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MiniReview

Interaction of viral proteins with metal ions: role in maintaining the structure and functions of viruses

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Abstract

Metal ions are integral part of some viral proteins and play an important role in their survival and pathogenesis. Zinc, magnesium and copper are the commonest metal ion that binds with viral proteins. Metal ions participate in maturation of genomic RNA, activation and catalytic mechanisms, reverse transcription, initial integration process and protection of newly synthesized DNA, inhibition of proton translocation (M2 protein), minus- and plus-strand transfer, enhance nucleic acid annealing, activation of transcription, integration of viral DNA into specific sites and act as a chaperone of nucleic acid. Metal ions are also required for nucleocapsid protein-transactivation response (TAR)–RNA interactions. In certain situations more than one metal ion is required e.g. RNA cleavage by RNase H. This review underscores the importance of metal ions in the survival and pathogenesis of a large group of viruses and studies on structural basis for metal binding should prove useful in the early design and development of viral inhibitors.

Keywords: Viral protein; Virus; Nucleoprotein; Metal; Metalloprotein; Zinc

1. Introduction

Among the many roles of metal ions in biological processes are bridging distant residues or domains of proteins, mediating interactions between proteins and ligands, and serving in the active site as a nucleophilic catalyst and in transfer of electron. Biological processes are often metal ion specific, although more than one metal ion can play each of these roles. For example the coagulation cascade is Ca^{2+} specific, protein biosynthesis is primarily Mg^{2+} specific, several enzymes are Zn^{2+} ion specific and oxidative processes are often iron specific. The conformational changes induced by binding a metal ion are remarkable. Residue side chains, which are greater than 20 Å apart in the metal ion-free structure, may become constituents of the metal ion-binding site [1,2]. A number of trace metals are essential micronutrients and their deficiency and infectious diseases often coexist and exhibit complex interactions. Several trace metals such as zinc, copper and manganese, etc. influence the susceptibility to, the course and the outcome of a variety of viral infections. Deficiency of trace metals is known to alter the genome of the viruses and the grave consequences of this may be the emergence of new infections [3]. On the other hand some metals like hexavalent chromium may have toxic effects [4,5]. Metals are integral part of several viruses and are known to play an important role in their survival and pathogenesis. This paper briefly reviews the consequences of the interactions of various metal ions with proteins of different viruses. Due to constraint of space only a limited number of studies have been cited.

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1.1. Composition of virion

Viruses are the smallest infectious agents containing only one type of nucleic acid (DNA or RNA) covered by a protein coat, which may be surrounded by a lipid-containing membrane. A virion is composed of viral proteins, nucleic acid, lipids and carbohydrates. The structural proteins of viruses protect the viral genome, participate in attachment of virus to a susceptible cell, facilitate transfer of viral nucleic acid from one host cell to another and are antigenic determinants of the virus. Viral envelopes contain lipids and glycoproteins (gp), the lipids are derived from the host cell while the glycoproteins are virus-encoded. Surface glycoproteins attach the virus to a target cell and are important viral antigens. Viral proteins coded by nonstructural genes are known as nonstructural proteins (NS). Present in the virus-infected cells, these proteins appear to have regulatory roles during replication. To give a few examples, the hepatitis C virus (HCV) genes code for NS2, NS3, NS4A, NS4B, NS5A, and NS5B nonstructural proteins. The mature structural proteins are C, E1 and E2 [6].

1.2. Protein–metal interactions

An interaction between a protein and a metal ion is of different types. Metalloprotein is a generic term for a protein that also contains a metal cofactor. One-third of all proteins are ''metalloproteins''. The metal ions in metalloproteins are critical to the protein's function, structure, or stability and numerous essential biological functions [7]. Understanding and ultimately controlling the binding and activity of protein metal sites are of great biological and medical importance. The functions of metalloproteins having metals that bind with different viral proteins are presented in Table 1.

