Dimerization of Transcobalamin II Receptor

REQUIREMENT OF A STRUCTURALLY ORDERED LIPID BILAYER*

(Received for publication, August 14, 1995, and in revised form, March 5, 1996)

Santanu Bose‡, Jimmy Feix§, Shakuntla Seetharam, and Bellur Seetharam¶

From the Departments of Biochemistry, Biophysics, and Medicine, Medical College of Wisconsin and Veterans Administration Medical Center, Milwaukee, Wisconsin 53226

Transcobalamin II receptor (TC II-R) exists as a monomer and a dimer of molecular masses of 62 and 124 kDa in the microsomal and plasma membranes, respectively, and in vitro, pure TC II-R monomer dimerizes upon insertion into egg PC/cholesterol (molar ratio, 4/1) liposomes (Bose, S., Seetharam, S., and Seetharam, B. (1995) J. Biol. Chem. 270, 8152–8157 and Bose, S., Seetharam, S., Hammond, T., and Seetharam, B. (1995) Biochem. J. 310, 923–929). The current studies were carried out to define the mechanism of TC II-R dimerization. Both the mature TC II-R (62 kDa) and the enzymatically deglycosylated TC II-R (45–47 kDa) demonstrated optimal association and formed dimers of molecular masses of 95 and 124 kDa, respectively, at 22°C when bound to egg PC vesicles containing at least 10 mol % of cholesterol. Mature TC II-R dimerized upon insertion into synthetic phosphatidycholine vesicles of different fatty acyl chain length (dimyristoyl, dipalmitoyl, and disteroyl phosphatidycholine) in the absence or the presence of cholesterol at temperatures below or above their transition temperatures, respectively. Dimerization of TC II-R also occurred with vesicles prepared using lipid extract from the plasma but not microsomal membranes. Cholesterol depletion of native intestinal plasma membranes or its enrichment in the microsomal membranes resulted in the in situ conversion of the 124-kDa dimer to the 62-kDa monomer or of the monomer into the dimer form, respectively. Treatment of plasma membranes with phospholipase A₂ resulted in the conversion of the dimer form of the receptor to the monomer form and spin label studies using 1-palmitoyl, 12 doxysteryl phosphatidycholine revealed that interactions of TC II-R with PC vesicles increased order around the probe. Based on these results, we suggest that dimerization of TC II-R is mediated by its interactions with a rigid more ordered lipid bilayer membrane, is regulated in plasma membranes by cholesterol levels, and is independent of glycosylation-mediated folding.

Plasma transport of absorbed Cobalamin (Cbl; vitamin B₁₂)³

*This work was supported by Grant NIDDK-26638 from the National Institutes of Health and Grant 7816-01P from the Veterans Administration. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

||To whom all correspondence should be addressed: MACC Fund Center, Rm. 6061, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226; Tel.: 414-456-4655; Fax: 414-259-1533.

The abbreviations used are: Cbl, cobalamin (vitamin B₁₂); TC II, transcobalamin II; TC II-R, transcobalamin II receptor; PC, phosphatidylcholine; DMPC, dimyristoyl PC; DPCC, dipalmitoyl PC; DSPC, disteryl PC; PAGE, polyacrylamide gel electrophoresis; BLM, basolateral membranes; EPR, electron paramagnetic resonance.

Requirements of a Structurally Ordered Lipid Bilayer* 11718

The following chemicals were purchased as indicated: egg PC, dimyristoyl PC, dipalmitoyl PC, disteryl PC, 1-palmitoyl-12-docosyl-sphingosylglycerol-3-phosphocholine, and cholesterol (Avanti Polar Lipids Inc. Alabaster, AL); [³⁵⁰Co] cyanocobalamin (15 μCi/μg) and carrier-free Na²⁻¹³C [Amersham Corp.]; peptide N-glycosidase from Flavobacterium meningosepticum and O-glycosidase from Diploroccus pneumoniae (Boehringer Mannheim); phospholipase A₁ from bee venom, phospholipase C from Clostridium Wd and C from Lactobacillus. 11718
Clostridium Perifringens, dihydrocholesterol, 7-keto cholesterol, and protein A (Sigma).

