Post-translational Modification of the Hepatitis C Virus Core Protein by Tissue Transglutaminase*

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The hepatitis C virus (HCV) core protein is a structural protein that packages the viral genomic RNA. In this study, we demonstrate that a stable core protein dimer could be produced in liver cells. The production of this protein could be enhanced by calphostin C and serum deprivation. This protein was determined to be the core protein dimer because of its reactivity with the anti-core antibody, its similar electrophoretic mobility compared with that of the core protein dimer generated by cross-linking with glutaraldehyde, and its increase in size by a hemagglutinin tag fused to the core protein sequence. This core protein dimer was highly stable and resistant to SDS and β-mercaptoethanol. The enzyme that mediated the formation of this stable core protein dimer was determined to be the tissue transglutaminase (tTG) because, first, tTG could be activated by calphostin C and serum deprivation; second, the formation of this dimer was suppressed by monodansylcadaverine, a tTG inhibitor; and third, the core protein could be cross-linked by tTG in vitro. Thus, the HCV core protein represents the first known viral structural protein substrate of tTG. The post-translational modification by tTG reduced the RNA binding activity of the core protein, raising the possibility that tTG may regulate the biological functions of the HCV core protein.

Hepatitis C virus (HCV) is a member of the Flavivirus family (1, 2). This virus can cause severe liver diseases, including acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (3–5). The research with HCV has been hampered by the lack of a cell culture system for its propagation. Nevertheless, a large amount of information regarding HCV has been obtained through molecular cloning and the expression, and cell growth (22).

The HCV core protein can form homodimers and multimers. This higher order structure may be important for the formation of the virions (13, 23–26). In this report, we demonstrate that a fraction of the HCV core protein dimer is highly stable and cannot be dissociated by boiling in β-mercaptoethanol and SDS. Further analyses indicated that this dimer is generated by tissue transglutaminase (tTG), which is an enzyme that catalyzes the formation of a γ-glutamyl-ε-lysine isopeptide bond by joining the γ-carboxamidase group of glutamine to the amino group of lysine (27, 28). The modification by tTG reduces the RNA binding activity of the core protein, suggesting a possible regulation of the core protein activity by this enzyme.

EXPERIMENTAL PROCEDURES

Chemicals and Enzymes—Calphostin C (CalC), GF 109203X, and Ro 31-8220 were purchased from Calbiochem-Novabiochem. These chemicals were dissolved in Me2SO. Guinea pig liver tTG, monodansylcadaverine (MDC), and N,N’-dimethylcasein were purchased from Sigma. Bovine serum albumin (BSA) was from New England Biolabs Inc. (Beverly, MA). TT RNA polymerase, RNasin, and RNase-free DNase I were obtained from Promega (Madison, WI). [1,4-32P]GTP was purchased from ICN (Irvine, CA). The Supersignal chemiluminescence kit and the horseradish peroxidase-conjugated goat anti-rabbit antibody were purchased from Pierce.

Construction of DNA Plasmids—The construction of the DNA plasmids pCDEF/RC, pCDEF/H4A-RC, and pCMV-CC has been described previously (29, 30). pCDEF/RC contains the full-length core protein coding sequence of the HCV RH isolate (15). The expression of the core protein in this plasmid is under the control of the promoter of the
eukaryotic elongation factor-1a gene. pCDEF/HA-RC is identical to pCDEF/RC, with the exception that a hemagglutinin (HA) tag derived from the hemagglutinin of the influenza virus is fused to the 5′-end of the core protein coding sequence. pCDEF/HA-RC EIb contains nucleotides 1–1642 of the HCV RH coding sequence, including the entire coding sequence for the core protein. The pCDEF/HA-RC EIIb sequence contains 10% fetal bovine serum (FBS). Transfection was performed—