Three proteins have been identified in mammals, GLABROUS1 (GLI), GLI2, and GLI3, having a highly conserved zinc finger (ZF) domain and function as transcription factors in the vertebrate sonic hedgehog-patched signaling pathway [7]. During evolution some proteins have chosen Mg^{2+} as a natural cofactor. Mg-binding sites appear to be weak and can be replaced by other divalent metals like Zn^{2+}, and in some cases, inhibit enzymatic activity thereby. Therefore, it seems that the cell machinery governs the process of metal binding by regulating appropriate concentrations of Mg^{2+} and Zn^{2+}, etc. in various biological compartments. Zn^{2+} has a higher affinity for a protein ligand and strongly prefers a tetrahedral geometry. Consequently, rigid Zn^{2+}-binding sites appear to be more selective than Mg^{2+}-binding sites, and a protein can generally select Zn^{2+} against the background of a much higher Mg^{2+} concentration [8]. The commonest metal to bind with a virus-protein is zinc. Zinc is the second most abundant trace metal found in eukaryotic organisms, second only to iron. Zinc is required for essential catalytic functions in more than 300 enzymes, stabilization and induction of the folding of protein subdomains. The latter functions include the essential role of zinc in the folding of the DNA-binding domains of eukaryotic transcription factors, including the ZF transcription factors [9]. ZF are small protein domains in which zinc plays a structural role contributing to the stability of the domain and are small DNA-binding peptide motifs. The cysteine-rich zinc-binding motifs known as the RING and B-box are found in several unrelated proteins. ZF are structurally diverse and are present among proteins that perform a broad range of functions in various cellular processes, such as replication and repair, transcription and translation, metabolism and signaling, cell proliferation and apoptosis. ZF typically function as interaction modules and bind to a wide variety of compounds, such as nucleic acids, proteins and small molecules. Three of these fold groups comprise the majority of zinc fingers, namely, C2H2-like finger, treble clef finger and the zinc ribbon [10].

1.3. Binding of metal ions with different virion proteins

Bindings of metal ions with viral proteins and its consequences have been investigated with a number of the

Table 1
Functions of metalloproteins

Metals	Enzyme and protein	
	Classes	Example
Zinc	Transferases, hydrolases, lyases, isomerases, ligases, oxidoreductases, transcription factor	RNA polymerases, alcohol dehydrogenases, glucocorticoid receptor
Copper	Oxidoreductases	Superoxide dismutase, ferroxidase (ceruloplasmin)
Iron	Oxidoreductases	Cytochrome oxodase
Cobalt	Transferases	Haemocysteine methyl-transferases
Manganisium	Oxidoreductases, methyltransferase,	Superoxide dismutase, protoporphyrin
Selenium[a]	Oxidoreductases, transferases	Glutathione peroxidase
Nickel	Oxidoreductases, hydrolases	Urease

Modified from Chaturvedi et al. [3].
[a] It is not a true metal.

groups of viruses. The structurally unusual heterotricyclic compound named bananin (BN) has several roles including Zn^{2+} chelator, and a target of interest is HIV-1 ZF, HIV-1 RNA-binding NC7. In addition, the targets of BN could be adsorption, transcription and/or viral RNA replication of a interestingly wide range RNA viruses reviewed in [11]. The findings summarized in Table 2 show that various viral proteins can bind different metal ions.

1.4. Human immunodeficiency virus and other retroviruses

The replication process of human immunodeficiency virus (HIV) requires a number of nucleic acid annealing steps facilitated by the hybridization and helix-destabilizing activities of HIV nucleocapsid (NC) protein. The NCp of HIV-1 has two ZF, each containing the invariant metal ion binding residues CCHC (Figs. 1 and 2). Guo et al. [12] reported that mutations in the CCHC motifs are deleterious for reverse transcription in vivo.

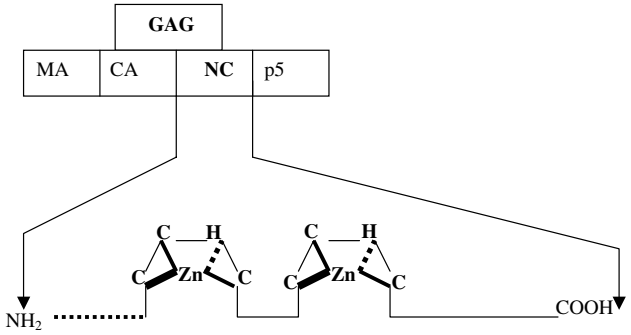

Fig. 1. Diagramatic presentation of two zinc fingers of a well-conserved $CX_2CX_4HX_4C$ on the HIV-1 nucleocapsid protein 7. (inspired from Berthoux et al. [39]).