Pure TC II-R was obtained from human placenta essentially as described earlier (4). Monospecific antiserum to TC II-R was prepared in rabbits as described earlier (4). The ligand TC II used in the TC II-R assays was partially purified from human plasma according to Lindemann et al. (7). TC II-R activity in the lipid vesicles was determined using human TC II-[57Co]Cbl (2 pmol) and the Triton X-100 extracts of human protein A (Sigma).

Isolation of Rat Intestinal Microsomal and Basolateral Membranes—Rat intestinal mucosal microsomes were prepared from a 5% (w/v) homogenate of mucosal mucosa prepared in 0.25 M sucrose containing 5 mM EDTA and 10 mM sodium phosphate buffer, pH 7.4. The post-mitochondrial supernatant was centrifuged at 150,000 × g for 2 h, and the pellet obtained was washed in the same buffer once and re-pelleted membranes were used as microsomes. The microsomal membrane contained less than 0.5–1% of the apical and basolateral markers, alkaline phosphatase, and Na+/K-ATPase, respectively. The basolateral membranes were prepared using differential and sorbitol gradient centrifugation according to Walters et al. (9). These membranes were enriched 12-fold for the marker Na+/K-ATPase with a recovery of 14% and contained <1–2% of the apical markers, alkaline phosphatase, and γ-glutamyl transpeptidase.

Lipid Extraction and Modulation of Lipid Composition of Microsomal and Basolateral Membranes—Microsomal or basolateral membranes (5–10 mg of protein) in 2 ml of 10 mM Tris-HCl buffer, pH 7.4, containing 140 mM NaCl and 0.1 mM phenylmethylsulfonyl fluoride was treated with 16 ml of chloroform-methanol (2:1), vortexed, and allowed to stand for 30 min. The mixture was centrifuged for 10 min at 3000 rpm, and the organic layer was removed for total phospholipid and cholesterol ester recovery. Total phospholipid was estimated following inorganic phosphates of the lipid extract. Cholesterol and phospholipid levels were estimated in the lipid extract using eithernativemembranesorlipidvesicle bound TC II-R (25–100 ng) were subjected to immunoblot analysis, the transfer to nitrocellulose membranes was carried out for either 45 or 90 min to detect optimal amounts of the monomer or the dimer species, respectively, present in these membranes (4). Immunoblotting experiments using either native membranes or lipid vesicles were carried out at least three times to confirm the results, particularly the relative mobilities of the two species of TC II-R. In all immunoblotting experiments, the distance between the dimer and the monomer forms of TC II-R was measured and was found to vary by less than 5%.

Elementary Charged Resonance (EPR) Spectroscopy—EPR spectroscopy was performed on a Varian Century Series E-109 spectrometer (Varian Associates Inc., Palo Alto, CA) equipped with a TESLA, rectangular cavity. Samples were contained in a quartz flat cell (Wilmad, Buena, NJ). Data acquisition was controlled by a PC utilizing the VIKING software package (C. C. Felix, Biophysics Research Institute, Milwaukee, WI). Spectra were obtained using 10 mW incident microwave power and a 100 kHz magnetic field modulation of 0.32 Gauss. Field modulation and sweep widths were calibrated with Frenzy's salt (potassium nitrosoisulfonate, Aldrich).

RESULTS

Immunoblot Analysis of TC II-R Monomer and Dimer Bound to Native Membrane or Lipid Vesicles—Reconstitution of pure TC II-R monomer and dimer forms, we first utilized the rather unusual property of TC II-R to stay as dimer when treated with SDS (4).

