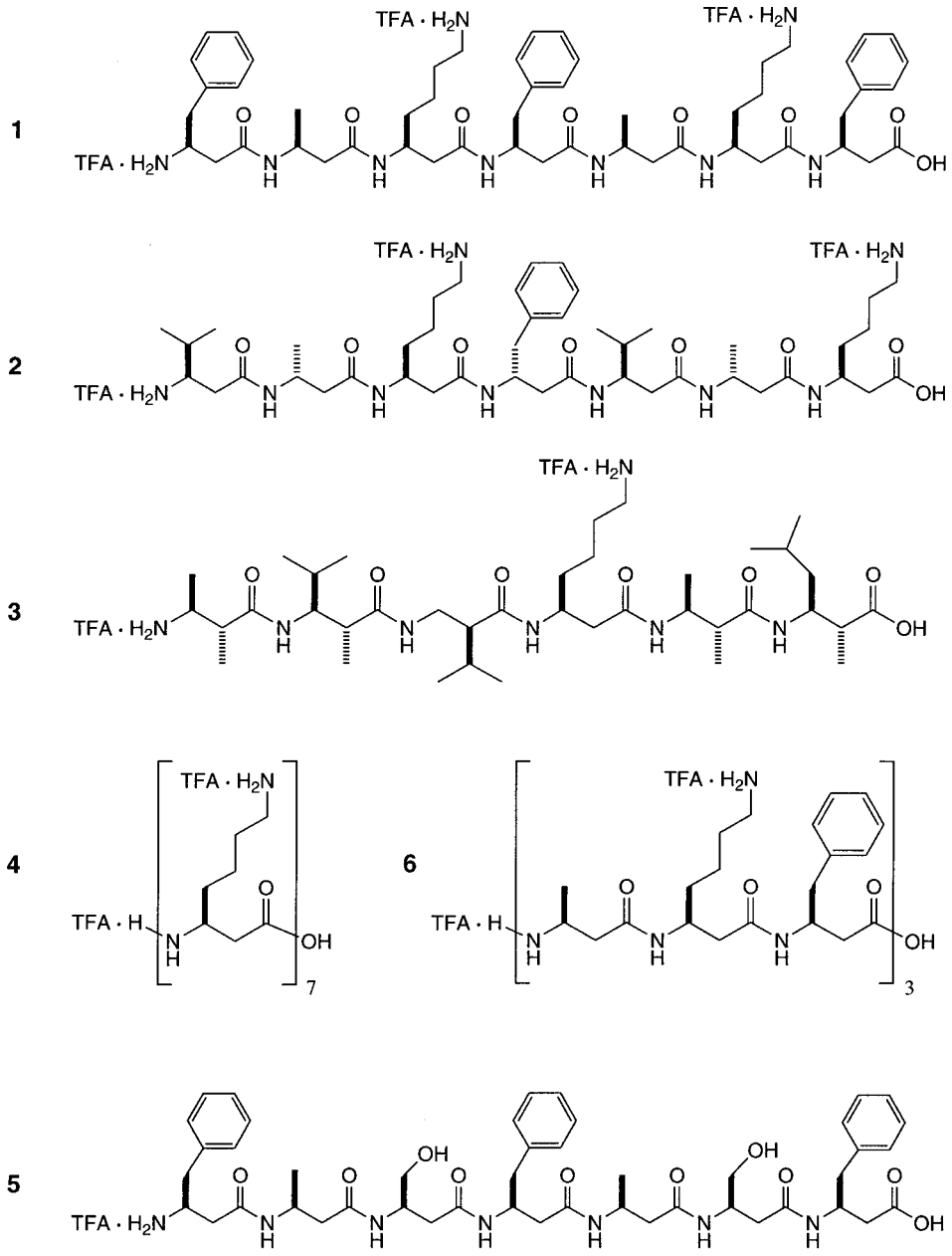
[5]) Convincing evidence has been presented from one of our laboratories showing that the uptake of both free and esterified cholesterol into BBMV is protein-mediated [1][7][12]. The use of cholesteryl ester as a representative sterol in lipid-uptake measurements has the advantage that, contrary to the use of free cholesterol, there is no contribution to the uptake process from simple passive diffusion. Radiolabeled cholesteryl oleoyl ether was used as a nonhydrolyzable analogue of cholesteryl oleoyl ester, and it was shown before [12] that cholesteryl ester and ether exhibit identical BBM-uptake kinetics.