**Establishment of Stable Cell Lines**—To establish Huh7/pCDEF and Huh7/HA-RC stable cell lines, Huh7 cells transfected with pCDEF or pCDEF/HA-RC were incubated in DMEM plus 10% FBS containing 400 μg/ml G418 for 2–3 weeks. The cell colonies were then pooled together for the establishment of Huh7/pCDEF and Huh7/HA-RC stable cell lines. Huh7/pCDEF and Huh7/HA-RC cell lines were derived from HepG2 cells, a well differentiated human hepatoblastoma cell line, and selected by the same procedures. HepG2/pCDEF cells contained the control pCDEF vector, and HepG2/RC cells expressed the core protein. For the serum deprivation experiment, stable cell lines were plated and maintained in DMEM containing 10% FBS. 18 h later, cells were rinsed with DMEM twice and incubated in DMEM without FBS. Huh7/pCDEF and Huh7/HA-RC cells were serum-deprived for 30 h, and HuhG2/pCDEF and HepG2/RC cells were serum-deprived for 48 h.

**In Vitro Cross-linking with Glutaraldehyde**—Huh7 cells transfected with pCDEF or pCDEF/RC were isolated 44 h after transfection and lysed in Tris-buffered saline containing 0.5% Nonidet P-40 and 1 mM phenylmethylsulfonyl fluoride. Cell lysates were electrophoresed on a 12.5% SDS-polyacrylamide gel and Western-blotted using the rabbit anti-core primary antibody and the horse-radish peroxidase-conjugated goat anti-rabbit secondary antibody. The signal was developed using the SuperSignal chemiluminescence kit. The tTG activity was measured by analyzing the incorporation of [1,4-14C]pulchresine dihydrochloride into proteins (31–33). Cells were treated with CaCl2 or serum-deprived and then lysed in Tris-buffered saline containing 0.5% Nonidet P-40 and 1 mM phenylmethylsulfonyl fluoride. After a brief centrifugation to remove the cell debris, a 20% (v/v) concentration in the supernatant was measured with the Bio-Rad protein kit. The tTG reaction was conducted at 37 °C for 1 h in a 150-μl reaction mixture containing 100 μg of cellular proteins, 150 mM Tris-HCl (pH 8.0), 90 mM NaCl, 10 mM dithiorthiole, 5 mM CaCl2, 2.5 mM MgCl2, N,N′-dimethylacrylamide, and 0.5 μCi of [1,4-14C]pulchresine. After the reaction, proteins were precipitated with 20% trichloroacetic acid and placed on ice for 15 min. The precipitates were collected by centrifugation in a microcentrifuge. The pellet was then successively washed with 10 and 5% trichloroacetic acid and resuspended in 1 N NaOH for liquid scintillation counting.

**Expression and Purification of the HCV Core Protein**—The expression of the HCV core protein was conducted as previously described (18). Briefly, the BL21(DE3) strain of Escherichia coli was transformed with pET-RC172. Cell colonies were then inoculated into 500 ml of LB medium, grown to an A600 of 0.6, and treated with 1 ml isopropyl-β-D-thiogalactopyranoside for 2 h for the induction of core protein expression. The cells were pelleted by centrifugation and subsequently lysed by sonication on ice in 10 ml of phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulfonyl fluoride. The cell lysates were then centrifuged in a Sorvall centrifuge to pellet the cell debris. The supernatants were subjected to a 15–60% sucrose gradient in PBS and centrifuged for 16 h at 26,000 rpm using a Beckman SW 28 rotor. The protein pellet was resuspended in PBS and purified on a 12.5% SDS-polyacrylamide gel. After electrophoresis in PBS containing 0.1% SDS, the protein was dialyzed against 100 mM NaH2PO4, lyophilized, resuspended in water, and stored in small aliquots in the freezer until used. The purity and identity of the core protein were verified by Coomassie Blue staining and Western blotting. The core protein truncated at amino acid 115 was also expressed in E. coli and subsequently purified. This protein was a gift of Dr. Gwo-Targ Shue (University of Southern California).