In order to optimize the conditions for the recovery of the monomer and dimer forms of TC II-R during immunoblotting, the proteins separated on SDS-PAGE were transferred to nitrocellulose membranes for 15–90 min (Fig. 1). Under identical conditions of electrophoresis and time of exposure to x-ray film, the times required for the optimal transfer of the dimer and the monomer forms were 90 (Fig. 1A, top) and 45 min (Fig. 1A, bottom), respectively. However, in order to examine whether both forms of TC II-R can be detected together in a single gel, a transfer time of 60 min was used for immunoblotting of intestinal mucosal total membrane proteins separated on SDS-PAGE. With a transfer time of 60 min, both the monomer and the dimer forms could be seen in the same gel (Fig. 1B). Quantitation of the immunoblots shown in Fig. 1A revealed that the dimer to monomer ratio was 8:1, whereas it was 2:1 for the data shown in Fig. 1B. These results show that a time frame chosen to detect both the monomer and the dimer in the same gel will not quantitatively reflect the actual amount of each species of TC II-R present in a given membrane fraction. Thus, in all subsequent immunoblot analysis, the transfer times employed were 45 and 90 min to visualize

Dimerization of Transcobalamin II Receptor

The results shown that
the monomer and the dimer forms of TC II-R, respectively.

Interactions of Pure TC II-R with Egg PC Vesicles: Effect of Cholesterol Concentration—Pure TC II-R of molecular mass of 62 kDa was reconstituted into egg PC liposomes containing increasing mol % of cholesterol to study the effect of cholesterol on the lipid vesicle association of TC II-R and its molecular mass following its association with these lipid vesicles. At 22°C in the absence of added cholesterol, there was no association of TC II-R activity with egg PC lipid bilayer. However, with the increase in the cholesterol content of the liposomes from 1 to 50 mol %, there was an increase from 25 to 80% of receptor activity associated with the lipid bilayer and as a consequence, TC II-R activity in the supernatant fraction decreased by similar amounts (Fig. 2a).

Upon SDS-PAGE and immunoblotting of the liposomally bound TC II-R (Fig. 3), the monomer form of 62 kDa was associated with the liposomes prepared with using 1 (Fig. 3a, lane 2) or 2 mol % (Fig. 3a, lane 1) of cholesterol (Fig. 3a) but not with the liposomes prepared using >10 mol % of cholesterol (Fig. 3a, lanes 3–5). On the other hand, the 124-kDa dimer was present only when the cholesterol content of the egg PC liposomes was >10 mol % (Fig. 3b, lanes 3–5) but not when the mol % of cholesterol was 1 or 2 (Fig. 3b, lanes 1 and 2). Quantitation of the immunoblots revealed that TC II-R protein association (Fig. 2b) increased by about 9-fold with an increase in cholesterol content of the liposomes from 1 to 50 mol %. These results suggested that the association of TC II-R with egg PC bilayers is dependent on the presence of cholesterol and that its optimal binding and dimerization occurred when the mol % of cholesterol was at least 10. In order to further examine the role of cholesterol in the lipid bilayer and its effect on the dimerization of TC II-R, reconstitution experiments were carried out using synthetic symmetrical PCs of different length fatty acyl residues in the presence and the absence of cholesterol at temperatures above and below their phase transition.

Interaction of Pure TC II-R with DMPC, DPPC, and DSPC Vesicles: Effect of Cholesterol and Temperature—Pure TC II-R associated and dimerized (Fig. 4a) in the absence of cholesterol at temperatures below the phase transition of DMPC (5°C, lane 1), DPPC and DSPC (22°C, lanes 2 and 3) vesicles. At the same temperatures, in the presence of cholesterol (50 mol %), there was no association of TC II-R with DMPC or DPPC or DSPC vesicles, and no dimer form could be detected (Fig. 4a, lanes 4–6). However, at temperatures above their phase transition (Fig. 4b), the association and dimerization of TC II-R occurred in the presence of 50 mol % of cholesterol with DMPC (37°C, lane 1), DPPC (45°C, lane 2), and DSPC (65°C, lane 3) vesicles but not in the absence of cholesterol (Fig. 4b, lanes 4–6). In order to further verify whether the presence of either a monomer or a dimer is solely dependent on the membrane cholesterol, further studies were carried out using native mi-
dimerization of TC II-R in the native membranes is dependent on the physical state of TC II-R. In liposomal membranes it exists as a dimer (5).