Table 2
Proteins of various groups of viruses that bind different metal ions

Virus	Metal	Site	Domain	Activity	References
HIV-1	Zn	NCP	2 ZF CCHC	Reverse transcription RNA Chaperone	[12] [13,14]
		TAR–RNA	NC	RNA Chaperone	[15]
		ZAS:C2H2 ZF	–	Growth and development	[16]
	Mg, Co	RNase H	–	RNA cleavage	[17]
	Mg, Ca	TAR–RNA	Bulge	Cleavage	
Hepatitis C Virus	Zn	NS3	C97, C99, C145, H149 N terminus	Structural stability, folding of NS3 serine protease	[18]
Hepatitis B Virus	Cu	Envelope protein	–	Argine NH_2 donor	[19]
Herpes Simplex Virus	Zn Zn Zn	ICPO, Vhs	Ring finger	Blocks IFN regulatory factor	[20]
	Zn	GL1	Protein activation domain	Transcription regulation	[7]
	Zn	E3 ubiquitin ligase			
Bovine Herpes Virus-1	Zn	ICPO	N terminus(C3HC4)	Aggregation of chromatin stimulating infection transactivating thymidine kinase	[21]
Pox-virus	Zn, Cu	protein	125-amino acid protein	Catalytic activity, and like Cu–Zn SOD	[22]
Rubella	Zn, Cd, Co	NS protease	N-terminus	Catalytic activity	[23]
Influenza	Cu, Ni, Pt, Zn	M2 protein	Transmembrane	Reversible inhibition of membrane current	[24]
	Zn, Mg, Mn, Co	M1 peptide linker	N and C terminal	Unfolding–refolding transition, virus uncoating, RNA endonuclease activity	[25]
	Zn	Peptide CCHH motif	–	Transcription inhibition	[26]
Corona virus	Zn	NS	Papain like fold	Papain like proteinase	[27,28]
		p66 HEL	Superfamily 1 helicase N terminus	Helicase	[29]
		NS13		Helicase	[30]
Human Papilloma Virus	Zn, Cd, Cu	E7	–	Destablizes agglomerates	[31,32]
Ebola virus	Zn	VP30	Cys(3)-His motif	Activation of transcription	[33]
Picorna and rhinovirus	Zn	Protein2C (ATPase)	C terminus	Structural role	[34]
Rota virus	Zn	VP6	3 fold molecular axis	–	[35]
		NS1	N and C terminus	IFN regulatory factor	[36]
		RoXan	C terminus	Translation regulation	[37]

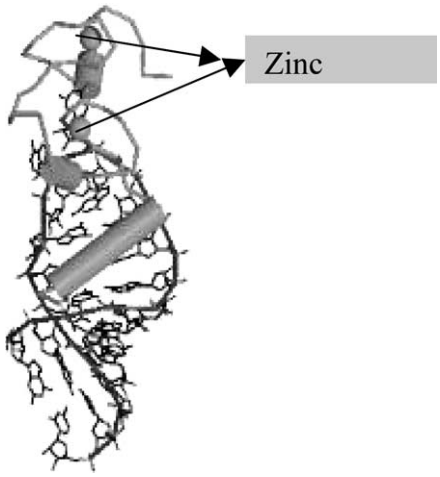

Fig. 2. Diagramatic presentation of binding sites of zinc on the nucleocapsid protein of HIV-1 (inspired from De Guzman et al. [40]).

ZF contribute to the role of NC in complete tRNA primer removal from minus-strand DNA during plus-strand transfer. Annealing of the primer binding site sequences in plus-strand strong-stop DNA to its complement in minus-strand acceptor DNA is not dependent on NC ZF. NC ZF are needed to unfold highly structured RNA and DNA strand transfer intermediates [12]. Thus, it appears that in these cases, ZF interactions are important components of NC nucleic acid chaperone activity. Heath et al. [38] suggested that ZF 1 has a greater role in unfolding of strong secondary structures, whereas ZF 2 serves an accessory role that leads to a further increase in the rate of annealing.

HIV-1 NCp consists of two basic amino acid regions which exhibit some activity for RNA chaperone and the two ZF enhance the efficiency of this activity [41]. In the minus-strand transfer step of HIV-1 reverse transcription, the NC promotes annealing of the 3′ 'R' (repeat) region of the RNA genome to its complementary sequence located in the newly synthesized minus-strand strong-stop DNA. The R region contains the highly stable transactivation response (TAR)–RNA hairpin. NC variants with mutations in their ZF domains have dramatically altered TAR–RNA binding interactions relative to wild-type NC [14]. This indicates that a specific ZF architecture is required for optimal TAR RNA binding, and help to explain the requirement for the ZF motifs of NC in its role as a nucleic acid chaperone in minus-strand transfer.

