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Gene regulation of provirus HIV-1: Involvement of redox regulation by thioredoxin.

Takuma Hayashi

Associate Professor, Dept. of Immunology and Infectious Disease, Shinshu University Graduate School of Medicine, Matsumoto, Nagano, Japan, **Business using Advanced Technology, Japan Science and Technology Agency (JST), Chiyoda, Tokyo, Japan,

ABSTRACT

Transcription from the human immunodeficiency virus type 1 (HIV-1) provirus is activated by a cellular factor, nuclear factor kappa B (NF- B), recognizing the tandemly repeated 10-base-pair sequences, termed the kappa B sequence, present in the enhancer region within the viral long terminal repeat (LTR). Using electrophoretic mobility shift assay (EMSA), which demonstrates specific DNA-protein interaction in vitro, we could demonstrate that reducto-oxidative modulation of NF- B dramatically changes its DNA binding activity and that a cellular physiological reducing catalyst, human thioredoxin (TRX) also known as adult T cell leukemia derived factor (ADF), fully restored the DNA-binding activity of the oxidized NF- B. We also observed that purified TRX/ADF protein could augment gene expression from HIV-1 LTR as demonstrated by in vitro transient chloramphenicol acetyltransferase (CAT) assay. These observations confirmed the previous notion that TRX/ADF might be an inducing factor of cellular interleukin-2 receptor alpha subunit (IL-2R alpha) through the kappa B sequence that is a common central cis-regulatory element in both IL-2R alpha and provirus HIV-1 gene expression. These observations indicate that reducto-oxidative regulation (or redox regulation) of a cysteine residue(s) on the NF- B molecule might play an important role in its specific DNA interaction and that it might provide a clue to the understanding of a pathway of cellular signal transduction to NF- B that is independent from the known pathways involving protein phosphorylation.

KEYWORDS : HIV-1, TRX, NF- B, Reduction/Oxidation

Introduction

NF-κB is a pleiotropic mediator of transcription for various promoters.1,2 It activates a wide variety of viral and cellular genes including human immuno-deficiency virus-1 (HIV-1).3,4 interleukin-2 receptor alpha (IL-2Rα), tumor necrosis factor-α (TNF-α) and Granulocyte Macrophage colony-stimulating Factor (GM-CSF).3,5,6,7,8 NF-κB is initially located in the cytoplasm as an inactive form associated with an inhibitory protein, IκB.9 The finding that phorbor esters induce nuclear translocation of NF-κB, and that NF-κB is released in vitro by treatment of the cytosol fraction with purified protein kinase C (PKC), have suggested that IκB may be inactivated by phosphorylation. However, one of the few known physiological inducers of NF-κB, TNF-α, was found to activate NF-κB even in the presence of protein kinase inhibitors, implying that a kinase independent mechanism was involved.10,11 Within the HIV-1 long terminal repeat (LTR), two tandemly repeated kappa-B binding sites have been identified in the region initially identified as enhancer. This c/s-regulatory element has been shown to regulate the transcriptional inducibility of provirus HIV-1 in activated T cells and in TNF-α-treated cells.2,4,11 Thus, NF-κB may serve as an intracellular mediator in activation of the latent provirus HIV-1 and thus participate in a critical step preceding the clinical manifestation of the acquired immunodeficiency syndrome (AIDS).

In T cells transformed by human T lymphotropic virus type 1 (HTLV-1), NF-κB is constitutrvely activated causing over expression of IL-2Rα on the cell surface and release of TNF-α and GM-CSF. 5,7,8,11 Moreover, replication of HIV-1 is greatly augmented in these cells and its augmentation is dependent on the kappa-B binding sites within LTR.3,11,12 Interestingly, recent reports demonstrated that a cellular factor, known as adult T cell leukemia-derived factor (ADF), which is released from cells transformed by HTLV-1 or Epstein-Barr virus and has activity to stimulate IL-2Rα expression, is the human homolog of thioredoxin (TRX).13,14 Human TRX/ADF is a 13 kDa thiol protein that is a strong catalyst of dithiol-disulfide exchange reactions. TRX/ADF participates in redox reactions through reversible oxidation of its active center dithiol to a disulfide. The actual role of TRX/ADF in inducing IL-2Rα is to be clarified, but there is much evidence to suggest that the reducto-oxidative conditions of sulfhydryl (SH) groups of some nucleic acid-binding proteins reversibly modulate their binding capabilities.15 Recently, several reports have indicated that Reduction/Oxidation (Redox) conditions modulated the DNA binding activity of NF-κB and AP1 by using non-physiological inorganic reagents.16,17,18 These findings prompted us to investigate whether redox regulation by the TRX/ADF system participates in the regulation of provirus HIV-1 gene expression.

