HIV-1 Rev or Tat fused fluorescent proteins its folding and emission by binding with corresponding RNA

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ABSTRACT

Rev and Tat peptide derived from HIV-1 forms α-helix and β-turn upon the binding with RRE or TAR RNA, respectively[1-2]. This peptide-RNA interaction could be used as a trigger that induced folding of whole protein. We have synthesized Tat or Rev peptide fused fluorescent proteins (FPs) for monitoring folding of the protein with their fluorescent emission enhancement by forming complex with corresponding RNA. Tat peptide sequence was fused into the green fluorescent protein (GFP) or two FPs were connected with Tat peptide as a linker with various length of spacer sequence of the repeated units of three glycines and a serine between Tat and GFPs. Among them, GFPTat7.5 that having seven and five repeat of spacer units on the upper and lower sides of Tat, respectively and GFPTat5.3 that having five and three repeat of spacer units exhibited obvious emission enhancement upon increasing concentrations of TAR RNA.

Cyan fluorescent protein for energy transfer, CyPet and yellow fluorescent protein for energy transfer, YPet[3] were conjugated together with Tat or Rev peptide as a linker with various lengths of spacer sequences. All the Tat or Rev fused double FPs enhanced or diminished its fluorescence upon binging with the corresponding RNA. Those Tat or Rev fused FPs could be enable to develop a novel probes for the drug discovery of anti HIV drug.

TAR-Tat interaction could be used as a regulator for the enzyme activity as well. Along this strategy, BioH, an esterase, and GFP were connected together with Tat peptide as a linker. The binding of TAR RNA with BioH-Tat-GFP may regulate its catalytic activity in the ester hydrolysis.

1. INTRODUCTION

Although over eighty percent of genomic information is translated to RNA, not all role of RNA is known. However, recently, it was found that some genomic RNA in the virus induces folding of the protein that constructs their body. RNA could be a chaperon for the protein folding.

When the single strand RNA has complementary, even if that is relatively short, it forms base pairing into secondary structure like a hairpin loop. Hairpin loop structure of RNA can be seen often as a protein-binding site. Discovery of specific hairpin RNA might enable the regulation of the protein folding and regulation of their function, as well.

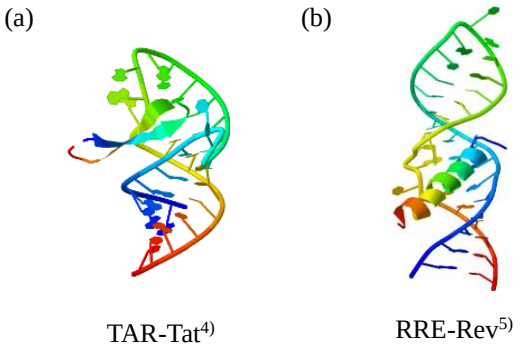


Figure 1. 3D structure of TAR-Tat complex (a), and RRE-Rev complex (b).

Human immunodeficiency virus type-1, (HIV-1) is the retrovirus that causes acquired immunodeficiency syndrome (AIDS). On the replication of HIV, two viral proteins, Rev and Tat, are known as activators that bind to the activator responsive region of the viral RNAs, RRE RNA and TAR RNA, respectively, then amplify the replication of HIV. The RNA binding site of these viral proteins, Rev and Tat peptide, forms α-helix and β-turn upon the binding with RRE or TAR RNA, respectively. This peptide-RNA interaction could be used as a trigger that induced folding of whole protein. We have synthesized Tat or Rev peptide fused fluorescent proteins

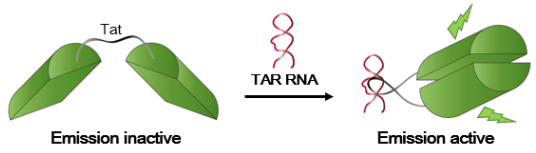
of which induced fluorescent emission with forming complex with corresponding RNA.