Reconstitution of Pure TC II-R in Liposomes Prepared Using Lipid Extracts from Native Membranes—The lipids extracted from the rat intestinal basolateral and microsomal membranes revealed a phospholipid to cholesterol ratio of 1.6:1 and 26:1, respectively. When pure TC II-R was reconstituted with liposomes prepared using 2 μmol of phospholipid in each case, the liposomally bound receptor (Fig. 5, lane 1), like in the native microsomes (Fig. 5, lane 2), was a monomer. In contrast, TC II-R, when reconstituted with liposomes derived from basolateral membrane lipids, was a dimer (Fig. 5, lane 7), like the receptor in the native basolateral membrane (Fig. 5, lane 8). TC II-R bound to microsomal lipids vesicles (Fig. 5, lane 5) or bound to native microsomal (Fig. 5, lane 6) membranes was a monomer and no dimer form of TC II-R could be detected. Similarly, the monomer form of TC II-R could not be detected when TC II-R was bound to basolateral membrane lipid derived liposomes (Fig. 5, lane 3) or in the native basolateral membranes (Fig. 5, lane 4). These studies again suggested that the dimerization of TC II-R in the native membranes is dependent upon its cholesterol content. In order to directly test this hypothesis, cholesterol levels of these native membranes were altered to determine whether the physical state of TC II-R would change in situ without its solubilization from the membranes.

Effect of Cholesterol Depletion and Enrichment in Native Membranes on the Physical State of TC II-R—Treatment of the basolateral membranes with digitonin resulted in the depletion of membrane cholesterol altering the phospholipid/cholesterol ratio from 1.6:1 to 27:1. Untreated, cholesterol-depleted, and Triton X-100-treated basolateral membranes were subjected to immunoblot analysis (Fig. 6, top panel) and studied for the presence of TC II-R monomer (panel a) or the dimer (panel b). Native untreated BLM contained only the TC II-R dimer (Fig. 6b, lane 6) but not the monomer (Fig. 6a, lane 1). Digitonin-treated BLM contained only the monomer (Fig. 6a, lane 2), and all the dimer in digitonin-treated membranes was converted to the monomer form (Fig. 6b, lane 7). The molecular mass of TC II-R monomer formed by digitonin treatment of the BLM was 62 kDa, exactly the same size as the TC II-R monomer present in the native microsomal membrane (Fig. 6a, lane 3) or the TC II-R present in the Triton X-100 solubilized extract from the BLM (Fig. 6a, lane 4) or microsomes (Fig. 6a, lane 5). The absence of TC II-R dimer in native microsomal membranes or Triton X-100-treated BLM and microsomes are shown in Fig. 6b, lanes 8, 9, and 10, respectively.

In contrast to the in situ conversion of the TC II-R dimer to the monomer form upon cholesterol depletion of the basolateral membranes, immunoblot analysis (Fig. 6, bottom panel) of the microsomal membranes enriched in cholesterol resulted in the conversion of the monomer form (Fig. 6a, lane 1) to the dimer form (Fig. 6b, lane 10). The conversion in the physical state of
Importance of Fatty Acyl Resides in Membrane Interactions and Dimerization of TC II-R—In order to explore the possibility that the cholesterol effects on TC II-R dimerization may be mediated by its interaction with the fatty acyl residue of phospholipid, the following experiments were carried out. When the native basolateral membranes were digested with various phospholipases (Fig. 7), a total in situ conversion of the dimer form of TC II-R (Fig. 7a, lane 1) to the monomer form occurred following treatment with phospholipase A2 (Fig. 7, a, lane 4, and b, lane 8) but not phospholipase C (Fig. 7, a, lane 2, and b, lane 6) or phospholipase D (Fig. 7, a, lane 3, and b, lane 7). Direct evidence for the interaction of TC II-R with the 2-fatty acyl residue was obtained with spin label studies (Fig. 8).

