THE INHIBITION OF PROTEIN KINASE C BY HEPATITIS C VIRUS NON-STRUCTURAL PROTEIN 3 DEPENDS ON ITS CONFORMATIONAL STATUS

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A broad range of biological and biochemical studies document that disturbing protein kinase C (PKC) function induces tumor promotion and is a cause of carcinogenesis. We have previously demonstrated that nonstructural protein 3 (NS3) of hepatitis C Virus (HCV) interacts directly with PKC. The interaction results in reduction of the catalytic activity of the PKC and inhibition of its shuttling between cell compartments. The inhibition is mainly mediated by a short arginine-rich amino acid stretch of NS3 (motif VI) that strongly resembles the autoregulatory domain of PKC.

According to crystallographic studies, the exposition of this NS3 domain to solvent is modified during the hydrolysis of NTP and/or the unwinding reaction. This may change the accessibility of this domain for PKC and, in consequence, change the inhibitory potential of NS3 towards PKC.

To test this possibility, we employed N7-chloroethyl guanine, a compound that stimulates the unwinding activity of the NTPase/helicase by an allosteric mechanism. Binding studies demonstrated that N7-chloroethyl guanine significantly enhances the affinity of the NTPase/helicase for an immobilized synthetic peptide with an amino acid sequence corresponding to the arginine-rich motif. Thus, the compound supports the “open” status of the enzyme and enhances the accessibility of the motif VI for PKC. Results of in vitro inhibition studies are in good agreement with this observation. The enzymic activity of the protein kinase (purified from rat brain - mixture of isoforms) was more effectively inhibited by HCV NTPase/helicase and by the entire NS3 protein in the presence N7-chloroethyl guanine. Interestingly, the results could not be reproduced with smaller fragments of the NTPase/helicase. The inhibitory potential of polypeptides corresponding to domains 1 and 2 or domain 2 of the enzyme could not be enhanced in the presence of N7-chloroethyl guanine. This strongly suggests that the binding site of N7-chloroethyl guanine is localized within domain 3 or in the clefts between domains 1 and 3, or 2 and 3. On the other hand it appears that the compound requires intact domain 3 for its PKC inhibitory and helicase activating effect.

The mechanism of action of the compound remains unclear. The mentioned 3D structure implies however, that the arginine-rich motif could be masked, at least partly, by domain 1. Indeed a polypeptide corresponding to domain 2 of the NTPase/helicase inhibits PKC more effectively than a polypeptide consisting of domains 1 and 2. The same crystallographic studies revealed a loop, within
domain 2, that might mask motif VI and therefore reduce the inhibitory potential of HCV NS3 protein towards PKC. To verify this option, a deleted version of domain 2 was constructed in which the loop was omitted and the polypeptides (wild type domain 2 and its deleted version) were compared for their inhibitory potential towards PKC. In vitro phosphorylation studies demonstrated unambiguously that the loop, similar to domain 1, reduces the extent of inhibition of PKC activity by domain 2, probably due to masking of motif VI. It remains to be clarified by which mechanism (domain 1, or loop-dependent) N7 chloroethyl guanine modulates the NS3 conformation. Summing up, it appears clear that inhibition of PKC by NS3 is dependent on the conformational status of the viral protein.