**Preparation of the Radiolabeled DNA Probe and Gel-shift Assay**—An RNA probe containing the entire coding sequence of the core protein plus 94 nucleotides of the 5′-noncoding region (nucleotides 247–913) was synthesized in vitro using Xbal-linearized pCMV-CC as the template. DNA Transfection and Western Blot Analysis—Huh7 cells transfected with pCDEF or pCDEF/HA-RC were incubated in DMEM plus 10% FBS containing 400 μg/ml G418 for 2–3 weeks. The cell colonies were then pooled together for the establishment of Huh7/pCDEF and Huh7/HA-RC stable cell lines. Huh7/pCDEF and Huh7/HA-RC cell lines were derived from HepG2 cells, a well differentiated human hepatoblastoma cell line, and selected by the same procedures. HepG2/pCDEF cells contained the control pCDEF vector, and HepG2/RC cells expressed the core protein.

**In Vitro Cross-linking with Glutaraldehyde**—Huh7 cells transfected with pCDEF or pCDEF/RC were isolated 44 h after transfection and lysed in Tris-buffered saline containing 0.5% Nonidet P-40 and 1 mM phenylmethylsulfonyl fluoride. Cell lysates were electrophoresed on a 12.5% SDS-polyacrylamide gel and Western-blotted using the rabbit anti-core primary antibody and the horse-radish peroxidase-conjugated goat anti-rabbit secondary antibody. The signal was developed using the SuperSignal chemiluminescence kit.

**RESULTS**

**Induction of HCV Core Protein Dimerization by Calphostin C**—In our previous studies on the expression of the HCV core protein in insect and mammalian cells, we frequently observed that a protein, approximately twice the size of the core protein, could cross-react with the anti-HCV core protein antibody in the Western blot analysis. During our recent studies on the phosphorylation of the HCV core protein, we found that the production of this protein species in Huh7 cells, a well differentiated human hepatoma cell line, could be enhanced by calphostin C, a protein kinase C inhibitor. An example of such studies is shown in Fig. 1A. In this study, Huh7 cells were transfected with either the control pCDEF vector or pCDEF/RC, a plasmid that expresses the HCV core protein of the RH strain. Two days after transfection, cells were treated with different concentrations of calphostin C for 1 h and then lysed for Western blot analysis using the anti-core antibody. As shown in Fig. 1A, a protein band with a relative molecular mass of ~38 kDa was detected. The intensity of this protein band increased with increased concentrations of calphostin C.
This protein band could also be detected in cells not treated with calphostin C, although a longer exposure of the film was required to reveal the signal (Fig. 1B).

This 38-kDa protein, which was approximately twice the size of the core protein and reacted with the anti-core antibody, was likely the core protein dimer. This possibility was supported by three lines of evidence. First, this protein had an electrophoretic mobility similar to that of the core protein dimer (lanes 3–6). 44 h after transfection, cells were treated with Me2SO (DMSO; lanes 2 and 3) or 0.1 µM (lane 4), 0.5 µM (lane 5), or 1.0 µM (lanes 2 and 6) CalC for 1 h. MeSO was used to prepare CalC solutions. Cells were placed under light during the first 5 min of treatment for the activation of CalC. After the treatment, cells were lysed, and the cell lysates were Western-blotted with the anti-core antibody. A, an experiment similar to the one shown in A was repeated. The film was exposed for a longer period of time to reveal the putative core protein dimer band in cells not treated with CalC. Lane 1, cells transfected with the control vector pCDEF; lane 2, cells transfected with pCDEF/RC, followed by treatment with Me2SO; lane 3, cells transfected with pCDEF/RC, followed by treatment with 1.0 µM CalC for 1 h. The arrow denotes the location of the putative core protein dimer. The locations of the molecular mass markers are also indicated. K, kDa.