EPR spectra of the phospholipid spin label, 12PcSL, in liposomes prepared from either basolateral or microsomal lipids and with and without TC II-R are shown in Fig. 8. Motion of the spin label was significantly restricted in basolateral liposomes containing TC II-R (Fig. 8B) relative to the other three systems, as indicated by the increased line widths and appearance of a strongly immobilized high field component in the EPR spectrum. Given the low protein content (protein/lipid, 1:3000) in the reconstituted system, this strongly suggests direct interaction of the dimeric receptor with the sn-2-acyl chain of PC that bears the nitroxide label. No such interaction was observed in TC II-R-containing liposomes composed of microsomal lipids, where the receptor is monomeric (Fig. 8D). The immobilization was not due to differences in the lipid phase alone, because the rotational motion of the spin label remained fast in both basolateral and microsomal lipid membranes lacking TC II-R (Fig. 8, A and C). Similar effects were observed in purely model systems and correlated well with dimer formation as observed by SDS-PAGE (e.g., Figs. 4 and 5), where the EPR spectrum of 12PcSL in DMPC/cholesterol liposomes (4:1) at 37°C, containing TC II-R was significantly broadened relative to that in DMPC/cholesterol (4:1) alone at 37°C or to that of DMPC vesicles prepared at 37°C with or without TC II-R (at 37°C) (data not shown).

Lack of Effect of TC II-R Glycosylation on Its Dimerization—The post-microsomal dimerization of TC II-R may be facilitated by the folding alterations due to maturation of its glycan chain(s) during its post-microsomal trafficking. In order to determine the extent to which, if at all, the glycan chains influence folding of TC II-R and thus its dimerization enzymatically deglycosylated mature TC II-R was inserted into egg PC/cholesterol vesicles. The results show mature TC II-R (Fig. 9, lane 1) or TC II-R treated with N-glycanase (Fig. 9, lane 2) when inserted in egg PC/cholesterol (4:1) liposomes demonstrated a molecular mass of 124 kDa. It was difficult to detect the size difference of 2 kDa due to the removal of a single N-linked sugar chain. However, upon treatment with sialidase and O-glycanase (lane 3) or with N-glycanase followed by sialidase and O-glycanase (lane 4), the receptor demonstrated a molecular mass of around 95 kDa, a size that would be predicted for the molecular mass of a dimer formed from a fully
mediated by its strong interactions with the membrane lipids. Results clearly indicated that the dimerization of TC II-R is
branes with 2:1 mixture of chloroform and methanol. These
without its solubilization by delipidation of the plasma mem-

native plasma membranes, TC II-R that exists as a dimer of
mechanism of TC II-R dimerization in phospholipid bilayers
and innativemembranes. Previously (4) we haveshown that in

optimal transfer of the monomer and the dimer form has been

In previous studies (4–6), an immunoblotting procedure to
detect and quantify the monomer and the dimer forms of TC
II-R was used. This method used a transfer time of 45 min to
detect the monomer and 90 min to detect the dimer, respec-
tively. In the current studies, the time of transfer used for the
optimal transfer of the monomer and the dimer form has been
validated (Fig. 1A). The data shown in Fig. 1A when quantified
demonstrated a monomer to dimer ratio of 1:8, a value similar
to that obtained earlier for the distribution of TC II-R monomer
and dimer forms in several rat tissue total membranes (5). A
single transfer time of 60 min (Fig. 1B) can be used to detect
both the species of TC II-R in a single gel. However, it will not
accurately reflect the absolute steady state amounts of the two
species present in any given membrane or the interconversion
of one form to the other during experimental modulation of the
membranes. When the data shown in Fig. 1B was quantified,
the ratio of monomer to dimer was 1:2. This value is different
than the value of 1:8 obtained using two different transfer
times, 45 min to transfer the monomer and 90 min to transfer
the dimer. The lower ratio reflects only partial transfer of the
dimer and a partial loss of the monomer during the 60-min
transfer of the protein from the gel. The decreased transfer of
the dimer could be visualized during the 60-min transfer of
pure TC II-R bound to PC/cholesterol vesicles by staining the
gel for protein. The loss of TC II-R monomer during the 60-min
transfer could also be visualized when pure TC II-R was
immunoblotted onto a second nitrocellulose filter (data not
shown). The likely explanation for the 2-fold time differential
for the optimal transfer from SDS-PAGE of the two forms of
TC II-R, the results are clarified by this phenomenon is related to the receptor status, i.e., a monomer or a
dimer.