The value of the *Hill* coefficient $n$ indicates that the interaction of the $\beta$-peptide with the BBM, or presumably with SR-B of this membrane, is non-cooperative.

The inhibitory activities of various short-chain $\beta$-peptides are compared in *Table 1*. The main conclusion we can draw from this table is that $\beta$-peptides capable of forming a

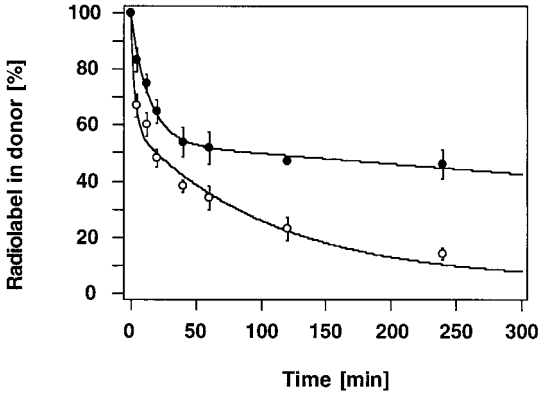



Fig. 2. *Kinetics of cholesteryl oleoyl ester uptake into rabbit BBMV.* Small unilamellar vesicles of egg phosphatidylcholine containing 1 mol-% cholesteryl oleoyl ester radiolabeled with [1$\alpha$,2$\alpha$(N)-$^3$H]cholesteryl oleyl ether (37 Ci/mmol) as the donor (0.05 mg total lipid/ml) and BBMV as the acceptor (5 mg protein/ml; 3 mg total lipid/ml), both dispersed in phosphate-buffered saline pH 7.4 (1.5 mM $KH_2PO_4$, 8.5 mM $Na_2HPO_4$, 0.12M NaCl, 2.7 mM KCl), were mixed at time zero and incubated at room temperature. After timed intervals, BBMV were separated from the donor by centrifugation at 115000 g for 2 min in a *Beckman* airfuge, and the radioactivity present in both pellet (BBMV) and supernatant (donor) was determined in a *Beckman LS 7500* liquid-scintillation counter. The transfer of radiolabeled cholesteryl ester from small unilamellar lipid vesicles to BBMV was measured in the absence (○) and in the presence (●) of 1 mg/ml of $\beta$-peptide 1. The solid lines represent curves fitted to the experimental data points (average ± SD, $n = 3$) with a double-exponential function [12]. Error bars smaller than the symbol size were omitted.

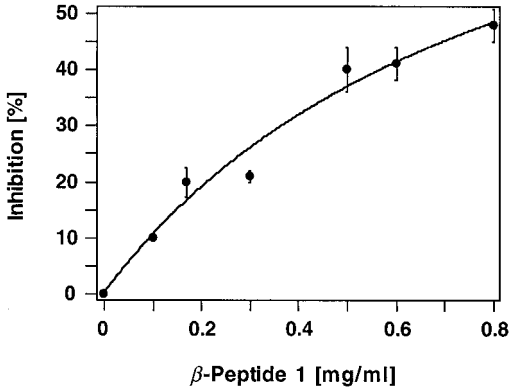



Fig. 3. *Dose-response curve of the inhibition of sterol uptake* (expressed as %) *as a function of the total concentration of $\beta$-peptide* **1**. The rate of cholesteryl oleoyl ester uptake into BBMV was determined by incubating donor (small unilamellar lipid vesicles at 0.05 mg total lipid/ml) and acceptor (5 mg protein/ml) for 20 min at room temperature with and without $\beta$-peptide **1**. The experimental conditions were identical to those described in the legend to *Fig. 2*. The cholesteryl ester uptake measured for 20 min in the absence of inhibitor was taken as 100%. The reduced uptake of cholesteryl ester measured in the presence of increasing amounts of **1** was expressed as % inhibition. The data points of the resulting dose-response curve were fitted by a modified *Hill* equation [13] $y = 1/[1 + (IC_{50}/x)^n]$ where $y$ is % inhibition, $x$ the total concentration of **1** (mg/ml), and $n$ the *Hill* coefficient.

left-handed $3_{14}$ helix in MeOH (see *Fig. 4*) are active as inhibitors of sterol uptake, while $\beta$-peptides **2** and **3**, which are known to form no helical structures, are inactive. The same is true for $\beta$-peptide **4**, which may be able to form a $3_{14}$ helix, but the helical structure would lack amphipathic character (see the comments in the caption of *Fig. 4*).