The 38-kDa core protein dimer was highly stable and not generated by disulfide bonds, as the cell lysates had been boiled in Laemmli buffer that contained 1% SDS and β-mercaptoethanol prior to gel electrophoresis and Western blotting. The production of this stable core protein dimer was independent of the downstream E1 envelope protein sequence. As shown in Fig. 3, the production of this core protein dimer could still be stimulated by calphostin C when the core protein was expressed in the presence of its downstream E1 envelope protein sequence. The expression of the E1 protein was verified by the radioimmunoprecipitation assay.2

Protein kinase C-independent Dimerization of the Core Protein—Calphostin C is a protein kinase C inhibitor (41).2 Thus, the formation of the stable core protein dimer might be due to the suppression of the protein kinase C activity. To investigate this possibility, Huh7 cells transfected with the core protein-expressing plasmid were treated with calphostin C or two other agents.

FIG. 1. Detection of a putative HCV core protein dimer. A, Huh7 cells were transfected with either the control vector pCDEF (lanes 1 and 2) or the core protein-expressing plasmid pCDEF/RC (lanes 3–6). 44 h after transfection, cells were treated with Me2SO (DMSO; lanes 1 and 3) or 0.1 µM (lane 4), 0.5 µM (lane 5), or 1.0 µM (lanes 2 and 6) CalC for 1 h. Me2SO was used to prepare CalC solutions. Cells were placed under light during the first 5 min of treatment for the activation of CalC. After the treatment, cells were lysed, and the cell lysates were Western-blotted with the anti-core antibody. B, an experiment similar to the one shown in A was repeated. The film was exposed for a longer period of time to reveal the putative core protein dimer band in cells not treated with CalC. Lane 1, cells transfected with the control vector pCDEF; lane 2, cells transfected with pCDEF/RC, followed by treatment with Me2SO; lane 3, cells transfected with pCDEF/RC, followed by treatment with 1.0 µM CalC for 1 h. The arrow denotes the location of the putative core protein dimer. The locations of the molecular mass markers are also indicated. K, kDa.

FIG. 2. Characterization of the putative core protein dimer. A, glutaraldehyde cross-linking experiment. Huh7 cells were transfected with pCDEF/RC (lanes 1–5) or pCDEF (lanes 6 and 7) and lysed for Western blotting as described in the legend to Fig. 1. Lane 1, cell lysates treated with 1.0 µM calphostin C for 1 h; lanes 2–5, cell lysates (without calphostin C treatment) treated with 0.01% glutaraldehyde (Glut) for 0, 1, 5, and 10 min, respectively, prior to gel electrophoresis and Western blotting; lanes 6 and 7, cell lysates treated with glutaraldehyde for 0 and 10 min, respectively. The arrow marks the location of the core protein dimer. B, expression of the core protein and the HA-tagged core protein in Huh7 cells. Lanes 1, 3, 5, and 7, Huh7 cells treated with Me2SO (DMSO); lanes 2, 4, 6, and 8, Huh7 cells treated with 1 µM CalC. Lanes 1 and 2, cells transfected with pCDEF; lanes 3 and 4, cells transfected with pCDEF/RC; lanes 5 and 6, cells cotransfected with an equal amount of pCDEF/RC and pCDEF/HA-RC; lanes 7 and 8, cells transfected with pCDEF/HA-RC. The arrows from bottom to top mark the locations of the putative core protein homodimer, the core protein and the HA-core protein heterodimer, and the HA-core protein homodimer, respectively. K, kDa.

FIG. 3. E1-independent production of the stable HCV core protein dimer. Huh7 cells were transfected with the control pCDEF vector (lane 1) or pCDEF/HA-RCEβ (lanes 2 and 3). Two days after transfection, cells were treated with Me2SO (DMSO; lanes 1 and 2) or 1.0 µM calphostin C (lane 3) for 1 h. The production of the stable core protein dimer could still be enhanced by calphostin C in the presence of its downstream E1 protein sequence. K, kDa.
protein kinase C inhibitors, GF 109203X and Ro 31-8220. As shown in Fig. 4, although calphostin C was able to induce the formation of the stable core protein dimer, neither GF 109203X nor Ro 31-8220, which could suppress the protein kinase C activity in Huh7 cells under this treatment condition in our previous studies, was able to do so. This result indicated that the induction of the core protein dimer was not likely due to the suppression of the protein kinase C activity in cells.