The two highly conserved ZF motifs of the HIV-1 NCp7, strongly bind Zn^{2+} through coordination of one His and three Cys residues (Fig. 1). Bombarda et al. [42] investigated the Zn^{2+} binding and acid–base properties of four single-point mutants of a short peptide corresponding to the distal finger motif of NCp7. In each mutant, one Zn^{2+}-coordinating residue is substituted with a noncoordinating one. Using the spectroscopic properties of Co^{2+}, it was establish that the four mutants retain their ability to bind a metal cation. The mutations do not affect the acid–base properties of the Zn^{2+}-coordinating residues. The apparent Zn^{2+} binding constants of the four mutants are strongly reduced compared to those of the native peptide but are similar to those of various host Zn^{2+} binding proteins. As a consequence, the loss of viral infectivity following the mutation of one Zn^{2+}-coordinating residue in vivo may not be related to the total loss of Zn^{2+} binding. The pH dependence of Zn^{2+} binding indicates that the coordinating residues bind Zn^{2+} stepwise and that the free energy provided by the binding of a given residue may be modulated by the entropic contribution of the residues already bound to Zn^{2+} [42]. This implies that Cys49 may act as a switch for Zn^{2+} dissociation in the distal finger motif of NCp7, a feature that may contribute to the high susceptibility of Cys49 to electrophilic attack.

HIV RNase H activity is essential for the synthesis of viral DNA by HIV reverse transcriptase (HIV-RT). RNA cleavage by RNase H requires the presence of divalent metal ions. Polymerase-independent HIV RNase H is similar to, or more active with Mn^{2+} and Co^{2+} compared with Mg^{2+} [16]. Activation of RNase H by these metal ions suggests cooperative metal ion binding. These results are consistent with a two-metal ion mechanism of RNA cleavage. Specific ZF architecture is required for HIV-1 NC's nucleic acid chaperone function [43] while Wu [15] has reported that ZAS: C2H2 ZF proteins are involved in growth and development. Studies of Bergstrom et al. [44] have shown that polysulfonates derived from metal thiolate complexes as inhibitors of HIV-1 and various other enveloped viruses in vitro. HIV-1 nucleocapsid ZF are required for efficient reverse transcription, initial integration processes, and protection of newly synthesized viral DNA [13]. HIV-1 Tat protein directly activates neuronal *N*-methyl-D-aspartate receptors at a zinc-sensitive allosteric site [45]. McGrath et al. [46] have reported that human cellular nucleic acid-binding protein ZFv support replication of HIV-1 when they are substituted in the NC. Lee et al. [14] have described ZF-dependent HIV-1 NC–TAR–RNA interactions.

Olejniczak et al. [17] analyzed binding of Mg^{2+}, Ca^{2+} and Co^{2+} to the HIV-1 TAR–RNA in solution. The Mg^{2+} and Ca^{2+} ions indicate specific weak binding at the bulge region. In the lead (Pb^{2+})-induced TAR–RNA cleavage experiment, strong and selective cleavage of the C24-U25 phosphodiester bond is observed, while Mg^{2+} and Ca^{2+} induced cuts at all 3-nt residues of the bulge. The inhibitions of Pb^{2+}-specific TAR cleavage by di- and trivalent metal ions reveal a binding specificity at the bulge site. The bulge region is mostly targeted by magnesium cations.

NC from Mason-Pfizer monkey virus contains two evolutionary invariant retroviral-type ZF structures,

where the Cys and His residues provide ligands to a tetrahedrally coordinated Zn^{2+}. All Co^{2+}-substituted peptide complexes adopt tetrahedral ligand geometries and have S–>Co^{2+} ligand-to-metal charge-transfer transition intensities consistent with three Co^{2+}–S bonds for F1-SC and F1-CS [47]. ZF domains in retroviral NCp usually contain one histidine per metal ion coordination Cys complex. Visna virus NC8, has two additional histidines (in the second of its two ZFs) that could potentially bind metal ions. Absorption spectra of cobalt-bound ZF2 peptides are altered by Cys alkylation and mutation, but not by mutation of the extra histidines. Further, visna p8 ZFs involve three Cys and one His in the canonical spacing in metal ion coordination, and that the two additional histidines appear to interact with nucleic acid bases in p8-DNA complexes [48]. Glutathione peroxidase (GPx) is the prototypical eukaryotic selenoprotein, with the rare amino acid selenocysteine at the enzyme active site, encoded by the UGA codon in RNA. Selenium-dependent GPx modules are encoded in a number of RNA viruses, including potentially serious human pathogens like HIV-1, HIV-2, coxsackievirus B3 and measles virus [49]. Tan et al. [50] prepared proteins consisting of wild type or truncated HIV-1 integrase fused to the synthetic polydactyl ZF protein E2C. The purified fusion proteins bind specifically to the 18-bp E2C recognition sequence. Thus, the integrase-E2C fusion proteins offer an efficient approach and a versatile framework for directing the integration of retroviral DNA into a predetermined DNA site.