Table 1. *SR-BI Inhibition and Secondary Structures of β-Peptides* **1**–**6**. $IC_{50}$ Values of the $\beta$-peptides as inhibitors of cholesteryl oleate uptake from unilamellar lipid vesicles into brush-border membrane vesicles (BBMV; *cf. Figs. 2* and *3*, and *Table 2*) and their ability to fold into $3_{14}$ helices with amphipathic character (*cf. Fig. 4*).

| $\beta$-Peptide | Able to form a $3_{14}$ helix with amphipathic character? | $IC_{50}$ [mg/ml] |
|---|---|---|
| **1** | yes | $0.85 \pm 0.08$ |
| **2** | no | inactive |
| **3** | no | inactive |
| **4** | no | inactive |
| **5** | yes | $0.95 \pm 0.1$ |
| **6** | yes | $0.59 \pm 0.06$ |

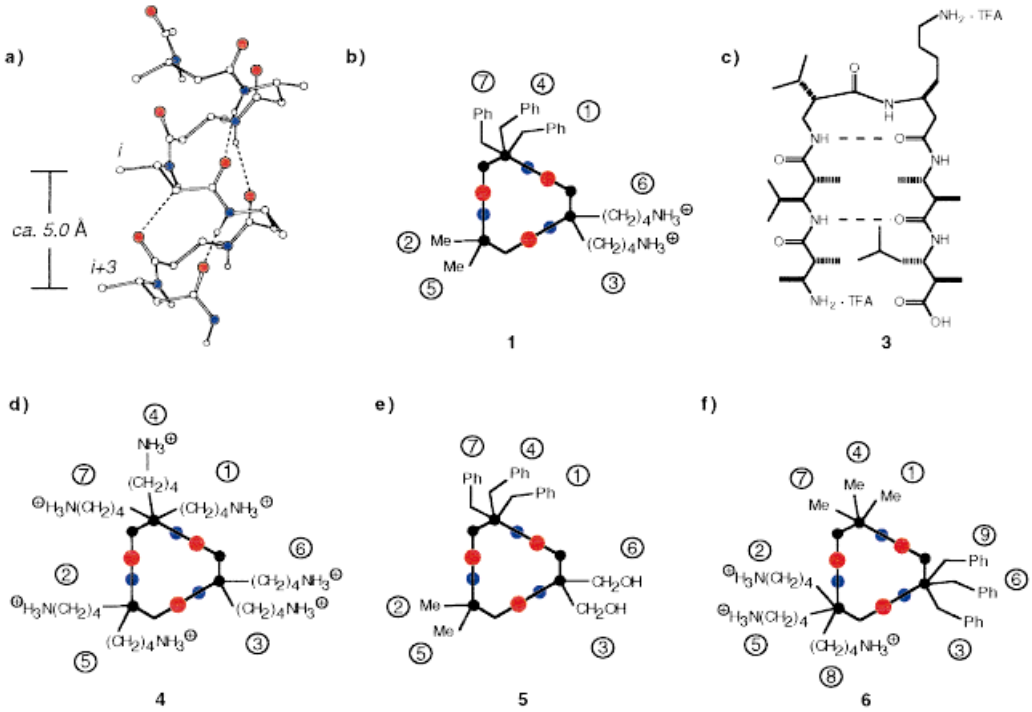



Fig. 4. *Possible* $3_{14}$*-helical and hair-pin structures of β-peptides* **1** *and* **3**–**6**. *a*) (*M*)-$3_{14}$-Helix of a $\beta$-peptide with pitch of *ca.* 5 Å and the side chains on residues *i* and $i+3$ in lateral juxtaposition. *b*), *d*)–*f*) Schematic presentation of a view along the $3_{14}$-helix axis for a $\beta$-peptide with the side chains as in **1**, **4**, **5**, and **6**, respectively. c) Hair-pin structure of $\beta$-peptide **3** in MeOH (by NMR analysis). The $\beta$-peptides **2** and **3** cannot possibly form a $3_{14}$-helical secondary structure [8b]. No secondary structure could be detected in MeOH solutions of $\beta$-peptide **2**. We, therefore, consider it possible that the particular configurational pattern of the $\beta$-amino acids in this $\beta$-peptide leads to a random-coil structure.