Post-translational Modification of the Core Protein by tTG—In addition to suppressing the protein kinase C activity, calphostin C can also stimulate the activity of tTG (34, 35). tTG belongs to a family of enzymes that catalyze the formation of the \(\gamma\)-glutamyl-\(\epsilon\)-lysine bond by linking the \(\gamma\)-carboxamide group of a peptide-bound glutamine residue to the amino group of a peptide-bound lysine (27, 28). Since this linkage is highly stable and resistant to denaturation and reduction (27, 28), the stable core protein dimer detected might be due to the cross-linking by tTG. As a first step to investigate whether tTG is indeed responsible for the dimerization of the stable core protein dimer, we examined whether calphostin C can activate tTG in Huh7 cells. Huh7 cells were stably transfected with the HCV core protein-expressing plasmid pCDEF/HA-RC or its control vector. The expression of the core protein in cells transfected with pCDEF/HA-RC was verified by Western blotting (see below). These stable cells were treated with calphostin C for various lengths of time and then lysed and assayed for the tTG activity. As shown in Fig. 5, there was a time-dependent increase in the tTG activity by calphostin C. This increase was independent of core protein expression. Next, we investigated whether tTG is responsible for the dimerization of the core protein by treating the cells with the tTG inhibitor MDC (34, 35). As shown in Fig. 6 (lanes 1 and 2), treating the cells with MDC in the absence of calphostin C reduced the basal core protein dimer signal on the gel to an undetectable level. In agreement with the results shown in the previous figures, treating the cells with calphostin C resulted in a significant increase in the core protein dimer signal (lanes 3 and 4). This signal was reduced when cells treated with calphostin C were also treated with MDC (lanes 5 and 6). There was an inverse relationship between the amount of MDC used and the amount of core protein dimer on the gel. Thus, the results shown in Fig. 6 support a role of tTG in the generation of the stable core protein dimer.

To further investigate whether tTG can indeed post-translationally modify the core protein, we performed the \textit{in vitro} cross-linking experiment. The HCV core protein truncated at either amino acid 115 or 172 was expressed in \textit{E. coli} and subsequently purified. The full-length core protein, which contained a hydrophobic carboxyl-terminal tail, was not used for these studies due to the difficulty of its expression in \textit{E. coli}.

These two truncated core proteins were treated with BSA or tTG and then analyzed by Western blotting using the anti-core antibody. As shown in Fig. 7A, treatment of either of the truncated core proteins with tTG resulted in the conversion of the monomeric form to a stable high molecular mass species, which was presumably the multimeric form. This post-translation modification of the core protein by tTG was specific, as the HBV core protein could not be multimerized by tTG in a similar experiment (Fig. 7B).

The efficient formation of the multimeric core protein \textit{in vitro} by tTG was likely because the truncated core proteins purified from \textit{E. coli} form capsid-like particles (24). Indeed, in our experiments, we found that the HCV core protein expressed in \textit{E. coli} sedimented readily into sucrose gradients, suggesting the formation of high molecular mass complexes. This multimeric core protein was apparently also produced in Huh7 cells expressing the core protein. As shown in Fig. 8, with a longer exposure of the film, the anti-core antibody could detect the stable core protein dimer and a protein band larger than the 185-kDa protein marker in Huh7 cells. Note that, in agreement with the \textit{in vitro} experiments shown in Fig. 7B, the HBV core protein expressed in Huh7 cells could not form dimers or multimers on the gel whether the cells were treated with calphostin C.
phostin C or not (data not shown).