1.5. Hepatitis viruses

The NS3 region of the hepatitis C virus (HCV) encodes for a serine protease activity, which is necessary for the processing of the nonstructural region of the viral polyprotein. The minimal domain with proteolytic activity resides in the N terminus, where a structural tetradentate zinc-binding site is located. The zinc-binding site has a role in maintaining the structural stability and guiding the folding of the NS3 serine proteinase domain. The ligands have been identified by X-ray crystallography as three cysteines and one histidine residue, postulated to coordinate the metal through a water molecule. The structure of NS3 proteinase from HCV BK strain has a substrate-binding site consistent with the cleavage specificity of the enzyme. Novel features include a structural zinc-binding site and a long N-terminus that interacts with neighboring molecules by binding to a hydrophobic surface patch [51]. Evidence for rearrangements of the metal coordination geometry induced by complex formation with an NS4A peptide cofactor have been reported [18]. The HCV NS3 protein N-terminal domain has a zinc-binding site exposed on the surface [52,53]. The HCV enzyme contains Zn^{2+} with NS3 ligation and that the metal is required for structural integrity and activity of the enzyme. HCV polyprotein processing is activated by Zn^{2+} and, to a lesser degree, by Cd^{2+}, Pb^{2+}, and Co^{2+} and is inhibited by Cu^{2+} and Hg^{2+} ions [54]. Zn^{2+} is not directly involved in catalysis but may have a structural role [55]. It is suggested that the three cysteines, and the histidines coordinate the structural zinc in the HCV NS3 proteinase at a Zn^{2+}-binding site while dengue 2 virus NS3 protease, though lot of homology, does not contain a Zn^{2+}-binding site [56]. Zn^{2+}-dependent HCV NS3 inhibition is relatively insensitive to the structural variations but dependent on the presence of negatively charged functionality [57]. This can be interpreted in the context of an initial electrostatic interaction between protease and inhibitor that is subsequently consolidated by Zn^{2+}, with binding facilitated by the featureless active site and proximal regions of the HCV NS3 protein. NS5A gene product displays a multitude of activities related to enhancement of viral pathogenesis. A complex multimechanistic role of NS5A in promoting viral persistence, pathogenesis and, indirectly, viral-related hepatocarcinogenesis indicates its key role in HCV pathobiology [58]. Potentiometric and spectroscopic data have shown that a fragment of envelope proteins of the hepatitis B virus could be a very specific binding molecule for Cu^{2+} ions using arginine lateral NH2 donor sites. The presence of Pro and Asp residues makes Arg binding not only very specific, but also very efficient [19].

1.6. Herpes simplex virus

Lin et al. [20] have reported that the herpes simplex virus, HSV-1, infected cell proteins (ICP0) and virion host shutoff protein (vhs) function in concert to disable the host antiviral response. ICP0 also blocks IFN regulatory factor IRF3- and IRF7-mediated activation of IFN-stimulated genes and that the RING finger domain of ICP0 is essential for this activity. Furthermore, HSV-1 modifies the IRF3 pathway in a manner different from that of the small RNA viruses most commonly studied [20]. The GLI-induced transcriptional activation requires the carboxyl-terminal amino acids. The presence of this region in the GLI activation domain provides a mechanism for GLI-induced transcriptional regulation [7]. Proteasome-dependent degradation of ubiquitinated proteins plays a key role in many important cellular processes. One class of E3 ubiquitin ligases has been shown to contain a common zinc-binding RING finger motif. It has been shown that HSV1 ICP0, itself a RING finger protein, induces the proteasome-dependent degradation of several cellular proteins and induces the accumulation of colocalizing conjugated ubiquitin in vivo. Mutations within the RING finger region that abolish the in vitro ubiquitination activity also cause severe reductions in ICP0 activity in other assays [59]. It shows that ICP0 has the potential to act as an E3 ubiquitin ligase during

viral infection and to target specific cellular proteins for destruction by the 26S proteasome.