The inhibitory activity of naturally occurring, amphipathic proteins such as apolipoprotein A-I and the lipidated forms of this protein, *e.g.*, human $HDL_3$ and reconstituted HDL particles, as well as of amphipathic $\alpha$-peptides such as Ac-$(Xaa)_{18}$-$NH_2$ (*cf. Fig. 1*), is compared to the activity of $\beta$-peptides in *Table 2*. From an inspection of this table, it is immediately clear that the activity of the $\beta$-peptides as inhibitors of sterol uptake is 2 to 3 orders of magnitude smaller. The activity of the $\alpha$-octadecapeptide Ac-$(Xaa)_{18}$-$NH_2$ (*Fig. 1* and *Table 2*) still exceeds that of the $\beta$-peptides by a factor of 20–60.

Table 2. *Comparison of the Inhibitory Effect of α-Peptides and Proteins, and β-Peptides*

| Inhibitor | $IC_{50}$ [a] [b] | |
|---|---|---|
| | [μg/ml] | μM |
| Human apolipoprotein A-I | $11 \pm 1$ | $0.4 \pm 0.04$ |
| Human $HDL_3$ | $29 \pm 2$ | $1.0 \pm 0.1$ |
| Reconstituted HDL particles | $25 \pm 5$ | $0.9 \pm 0.2$ |
| Ac-$(Xaa)_{18}$-$NH_2$ [c] | $33 \pm 2$ | $15 \pm 1$ |
| $\beta$-Peptide **1** | $850 \pm 80$ | $890 \pm 90$ |
| $\beta$-Peptide **6** | $590 \pm 60$ | $360 \pm 40$ |

[a]) Cholesteryl oleoyl ester uptake was measured in the absence and in the presence of inhibitor with unilamellar lipid vesicles as the donor and BBMV as the acceptor. Conditions were as described in the legend of *Fig. 2*. [b]) The $IC_{50}$ values for proteins, lipoproteins, and $\alpha$-peptides were taken from [7]. [c]) Ac-$(Xaa)_{18}$-$NH_2$: Ac-Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe-$NH_2$.

The 'acid test' was the investigation of cholesterol transport through CaCo-2 cells, which is not inhibited by $\alpha$-peptides[6]). *Fig. 5* shows the kinetics of cholesterol absorption by a tightly packed monolayer of CaCo-2 cells in the absence and presence of $\beta$-nonapeptide **6**. HDL Particles reconstituted from human apolipoprotein A-I and dimyristoyl phosphatidylcholine were used as the donor[7]). From an inspection of *Fig. 5*, it is clear that, in the presence of the $\beta$-peptide, the absorption of cholesterol was slowed down markedly. In the absence of inhibitor, the biphasic curve (*Fig. 5*) was fitted best by a double-exponential function, yielding half-lives on the order of 1 min for the initial fast phase and $t_{1/2} = 102 \pm 15$ min for the ensuing slower phase. In the presence of $\beta$-peptide **6** (1 mg/ml), the kinetic curve was monophasic, and curve-fitting (with a single-exponential function) yielded a half-life of $t_{1/2} = 2.6 \pm 0.2$ h. We can conclude that in the presence of the $\beta$-peptide, the initial fast phase of cholesterol

6) The enantiomer (from D-amino acids) of the octadecamer shown in *Fig. 1* and included in *Table 2* is protected from proteolysis, and thus, active in the CaCo-2 cell test [1].