Tat peptide sequence was fused into the green fluorescent protein (GFP) with various length of spacer sequence of the repeated units of three glycines and a serine as spacers between Tat and partial GFPs. While GFP-Tatm,n are emission inactive in the absence of TAR RNA, they are emission active in the presence of TAR RNA by the folding of Tat peptide as a β-turn with the binding of TAR RNA.

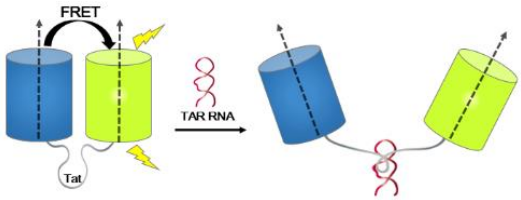
CyPet and YPet were connected with Tat or Rev peptide as a linker with various length of spacer sequence of the repeated units of three glycines and a serine (C-Tat-Yn.n or C-Rev-Yn.n). While C-Tat-Yn.ns exhibits weaker FRET emission in the presence of TAR RNA, by the folding of Tat peptide as a β-turn with the binding of the RNA, C-Rev-Yns exhibits enhanced FRET in the presence of RRE RNA by the folding of Rev peptide as a α-helix with the binding of the RNA.

Also, hydrolytic enzyme, BioH and GFP were connected with Tat peptide, so that the catalytic activity of the enzyme could be modulate by the binding with TAR RNA.

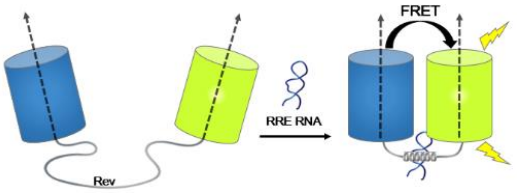
(a) **GFPTat$_{m.n}$**



(b) **C-Tat-Y$_{n.n}$**



(c) **C-Rev-Y$_{n.n}$**



(d) **GFP-Tat-BioH$_{n.n}$**

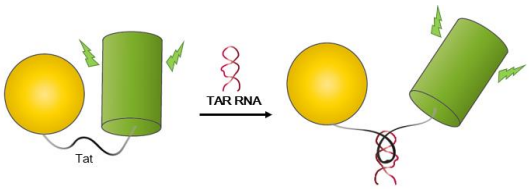


Figure 2. Design of Tat or Rev fused fluorescence protein. (a) GFPTat$_{m.n}$ (b) C-Tat-Y$_{n.n}$ (c) C-Rev-Y$_{n.n}$ (d) GFP-Tat-BioH$_{n.n}$

2. EXPERIMENT

2.1 Design of the vectors

The designed vectors in this study were summarized in figure 3. Histidine affinity tag was employed for the purification of each proteins, the appropriate amino acid sequence of Tat or Rev fused FPs were followed. The amino acid sequence of (GGGS)m-RKKRRQRRR -(GGGS)n (m and n are 3.3, 5.3 or 7.5) was inserted into the GFP between 157 and 158 as amino acid number of GFP. Tat or Rev with spacer sequences was placed between CyPet and YPet. GFP and BioH were connected with Tat peptide as a linker with the spacer sequence of three times repeated units of three glycines and a serine.

Figure 3. Vector design of the Tat or Rev fused FPs. Tat peptide sequence (-RKKRRORRR-) was placed between GFP1-157 and GFP158-238 in GFPTat$_{m.n}$ (m.n =1.1, 3.3, 5.3, 7.5), and Tat or Rev peptide sequence (-TRQRERWRRRRNRRAQR-) was placed between CyPet and YPet. The repeated amino acid sequence of three glycines and a serine was placed both side of either Rev or Tat sequence.

2.2 Vector construction and the protein synthesis.

Each vector was constructed on pHAT20 (Clontech). The vectors were transformed into E. coli strain BL21(DE3), then the desired proteins were harvested and purified by histidine affinity chromatography and gel filtration, then the purity was confirmed by PAGE analysis.