The bICP0 protein encoded by bovine herpesvirus 1 (BHV-1) activates transcription and consequently productive infection. bICP0 is believed to be functionally similar to the HSV1-encoded ICP0, the only protein domain that is well conserved is a C3HC4 ZF RING located near the N terminus of both proteins. Site-specific mutagenesis of the ZF RING of bICP0 demonstrates that it is important for inducing aggregated chromatin structures in transfected cells and toxicity. The ZF RING is also required for stimulating productive infection in bovine cells and for trans-activating the thymidine kinase (TK) promoter of HSV1 [21]. Taken together, these studies indicated that bICP0 has several functional domains, including the ZF RING, which stimulate productive infection and influence cell survival.

1.7. Poxviruses

Almazan et al. [22] have reported that the open reading frame of the A45R gene from vaccinia virus (VV) strain encodes a 125-amino-acid protein with 39% amino acid identity to copper–zinc superoxide dismutase (Cu–Zn SOD). Sequencing of the A45R gene from other orthopoxviruses shows that the protein is highly conserved in all viruses sequenced, including 16 strains of VV, 2 strains of cowpox virus, camelpox virus, and 4 strains of variola virus (reviewed in [22]). In all cases the protein lacks key residues involved in metal ion binding that are important for the catalytic activity. A45R is unusual that affects neither virus replication nor virulence. Molluscum contagiosum, a DNA virus, has been shown to encode a functional selenium-dependent GPx enzyme [49]. Many Chordopoxviruses encode catalytically inactive homologs of cellular Cu–Zn SOD. The biological function of these proteins is unknown, although the proteins encoded by Leporipoxviruses have been shown to promote a slow decline in the level of SOD activity in virus-infected cells. Teoh et al. [60] have reported that Shope fibroma virus SOD retains the zinc binding properties of its cellular homolog, but cannot bind copper. Further, recombinant Shope fibroma virus SOD forms very stable complexes with cellular copper chaperones for SOD. Similar viral SOD/chaperone complexes are formed in cells infected with a closely related myxoma virus. These poxviral SOD homologs do not form stable complexes with cellular Cu, Zn-SOD or affect its concentration [60].

1.8. Rubella virus

The rubella virus (RUB) NS protease is a papain-like cysteine protease (PCP) located in the NS-protein open reading frame (NSP-ORF) that cleaves the NSP-ORF translation product at a single site to produce two products, P150 (the N-terminal product) and P90 (the C-terminal product). The RUB NS protease does not function following translation in vitro in a standard rabbit reticulocyte lysate system, although all of the other viral PCPs do so. However, in the presence of divalent cations such as Zn^{2+}, Cd^{2+}, and Co^{2+}, the RUB NS protease function efficiently, indicating that these cations are required either as direct cofactors in catalytic activity or for correct acquisition of three-dimensional conformation of the protease. Further, trans cleavage is detected in vitro, indicating that the protease present in the mutated construct cleaves the catalytic-site mutant precursor and the RUB NS protease can function in trans [23]. But in vivo studies do not show such trans cleavage activity [61].

1.9. Influenza virus

The homotetrameric M2 membrane protein of influenza virus forms a proton-selective ion channel. Cu^{2+} strongly and reversibly inhibits membrane currents with biphasic reaction kinetics. Binding of Cu^{2+} to the high affinity site results in an approximately equal inhibition of both inward and outward currents. The wild-type protein has very high specificity for Cu^{2+} and is only partially inhibited by 1 mM nickel (Ni^{2+}), platinum (Pt^{2+}), and Zn^{2+} [24]. The M1 protein has a peptide linker (M1Lnk). The pH-dependent conformational transition of M1Lnk strongly suggests that the inter domain linker region of M1 also undergoes a pH-dependent unfolding–refolding transition in the presence of Zn^{2+}. A small but significant portion of the M1 protein is bound to Zn^{2+} in the virion. The Zn^{2+}-bound M1 molecule may play a special role in virus uncoating by changing the disposition of the N- and C-terminal domains upon acidification of the virion interior [25]. The influenza virus RNA-dependent RNA polymerase protein complex contains an associated RNA endonuclease activity, which is dependent on the presence of divalent metal ions. Virus-specific RNA cleavage by endonuclease is observed with various metal ions, and maximum cleavage activity is obtained with Mn^{2+} or Co^{2+} and is much less with Mg^{2+}. Synergistic activation of cleavage activity is observed with combinations of different metal ions at varying concentrations [25]. These results support a two-metal ion mechanism of RNA cleavage for the influenza virus cap-dependent endonuclease.