**Activation of tTG by Serum Deprivation**—tTG can be activated by a variety of external stimuli, including serum deprivation and retinoic acid (34, 42). Thus, if the production of the stable core protein dimer were indeed due to tTG, then this production should also be enhanced by these stimuli in addition to calphostin C. For this reason, we tested whether serum deprivation would similarly enhance the production of the stable core protein dimer. As the first step, we investigated whether serum deprivation would result in the activation of the tTG activity.

**Reduction of the RNA Binding Activity of the Core Protein by tTG**—The HCV core protein is a structural protein that packs the viral RNA. To investigate the possible effect of tTG on its RNA binding activity, we performed the gel-shift experiment using an HCV RNA probe containing the entire core protein coding sequence and part of the 5′-noncoding sequence. This RNA fragment has been used for core protein binding assays in previous studies (39, 43). As shown in Fig. 9, incubation of the RNA probe with BSA did not generate any shifted band. However, incubation of the RNA probe with the core protein truncated at amino acid 115 resulted in retention of the RNA probe in the well of the gel (lane 3). This result was consistent with previous reports indicating that the truncated core protein can bind to RNA to form capsid-like particles and to retain the RNA probe in the well (24, 39). Incubation of the truncated core protein with tTG at room temperature for 20 min prior to the RNA binding reaction suppressed the RNA binding activity of the core protein (lane 4). This suppression was even more prominent if the core protein was treated with tTG at 37 °C for 1 h (lane 5).

**Fig. 7. Cross-linking of HCV and HBV core proteins by tTG in vitro.** A, cross-linking studies with the HCV core protein. The HCV core protein was expressed in E. coli and purified as described under “Experimental Procedures.” The core protein was then cross-linked with tTG at 37 °C for 1 h. Lanes 1 and 2, the control lysates prepared from E. coli transformed by the pET3a vector; lanes 3 and 4, the core protein truncated at amino acid 115 and 172 (core115 and core172) are also indicated. B, cross-linking studies with the HBV core protein. 0.7 μg of purified HBV core protein was treated with 2 μg of tTG (lane 1) or BSA (lane 2) for 1 h at 37 °C. The location of the HBV core protein is marked. K, kDa.

**Fig. 8. Detection of the stable HCV core protein multimer in Huh7 cells treated with calphostin C.** Huh7 cells transfected with the control vector (lanes 1 and 2) or with pCDEF/HA-RC (lanes 3 and 4), which expresses the HA-tagged core protein, were treated with calphostin C and lysed for Western blotting using the anti-core antibody. Lanes 1 and 3, cells treated with Me2SO (DMSO); lanes 2 and 4, cells treated with calphostin C. The locations of the core protein dimer and multimer are marked by the arrowhead and arrow, respectively. The locations of the protein markers are also indicated. Huh7 cells transfected with pCDEF/RC, which expresses the core protein without the HA tag, generated similar results (data not shown). K, kDa.

**Fig. 9. Activation of the tTG activity by serum deprivation.** Stable Huh7 cells (Huh7/pCDEF and Huh7/HA-RC) were incubated in medium with or without serum for 30 h. Cells were then lysed and analyzed for the tTG activity as described under “Experimental Procedures.” The tTG activity of Huh7/pCDEF cells with FBS was arbitrarily defined as 1. Huh7/pCDEF and Huh7/HA-RC were treated with the indicated reagents, and the tTG activity was assessed by Western blotting with the anti-tTG antibody. Relative tTG activity was determined by densitometric analysis of the Western blots.