In the present work we have addressed issues related to the
mechanism of TC II-R dimerization in phospholipid bilayers
and in native membranes. Previously (4) we have shown that in
native plasma membranes, TC II-R that exists as a dimer of
molecular mass of 124 kDa could be converted to a 62-kDa
monomer with solubilization of the receptor by Triton X-100 or
without its solubilization by delipidation of the plasma mem-
branes with 2:1 mixture of chloroform and methanol. These
results clearly indicated that the dimerization of TC II-R is
mediated by its strong interactions with the membrane lipids.

Some insight into nature of the membrane that may mediate
the dimerization of TC II-R was obtained from further studies
(5) aimed at understanding the intracellular distribution of TC
II-R in the rat kidney. These studies (5) demonstrated that
under identical conditions of immunoblotting (except the trans-
fer time), the monomer form of TC II-R with a molecular mass
of 62 kDa was detected only in the microsomal membranes,
whereas the dimer form of TC II-R with a molecular mass of
124 kDa was the only species present in the plasma mem-
branes. In addition, these studies showed that in many rat
tissues, the dimer form of TC II-R was the predominant form
present.

One major difference between the plasma and microsomal
membranes of many mammalian tissues is the molar ratio
of phospholipid to cholesterol. In the plasma membranes the ratio
is about 1–1.2:1 (14, 15), whereas it is about 22:1, 20:1, and 26:1
in the microsomal membranes isolated from rat kidney, pla-
centa, and intestinal mucosa, respectively.2 Because TC II-R
exists in two different physical states in these two membranes,
we hypothesized that TC II-R dimerization in the native
plasma membranes may be related to the presence of higher
amount of cholesterol in these membranes. This hypothesis
was further based on our earlier observation that TC II-R is
able to dimerize with egg PC vesicles that contained 25 mol %
of cholesterol (4). In order to explore this hypothesis, additional
studies were carried out using both phospholipid vesicles and
native microsomal and plasma membranes isolated from rat
intestinal mucosa.

Based on the experimental evidence from the current study,
we conclude that the single most important factor affecting the
dimerization of TC II-R in native plasma membranes and lack
of dimerization in the microsomal membranes is the difference
in their relative cholesterol content. This conclusion is based
on the three lines of evidence. First, the association (Fig. 2) and
the ensuing dimerization of TC II-R with egg PC occurred with
a minimum cholesterol content of 10 mol % (Fig. 2). At choles-
terol levels of 1 or 2 mol %, TC II-R associated poorly with egg
PC vesicles and stayed as a monomer. Second, mature TC II-R
dimerized upon insertion into lipid vesicles prepared using
total lipids from intestinal basolateral but not microsomal
membranes (Fig. 5). The phospholipid to cholesterol ratios in
these extracts were 1:2:1 and 26:1, respectively (data not
shown). Third, changing the phospholipid/cholesterol ratio
from 1:2:1 to 26:1 in the basolateral plasma membranes by
cholesterol depletion with digitonin treatment (Fig. 6, top
panel) or from 26:1 to 10:1 in the microsomal membranes by
cholesterol enrichment (Fig. 6, bottom panel) resulted in the in-
situ conversion of the dimer form to the monomer form and
from the monomer form to the dimer form, respectively.

How does cholesterol influence the interaction between the
two monomers of TC II-R in order to facilitate the formation
of noncovalent dimers in the native membranes? The answer
may be related to the potential function(s) of cholesterol in mem-
branes. Cholesterol could play a role in facilitating the hydro-
phobic match between the lipid bilayer and TC II-R, thus
leading to its association and dimerization. Hydrophobic
matching is a well established model for lipid-protein interac-
tions in the membranes (16–18). This model states that in
order to accommodate or match the hydrophobic region of a
protein with that of the hydrophobic core of lipid bilayer, asso-
ciation of a protein is accompanied by alterations in the thick-
ness of lipid bilayers. Earlier studies (19–21) have confirmed
that physiological membrane functions are controlled by hydro-
phobic membrane thickness. For example, optimal activity of