A peptide containing the CCHH motif, binds zinc in a one-to-one complex with the characteristics of a typical zinc-binding peptide. Intact influenza virus also contains zinc bound to the M1 protein, but the amount varies in different strains. The zinc content has no influence on the RNA binding and transcription inhibition activities of various M1 proteins [26]. This suggests that

the zinc in M1 has a structural role in the virion other than nucleic acid binding. A peptide synthesized to the ZF region of the M1 sequence of influenza virus strain A/PR/8/34 is effective as a polymerase inhibitor. This peptide represents a ZF and has antiviral activity. Reduction in the number of residues involved in coordination of Zn^{2+} abolishes antiviral activity. In addition, this protein has antiviral activity against other type A influenza viruses (H1N1, H2N2, and H3N2 subtypes), influenza B and vesicular stomatitis viruses [62]. It was expected that ZF or analogs of ZF may provide a new class of antiviral agents effective against influenza virus and possibly other viruses, but has not happened so far. The data of Hui et al. [63] show that the CCHH motif does not provide a critical function in the influenza virus life cycle in cell culture and that the zinc-binding function may not be involved in virus biology.

1.10. Corona viruses

The NS polypeptide encoded by the ORF1b of human corona virus 229E (HCoV-229E) is a zinc-binding protein [27]. A HcoV-229E papain-like proteinase and its corona viral relatives have a poorly conserved ZF connecting the left and right hand domains of a papain-like fold. In denaturation/renaturation experiments using the recombinant protein, its activity is strongly dependent upon Zn^{2+} [28]. The 229E replicase gene encodes a protein, p66HEL, that contains a putative ZF structure linked to a putative superfamily 1 helicase [29]. It also encodes NS13 containing an N-terminal zinc-binding domain and a C-terminal superfamily 1 helicase domain [30]. Corona virus replication and transcription are highly specialized processes of cytoplasmic RNA synthesis that localize to virus-induced membrane structures. The enzymatic activities of a recombinant form of the severe acute respiratory syndrome corona virus (SARS-CoV) helicase NS13; a superfamily 1 helicase with an N-terminal zinc-binding domain has been characterized. NS13 has both RNA and DNA duplex-unwinding activities [64].

1.11. Papillomaviruses and Ebola virus

The purified oncogenic E7 proteins of human papilloma virus (HPV 16) and of cottontail rabbit papilloma virus (CRPV) contain one tightly bound Zn^{2+} per molecule. The metal site shows facile exchange with either cadmium (Cd^{2+}) or Cu^{1+}. The HPV 16 E7 maximally binds one Cd^{2+} or two Cu^{1+} ions, while the CRPV E7 binds two Cd^{2+} or three Cu^{1+} ions [31]. The E6 protein of HPV 16 has two putative Zn^{2+} binding sites crucial for its function. A specific chelating agent, which functionally mimics a metallochaperone, stabilizes the soluble monomeric form of E6 and inhibits multimerization in vitro [32]. It may be that chelating agents of appropriate strength could assist zinc delivery to recombinant metalloproteins in vitro and may even destabilize existing agglomerates.

VP30 is an essential activator of Ebola virus transcription. A conspicuous structural feature of VP30 is an unconventional zinc-binding Cys(3)-His motif that stoichiometrically binds Zn^{2+} in a one-to-one relationship. Substitution of the conserved cysteines and histidine within the motif leads to a complete loss of the capacity for zinc binding [33].

1.12. Picorna and rhinoviruses

Protein 2C (ATPase) of picornaviruses is involved in the rearrangement of host cell organelles, viral RNA replication, and encapsidation. The cysteine-rich motif near the carboxy terminus of poliovirus 2C (ATPase) is well conserved among enteroviruses and rhinoviruses displaying an amino acid arrangement resembling ZF motifs. A mutant virus that lacks the second of four potential coordination sites for zinc is temperature sensitive. The cysteine-rich motif is sufficient to bind zinc in vitro. The metal binding site is also conserved in the chymotrypsin-like 2A cysteine proteinases of picornaviruses [34]. The zinc atom is not directly involved in catalysis but rather may have a structural role. The coordination of the structural zinc in the HCV NS3 proteinase is mediated by Cys-97, Cys-99, Cys-145, and His-149. A similar metal binding motif is found in 2A proteinases of enteroviruses and rhinoviruses, suggesting that they are structurally related [55].