3. RESULTS AND DISCUSSION

The binding between Tat or Rev fused FPs and the corresponding RNA, folding and melting temperature of those fusion proteins were studied with fluorescence and circular dichroism (CD) spectroscopy.

3.1 TAR RNA induces folding and emission of GFPTatn.

GFPTat5.3 and GFPTat7.5 exhibited obvious emission enhancement depend on increasing concentrations of TAR RNA. Dissociation constants of GFPTat5.3 and GFPTat7.5 were determined by the fluorescent titration analysis with 1:1 stoichiometry, as 2.0 and 3.0 μM, respectively. The magnitude of emission enhancement for GFPTat5.3 was 2.0 times higher than that of GFPTat7.5. These results revealed that shorter spacer is better for the recovery of GFP emission. The melting temperature (Tm) of GFPTat7.5 and GFPTat5.3 were determined by the

temperature dependent CD experiment. The Tm of GFP was 76 Cº. On the other hand, those of GFPTat7.5 and GFPTat5 were 58 and 53 Cº, respectively. Tms of Tat fused proteins were nearly 20 degrees lower than that of native GFP. These results imply that the insertion of Tat sequence into GFP make it structurally more flexible than that of native GFP. These results demonstrated that the TAR-Tat interaction induced folding of not only Tat sequence but also whole Tat fused GFP, that result in brighter emission of the Tat fused GFP in the presence of TAR RNA.

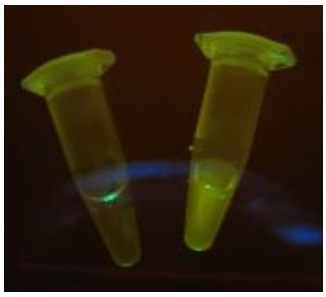


Figure 4. Test tubes including a solution of GFPTat7.5, in the absence (left) and in the presence (right) of TAR RNA.

3.2 RNA binding modulates emission of conjugated FRET FPs.

Rev fused FRET FPs、C-Rev-Y1.1 and C-Rev-Y5.5 exhibited emission enhancement depends on increasing concentrations of RRE RNA, then the dissociation constants were determined by the fluorescent titration analysis with 1:1 stoichiometry, as 3.7 and 0.9 nM, respectively. Upon the binding of C-Rev-Y1.1 and C-Rev-Y5.5 with RRE RNA, Rev peptide forms α-helix, the distance between CYpet and YPet was shortened, two fluorescent proteins placed closer each other, as a result, FRET emission increases with increasing concentration of RRE RNA.

Tat fused FRET FPs, C-Tat-Y3.3 exhibited emission decreasing depends on increasing concentrations of TAR RNA and dissociation constant was determined by the fluorescent titration analysis with 1:1 stoichiometry, as 495 nM. C-Tat-Y3.3, upon the binding with TAR RNA, Tat peptide forms β-turn, the dipole moment of those two fluorescent proteins may twist each other, as a result, FRET emission decreases with increasing concentration of TAR RNA.

CONCLUSION

Although both Tat fused GFPs, GFPTat5.3 and GFPTat7.3 has more relaxed structure than that of native GFP, binding with TAR RNA remarkably enhanced the emission of GFPTat5.3 and GFPTat7.5. These results demonstrated that TAR RNA induced folding of whole GFPTatm,n and that result in their emission enhancement. These Tat fused GFPs might be useful as a probe for drug discovery of TAR binding molecules.

While, binding of TAR RNA quenched the emission of C-Tat-Y3.3, that of RRE enhanced the emission of C-Rev-Y1.1 and C-Rev-Y5.5. TAR-Tat complex in C-Tat-Y3.3 may not favorable for FRET emission, it may twist the dipole moments of FPs.

Rev forms α-helix in RRE-Rev complex. This folding of Rev in the complex makes shorten the distance between CyPet and YPet in C-Rev-Ys. That might be favorable for FRET emission of C-Rev-Ys. GFPTats, C-Tat-Ys and C-Rev-Ys are useful as a probe for drug discovery of TAR or RRE binding molecules.

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