2 S. Bose and B. Seetharam, unpublished observations.
Ca\(^{2+}\)-ATPase (22, 23) and acetylcholine receptor (24, 25) function can be altered by the introduction of n-alkanes, which are known to increase hydrophobic thickness of the lipid bilayer. Like n-alkanes, cholesterol is also known to increase the hydrophobic thickness of lipid bilayers (26, 27). Therefore one possible explanation for the cholesterol induced effects of TC II-R association and the ensuing dimerization could be that it is due to the cholesterol-mediated thickening of lipid bilayer. During such a thickening, hydrophobic matching between TC II-R and the hydrophobic core of the membrane could facilitate two monomers of TC II-R to be spatially near to one another and dimerize encompassing a specific lipid milieu within the bilayer.

Based on experimental evidence, a more likely possible role of cholesterol in the dimerization of TC II-R is that it is due to interaction of cholesterol with the phospholipid. X-ray and neutron scattering studies have shown that cholesterol inserts normal to the plane of the bilayer with the \(\beta\)-OH group near the ester carbonyl of the lipid (28, 29). It is thought that this results in the development of hydrogen bonding between the \(\beta\)-hydroxyl group of cholesterol and the carbonyl oxygen linking the fatty acyl chains with its glycerol backbone (30). However, Raman spectroscopy indicates that no actual hydrogen bonding occurs with these carbonyls (31). Despite this uncertainty, what is generally agreed upon is that cholesterol has a substantial effect on the order parameters measured along the lipid hydrocarbon chain by \(^{2}H\)-NMR (32) and on the phase transition of the phospholipid (33). Fourier transform infrared spectroscopy studies (34) have shown that above the transition midpoint cholesterol decreases the fraction of gauche rotomers in the phospholipid hydrocarbon chain, whereas just the opposite is the effect below the transition midpoint.

Effect of cholesterol on the membrane interactions of TC II-R are mediated by increasing the order around the fatty acyl residues. Several lines of evidence support that the order around the 2-fatty acyl residue is important for the interactions of TC II-R with lipid bilayers. One, cholesterol analogues were around the 2-fatty acyl residue is important for the interactions of TC II-R and the hydrophobic core of the membrane could facilitate two monomers of TC II-R to be spatially near to one another and dimerize encompassing a specific lipid milieu within the bilayer.

In conclusion, the results of the present study have shown that the dimerization of TC II-R, an important nutrient receptor, is a post-microsomal event and is due to its strong interactions with a highly ordered lipid bilayer membrane. The membrane fluidity regulated dimerization of plasma membrane TC II-R noted in this study may represent an unique situation. Other factors such as ligand binding (40) and phosphorylation-dephosphorylation (41) are known to regulate the oligomerization of several receptors. Finally, it is not known what physiological advantage a cell might have with TC II-R dimers in their plasma membranes. However, because the dimers are functional in ligand binding and bind 2 mol of ligand/dimer, it is likely that the existence of dimers will help in the rapid uptake of circulating Cbl. Further studies are needed to identify the hydrophobic regions of TC II-R that mediate its interaction with the lipid bilayer, and such studies are possible once the sequence of TC II-R is known.