Here we present evidence which suggests that TRX/ADF might activate HIV-1 gene expression by a dithiol-disulfide exchange reaction of the cysteine residues on the NF-κB molecule. First, the present study has shown that free SH groups of NF-κB required for its specific DNA-binding activity. Oxidation of already 'activated' NF-κB prepared from human T lymphocyte MT2 cell nuclei inactivated its specific DNA-binding activity and subsequent reduction of the oxidized protein by TRX/ADF efficiently restored its activity. Moreover, the transient CAT gene expression assay has demonstrated that gene expression from HIV-1 LTR was augmented by the exogenously added TRX/ADF. Since this activity was abolished when the upstream c/s-regulatory region containing the NF-κB binding sites was deleted from the HIV-1 LTR, this in vivo effect of TRX/ADF might also be mediated through NF-κB. This kind of control mechanism involving sulfhydryts of cysteine residues is collectively called 'redox regulation'.14 Similar phenomena have been observed with other nucleic acid-binding proteins. For example, oxidation of the iron-responsive element binding protein (IRE-BP), which specifically interacts with the 5' untranslated region of the ferritin heavy-chain mRNA, increased its binding affinity to its RNA target (IRE) by a factor of over 100.15 On the other hand, in the case of the bacterial transcriptional regulatory protein OxyR, which regulates gene expression in response to oxidative stress, oxidation of a single essential cysteine changed its DNA-binding specificity and activated gene expression of a set of genes responsible for the resistance to oxidative environment.16 Furthermore, the DNA-binding activity of the Fox-Jun heterodimer has been shown to be modulated by the redox status of a single conserved cysteine residue in the DNA-binding domain of each of the two proteins.16 However, similar experiments with other transcription factors, namely CBP, Sp-1 and NF-1 transcriptional regulator did not show any effects of oxidation or reduction of sulfhydryls on their DNA binding activities.17 Thus, one of the physiological roles of the cellular redox regulation system might be that it serves as a novel modality in selective activation of a certain set of genes by modulating the activity of particular nucleic acid-binding proteins.

Besides the TRX/ ADF system, there are other cellular reducing systems such as the glutaredoxin (GSH) system.14,19 The TRX/ADF and Glutathione-SH (G-SH) systems are considered to have specific, but overlapping functions in many reduction systems. Since reduced glutathione, even in the presence of glutathione reductase, did not restore the activity of the oxidized NF-κB in vitro, TRX/ADF is more likely

to be important in the reduction of NF-κB. Recently, research group reported that N-acetyl-L-cysteine (NAC), acting both as a scavenger for oxygen radicals and as a precursor of GSH, blocked activation of provirus HIV-1 gene transcription by TNF-α through NF-κB when added at a very high concentration.19 It is possible that there are cross talks within the cells among different reducing systems. However, the in vivo effects of reducing agents, especially in the presence of oxidative stress, such as that evoked by TNF-α, need to be evaluated carefully. For example, the effect of TNF-α in inducing endogenous TRX/ADF may have been blocked in the presence of excess intracellular GSH because of the radical scavenger activity of GSH. Thus, cellular reducing systems, such as those of TRX/ADF and GSH, might have distinct roles in the signal transduction pathway involving NF-κB. In T cells infected with HTLV-1, where TRX/ADF as well as TNF-α is over expressed, NF-κB is known to be constitutively activated and replication of HIV-1 is greatly augmented.7,12 It is interesting to see if excessive amounts of the extracellular reducing agent such as NAC could inactivate NF-κB even in cells infected with HTLV-1. There are many cysteine residues in the putative DNA-binding domain of the NF-κB molecule, but the cysteine residue(s) required for DNA recognition has not yet been identified. Nevertheless, our results, together with others, suggest that a novel molecular mechanism involving the sulfhydryls has an important role in specific DNA recognition by transcriptional regulator.20,21 The concept of 'redox regulation' may not only provide an insight into a novel mechanism of DNA-binding by NF-κB but also may explain, at least partly, why gene expression of HIV and IL-2Rα is augmented in the cells transformed by HTLV-1. Further investigation will hopefully lead to development of a new strategy which can efficiently decelerate the pathological process of AIDS.

Conclusion

In view of therapeutic interventions, one can envisage the application of reductants, such as NAC and other thiol compounds, to restore the redox status to prevent AIDS development and its progression. It remains to be examined whether TRX/ADF plays a part in disease progression *in vivo* and whether reversion of the redox status is effective in blocking the induction of TRX/ADF.

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