7) Cholesterol uptake into CaCo-2 cells was also inhibited in the presence of $\beta$-peptide **6** when mixed bile-salt micelles (5 mM sodium taurocholate, 0.6 mM sodium oleate, 0.1 mM radiolabeled cholesterol) were used as the donor. At concentrations of the positively charged $\beta$-peptide **6** equivalent to the negative surface charge of the mixed bile-salt micelles, a precipitate formed, and no uptake was measured. The precipitate formed under these conditions is very likely due to electrostatic salting out of the mixed bile-salt micelles by the $\beta$-peptide at the point of charge neutralization. Clearly, the mechanism of inhibition applicable in this case is fundamentally different from that encountered in experiments documented in *Figs. 2* and *5*. Therefore, the mixed bile-salt micelles had to be replaced as the donor: in the experiment shown in *Fig. 5*, reconstituted HDL, the physiological ligand of SR-B, was used. Hence, the absorption experiment (*Fig. 5*) may be viewed as selective uptake of cholesterol from the HDL ligand to CaCo-2 cells.

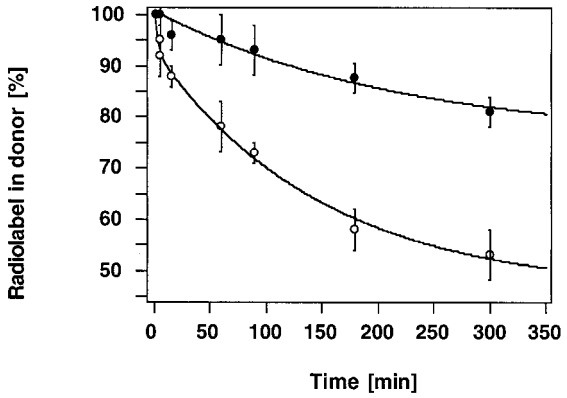



Fig. 5. *Kinetics of cholesterol absorption by CaCo-2 cells in the absence and presence of β-peptide* **6**. CaCo-2 Cells were grown to confluency in *Dulbecco*'s modified *Eagle* medium, and cholesterol absorption by the confluent monolayer of CaCo-2 cells was measured at 37° as previously described. Reconstituted HDL particles were used as the donor. For reconstitution, human apolipoprotein A-I (0.15 mg protein/ml) was interacted with dimyristoyl phosphatidylcholine and cholesterol radiolabeled with [4-$^{14}$C]cholesterol (50 Ci/mol) (total lipid concentration 0.3 mg lipid/ml; phospholipid/cholesterol mol ratio = 95 : 5) at 25° [17]. Transfer of radiolabeled cholesterol in the absence (○) and presence (●) of the β-peptide **6**. Data points are plotted as the average ± SD of two different measurements in triplicate. The solid lines represent curves fitted to the experimental data with double-exponential and single-exponential functions for the data in the absence (○) and presence (●) of β-peptide **6**, respectively.

absorption was abolished, and the residual absorption of cholesterol was reduced to a degree which is probably due to simple passive diffusion [14].

As was pointed out for α-peptides [1][7], the inhibition of sterol uptake reported in *Figs. 2*, *3*, and *5* could be an artefact arising from interactions of the β-peptides with either the donor particle or the acceptor membrane interfering with the uptake assays used. Control experiments were carried out analogously to those described for apolipoproteins and α-peptides [7]. Controls clearly show that the β-peptides used here have no effect on the size and integrity of donor and acceptor (data not shown). Therefore, we conclude that the inhibitory effect of β-peptides described here is genuine.

**Discussion, Conclusions, and Outlook.** – Short-chain β-peptides may inhibit small-intestinal cholesterol absorption.

With apolipoproteins and α-peptides as inhibitors, we showed previously that this inhibition is competitive (*i.e.*, the inhibitor interacts directly with the SR-BI protein [1]), and that the minimum structural requirement for inhibitory activity is an amphipathic α-helix, the structural motif common to all apolipoproteins: thus, the octadecapeptide Ac-$(Xaa)_{18}$-$NH_2$, shown in *Fig. 1* and *Table 2*, known to form a type A amphipathic α-helix [15][16], is a good inhibitor of sterol uptake in a cell-free model system, while the octadecapeptide Ac-Asp-Trp-Leu-Ala-Lys-Asp-Tyr-Phe-Lys-Lys-Ala-Leu-Val-Glu-Glu-Phe-Ala-Lys-$NH_2$ of the same amino-acid composition but with *randomized* sequence of amino acids is not. Due to degradation by proteinases released from or in CaCo-2 cells, neither the octadecamer nor apolipoprotein A-I is inhibiting lipid transport through a CaCo-2 cell monolayer [1].