REFERENCES

1. Quadros, E. V., Rothenberg, S. P., Pan, Y.-C. E., and Stein, S. (1986) J. Biol. Chem. 261, 15455–15460
2. Younghal-Turner, P., Risenberg, L. E., and Allen, R. H. (1978) J. Clin. Invest. 61, 133–141
3. Nissel, E., and Hollenberg, M. D. (1980) Biochim. Biophys. Acta 628, 190–200
4. Bose, S., Seetharam, S., and Seetharam, B. (1995) J. Biol. Chem. 270, 8152–8157
5. Bose, S., Seetharam, S., Hammond, T. G., and Seetharam, B. (1995) Biochem. J. 310, 923–929
6. Bose, S., Komorowski, R., Seetharam, S., Gilfix, B., Rosenblatt, D. S., and Stein, S. (1996) J. Biol. Chem. 271, 4355–4360
7. Lindemann, J., Van Koppel, J., and Abels, J. (1986) Scand. J. Clin. Lab. Invest. 46, 223–232
8. Seligman, P. A., and Allen, R. H. (1978) J. Biol. Chem. 253, 1766–1772
9. Walters, J. R., Horvath, P. J., and Weiser, M. M. (1986) Gastroenterology 91, 34–40
10. Barlett, G. R. (1959) J. Biol. Chem. 234, 466–468
11. Courchaine, A. J., Miller, W. H., and Stein, D. B. (1959) Clin. Chem. 5, 609–614
12. Low, M. G., and Zilversmit, D. B. (1980) Biochemistry 19, 3913–3918
13. Laemmli, U. K. (1970) Nature 269, 680–685
14. Molitoris, B. A., and Simon, F. R. (1985) J. Membr. Biol. 83, 207–215
15. Chapelle, S., Gilles-Baillien, M. (1983) In Biological Membranes (Chapman, D., ed) Vol. 5, pp. 1766–1772
16. Mouritsen, O. G., and Bloom, M. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 145–171
17. Engelman, D. M., and Zaccal, G. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5894–5898
18. Mouritsen, O. G., and Bloom, M. (1984) Biophys. J. 46, 141–153
19. Kinnunen, P. K. J. (1991) Chem. Phys. Lipids 57, 375–399
20. Sackmann, E. (1984) in Biological Membranes (Chapman, D., ed) Vol. 5, pp. 145–171
Dimerization of Transcobalamin II Receptor

105–143, Academic Press, New York
21. Sanderman, H. (1978) Biochim. Biophys. Acta 515, 209–237
22. East, J. M., Jones, O. T., Simmonds, A. C., and Lee, A. G. (1984) J. Biol. Chem. 259, 8070–8071
23. Johannsson, A., Kightley, C. A., Smith, G. A., Richards, C. D., Hesketh, T. R., and Metcalfe, J. C. (1981) J. Biol. Chem. 256, 1643–1650
24. Criado, M., Eibl, H., and Barrantes, F. J. (1984) J. Biol. Chem. 259, 9188–9198
25. Fong, T. M., and McNamee, M. G. (1986) Biochemistry. 25, 830–840
26. Ipsen, J. H., Mouritsen, O. G., and Bloom, M. (1990) Biophys. J. 57, 405–412
27. Vist, M. R., and Davis, J. H. (1990) Biochemistry. 29, 451–464
28. Worcester, D. L., and Franks, N. P. (1978) J. Mol. Biol. 100, 359–378
29. Gennis, R. B. (1989) Biomembranes: Molecular Structure and Function (Cantor, C. R., ed) pp. 73–76, Springer-Verlag New York Inc., New York
30. Oldfield, E., and Chapman, D. (1972) FEBS Lett. 23, 285–297
31. Oldfield, E., and Chapman, D. (1972) FEBS Lett. 23, 285–297
32. Oldfield, E., and Chapman, D. (1972) FEBS Lett. 23, 285–297
33. Mabrey, S., Mateo, P. L., and Sturtevant, J. M. (1978) Biochemistry 17, 2464–2468
34. Cortijo, M., Alonso, A., Gomez-Fernandez, J. C., and Chapman, D. (1982) J. Mol. Biol. 157, 597–618
35. Chapman, D. (1975) Q. Rev. Biophys. 8, 185–235
36. Chapman, D., Gomez-Fernandez, J. C., and Goni, F. M. (1979) FEBS Lett. 98, 211–223
37. Shimshick, E. J., and McConnell, H. M. (1973) Biochemistry 12, 2351–2360
38. Laderke, B. D., Williams, B. D., and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333–340
39. Searmark, T., Jacobsen, C., Magee, A., and Vilhardt, H. (1987) J. Mol. Endocrinol. 4, 51–59
40. Hurwitz, D. R., Emmanuel, E. L., Nathan, M. H., Sarver, N., Ulrich, A., Felder, S., Lax, K., and Schlessinger, J. (1987) J. Biol. Chem. 262, 22035–22043
41. Carlberg, K., and Rohrschneider, L. (1994) Mol. Biol. Cell 5, 81–95