1.13. Rotaviruses

Rotaviruses have a complex architecture. Its double-stranded RNA genome is composed of 11 segments that codes for 6 structural proteins and 6 NS. Several NS facilitate the subsequent processes of genome replication and packaging [65]. The structural protein VP6 forms the middle layer in the triple-layered viral capsid with a central Zn^{2+} located on the 3-fold molecular axis [35]. The properties of VP6 depend on the presence of the centrally coordinated Zn^{2+} in the trimer. The wild-type VP6 depleted of the zinc behaves like the mutant protein and its susceptibility to proteases is greatly increased in the absence of zinc [66]. These observations suggest that VP6 trimers present a structural flexibility that is controlled by the presence of a Zn^{2+}. The NS1 acts as IFN regulatory factor 3 (IRF-3). NS1 synthesized in rotavirus-infected cells binds IRF-3. The binding domain resides in the C terminus of NS1 and that the N-terminal conserved ZF is important but not sufficient to mediate binding to IRF-3 [36]. Therefore, the role for NS1 in rotavirus-infected cells appears to inhibit activation of IRF-3 and diminish the cellular IFN response.

Rotavirus mRNAs are capped but not polyadenylated, and viral proteins are translated by the cellular translation machinery. The cellular protein, named RoXaN (rotavirus X protein associated with NS3), contains a minimum of three regions predicted to be involved in protein–protein or nucleic acid–protein interactions. In the carboxy terminus, at least five ZF motifs are observed, suggesting the capacity of RoXaN to bind other proteins or nucleic acids. RoXaN is capable of interacting with NS3 in vivo and during rotavirus infection and has been implicated in translation regulation [37].

The nucleotide sequences for the simian rotavirus SA11 gene segment 5 codes for the NS53. There is a conserved region between amino acids 37–81 which contains a generalized motif for a metal binding domain. All eight cysteine and two histidine residues in this short sequence are conserved between the simian and bovine NS53 [67]. The conservation of this domain despite extensive sequence diversity in the remainder of the protein suggests that this region is functionally important. Brottier et al. [68] demonstrated that the recombinant genomic segment 5 of bovine rotavirus (RF strain) protein binds zinc and is an RNA-binding protein as are several other ZF proteins. The NS2 self-assembles into homomultimers, binds single-stranded RNA nonspecifically, possesses an Mg^{2+}-dependent nucleoside triphosphatase (NTPase) activity, and is a component of replication intermediates. The presence of Mg^{2+}, Zn^{2+} and other divalent cations inhibit by approximately one-half the activity of NS2, due to the increased stability of the duplex substrate brought on by the cations. In contrast, under conditions where NSP2 functions as an NTPase, its helix-destabilizing activity is less sensitive to the presence of Mg^{2+}, Zn^{2+}, suggesting that in the cellular environment the two activities associated with the protein, helix destabilization and NTPase, may function together. In contrast, under conditions where NS2 functions as an NTPase, its helix-destabilizing activity is less sensitive to the presence of Mg^{2+}, suggesting that in the cellular environment the two activities associated with the protein, helix destabilization and NTPase, may function together [69]. The octamers formed by the NS2 of rotavirus have RNA binding, helix unwinding, and Mg^{2+}-dependent NTPase activities and play a crucial role in assembly of viral replication factories, viroplasms [70]. NTPase activity of NS2 may have a role subsequent to the formation of viroplasms, consistent with its suspected involvement in RNA packaging and/or replication.

2. Conclusions

Although a wealth of information has been accumulated on metal-dependent enzymes and proteins, many questions remain. For example, it is not clear why metal ions tends to bind to viral nucleic acids indirectly via a water molecule whereas it tends to bind to proteins directly. The factors that define the energetically most favorable ligand coordination set and geometry for a given metal cation are ill defined. How does a viral protein select a specific metal cation from the mixture of ions in the surrounding fluids, for example why viral proteins and nucleoproteins choose mostly zinc ions? Is this selectivity due to (i) the natural abundance of the metal in the biological locality, or (ii) properties of the metal (e.g., its stereochemical and charge to size requirements), or (iii) properties of the protein (e.g., its unique set of amino acid residues forming the metal-binding pocket and the stereochemistry of this pocket)? Most of the studies have been done with zinc. There is a need to study interaction of various other metals with different viral proteins. Stray efforts have been made to use these approaches for the development of antiviral agents. A deeper understanding of these questions and lot more studies on structural basis for metal binding should prove useful in the early design and development of viral inhibitors that may lead to new drug discovery.

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