Three of the six $\beta$-peptides tested inhibit sterol uptake in the BBM model, and with $\beta$-nonapeptide **6** we have demonstrated inhibitory activity with CaCo-2 cells, confirming its stability towards proteolytic cleavage. There is a striking correlation between the structure of the $\beta$-peptides tested and their activity as SR-BI inhibitors: the only active $\beta$-peptides are those that could *possibly* form the characteristic $3_{14}$-helical secondary structure in such a way that the functionalized lysine (**1** and **6**) or serine (**5**) side chains would be in lateral juxtaposition on one side of the helix, and the non-polar Me and Bn groups of $\beta$-HAla and $\beta$-HPhe on the other side, which would render them somewhat amphipathic (see *Fig. 4*). The much lower activity of the $\beta$-peptides, compared to the reference amphipathic $3.6_{13}$-helical $\alpha$-peptide, is not surprising at all, when we consider how different the structures and shapes of a right-handed $3.6_{13}$-helical $\alpha$-octadecamer (*ca.* 27 Å long) and of a left-handed $3_{14}$-helical $\beta$-nonapeptide (*ca.* 15 Å long) actually are, with opposite directions of the resulting helix dipoles [8] (*cf. Figs. 1* and *4*). Rather, it is astonishing that the very simple analogs **1**, **5**, and **6** are really capable of mimicking the more complex $\alpha$-peptide 'originals'! Furthermore, it is important to point out that we have no proof at this point that helical structures are present in the $\beta$-peptides **1**, **5**, and **6** in *water*, other than characteristic patterns in the CD spectra (*i.e.*, weak *Cotton* effects) [9a][8]).

The identification of synthetic compounds such as $\beta$-peptides as peptidomimetics that interact with SR-B of the intestinal BBM and thus inhibit cholesterol and lipid uptake in general is important from a medicinal viewpoint. It raises the prospect of manipulating the small intestine's ability to absorb dietary cholesterol and triacylglycerols[9]), and thus for controlling hypercholesterolemia and obesity, two prominent risk factors of atherosclerosis. Synthetic compounds, such as the $\beta$-peptides described here, have an attractive advantage over proteins and natural $\alpha$-peptides as inhibitors of lipid absorption: they are resistant to pancreatic proteases, which makes the direct oral application of these compounds possible and obviates the need for encapsulation in a protective coat. Because of their importance, scavenger receptors may become an attractive target in the future, and the identification and/or development of synthetic compounds interacting with and activating or inhibiting these receptors will be highly desirable.

Animal experiments to test $\beta$-peptides in hypercholesterolemic mice are underway.

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## Experimental Part

Brush-border membrane vesicles (BBMV) were prepared routinely from rabbit duodenum and jejunum [17][18]. Published methods were used to prepare mixed bile-salt micelles and small unilamellar lipid vesicles [18], to reconstitute HDL particles from human apolipoprotein A-I and dimyristoyl phosphatidylcholine (lipid/protein wt. ratio 2:1) [15], to prepare human $HDL_3$ from fresh plasma of normal-lipidemic donors [19], to grow CaCo-2 cells in tissue culture, and to measure lipid absorption by a confluent monolayer of CaCo-2 cells [20].

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[8]) The structure of a $\beta$-peptide, as of any other natural or unnatural complex molecule capable of folding into secondary structures, in MeOH, in $H_2O$, and in the binding pocket of a protein may be quite different.

[9]) We have shown previously that apolipoprotein A-I interacts with SR-BI, and, as a result, inhibits the uptake of not only sterols but also phospholipids and triacylglycerols [1].

The permeability and integrity of the CaCo-2 cell monolayer were monitored throughout the lipid-absorption experiment with [1,2-$^3$H]polyethylene glycol (2 mCi/g, average $M_r = 900$) [20]. The viability of the CaCo-2 cells was checked at the end of the absorption experiment with Trypan Blue staining [20]. In a typical absorption experiment, CaCo-2 cells remained intact as judged from their permeability to polyethylene glycol, which was less than 0.5%, and their viability at the end of the experiment was greater than 99% [20].

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