stabilizes the viral RNA. To investigate the possible effect of tTG on its RNA binding activity, we performed the gel-shift experiment using an HCV RNA probe containing the entire core protein coding sequence and part of the 5′-noncoding sequence. This RNA fragment has been used for core protein binding assays in previous studies (39, 43). As shown in Fig. 11, incubation of the RNA probe with BSA did not generate any shifted band. However, incubation of the RNA probe with the core protein truncated at amino acid 115 resulted in retention of the RNA probe in the well of the gel (lane 3). This result was consistent with previous reports indicating that the truncated core protein can bind to RNA to form capsid-like particles and to retain the RNA probe in the well (24, 39). Incubation of the truncated core protein with tTG at room temperature for 20 min prior to the RNA binding reaction suppressed the RNA binding activity of the core protein (lane 4). This suppression was even more prominent if the core protein was treated with tTG at 37 °C for 1 h (lane 5). Similar results were obtained with the core protein truncated at amino acid 172. As shown in Fig. 11, the binding of the RNA probe by this protein retarded the mobility of the probe without trapping the probe in the well.
The HCV core protein is a structural protein that packages the viral genomic RNA (10, 13–16). Previous research indicated that this protein can dimerize (13, 23–25). This protein dimer was thought to serve as the fundamental building block for the formation of the viral nucleocapsid. In this study, we demonstrated that a small fraction of the core protein dimer was highly stable and resistant to denaturation and reduction by SDS and β-mercaptoethanol (Fig. 1B). The production of this stable core protein dimer could be significantly enhanced by treating the cells with calphostin C (Figs. 1–3) or serum deprivation (Fig. 10), which both stimulated the tTG activity in Huh7 cells (Figs. 5 and 9). The possible role of tTG in the production of the stable core protein dimer was further supported by two lines of evidence. First, the tTG inhibitor MDC could suppress the production of this stable core protein dimer (Fig. 6); and second, tTG could post-translationally modify the core protein to generate multimers in vitro (Fig. 7). The production of the stable core protein dimer was independent of the downstream E1 envelope protein sequence (Fig. 3). It was also specific, as the HBV core protein, a protein with a similar size and structural function, was unable to form this type of stable dimer or multimers in similar experiments (Fig. 7B).

tTG is a calcium-dependent enzyme that catalyzes the formation of the γ-glutamyl-ε-lysine bond (27, 28, 44). It is primarily an intracellular enzyme, but, in some cases, may be released into the extracellular space. tTG substrates include midkine, actin, annexin, troponin, histone H2B, and retinoblastoma protein. Some of these substrates are involved in cell cycle regulation, extracellular matrix remodeling, cellular differentiation, and apoptosis (28, 34, 35, 44, 45). For example, tTG has been shown to stabilize the dimer of midkine, a growth/differentiation factor, and to enhance its activity (44). In primary hepatocytes, tTG can negatively regulate the growth signal stimulated by epidermal growth factor (46). To our knowledge, this is the first report that describes the post-translational modification of a viral structural protein by tTG. The molecular details that mediate this post-translational modification are unclear. Since the core protein truncated at amino acid 115 could still be multimerized by tTG, the amino acid residues involved are likely located at the amino terminus of the core protein sequence. Indeed, an examination of the core protein sequence of the HCV RH strain revealed six glutamine residues that are all located upstream of codon 80. Similarly, all lysine residues but one are located upstream of codon 70. Thus, it is likely that the cross-linking by tTG involves only amino acids located at the amino terminus of the core protein sequence.

The finding that the core protein could be modified by tTG raises the possibility that the activities of the core protein may be regulated by tTG. This modification may be important for the maturation of the HCV capsid particle. Alternatively, since the RNA binding activity of the core protein could be suppressed by tTG in vitro (Fig. 11), tTG may play a role in the uncoating and release of the viral genome from the capsid during the early stage of infection. It is equally likely that tTG may represent a host response to suppress HCV replication. For example, since tTG can be activated by external stimuli (Figs. 5 and 9), it is possible that the host response such as the release of cytokines or oxygen radicals may stimulate tTG to modify the HCV core protein to suppress viral RNA packaging and maturation. In addition to its structural functions, the HCV core protein has also been shown to possess regulatory functions that can regulate cellular signaling pathways, gene expression, cell growth, and apoptosis (22). Thus, it is also conceivable that some of these activities may also be mediated by the stable core protein dimer generated by tTG. Further research, which will have to await the development of a cell culture system for HCV propagation, will be required to resolve these possibilities.

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