Binding Mode of [Ruthenium(II)(1,10-Phenanthroline)$_2$L]$^{2+}$ to Poly(dG)·poly(dC)·poly(dC)$^+$ Triplex DNA

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Binding geometries of [Ru(II)(1,10-phenanthroline)$_2$L]$^{2+}$ complexes (where L = dipyrido [3,2-a:2',3'-c]phenazine (DPPZ) or benzodipyrido[3,2-a:2',3'-c]phenazine (BDPPZ)) to poly(dG)·poly(dC)·poly(dC)$^+$ triplex DNA (CGC$^+$ triplex) has been investigated by linear dichroism and normal absorption spectroscopy. Analysis of the linear dichroism for the CGC$^+$ triplex and [Ru(II)(phen)$_2$BDPPZ]$^{2+}$ complex indicates that the extended ligand of the metal complex lie perpendicular to the polynucleotide helix axis. Together with strong hypochromism and red shift in the interligand absorption region, we concluded that the extended BDPPZ or DPPZ ligand intercalated between the bases of polynucleotide. The spectral properties of the metal complexes bound to CGC$^+$ triplex are similar to those bound to poly(dA)[poly(dT)]$_2$ triplex (Choi *et al.*, *Biochemistry* **1997**, *36*, 214), suggesting that the metal complex is located in the minor groove of the CGC$^+$ triplex.

Introduction

Interactions between the metal complexes containing planar aromatic ligands and various DNAs have been widely studied.[1] A representative metal complex in this group is octahedral ruthenium complexes such as [Ru(phen)$_2$L]$^{2+}$, which has been investigated as probes of nucleic acid structure,[2] as chemical nuclease,[3,4] as fluorescence probes[5] and in electron transfer system.[6,7] Various binding mode of the [Ru(phen)$_2$L]$^{2+}$ complex to DNA have been proposed.[6,8-14] Initially the [Ru(phen)$_3$]$^{2+}$ complex was suggested to bind to surface of the minor groove of DNA and intercalation from the major groove with one of its 1,10-phenanthroline sit between the DNA base pairs.[6,9] However, possibility of classic intercalation of any phenanthroline ligands has been rule out by viscosity measurements.[13,14] Although partial intercalation from the major groove was suggested by molecular modeling and energy minimization work,[10] a recent NMR and circular dichroism study showed that the interaction between the [Ru(phen)$_3$]$^{2+}$ complex and DNA occurs in the minor groove of DNA without separation of the stacked bases.[11,12] When one of the ligand of the [Ru(phen)$_3$]$^{2+}$ complex is replaced by either dipyrido[3,2-a:2',3'-c]phenazine(referred to as DPPZ) or benzodipyrido[3,2-a:2',3'-c]phenazine (referred to as BDPPZ), the extended ligand undoubtedly intercalates between the DNA base pairs.[8,15-19] Although intercalation from the major groove has been suggested for [Ru(phen)$_2$DPPZ]$^{2+}$ from NMR studies with a hexamer, recent spectroscopic studies with triple helical poly(dA)[poly(dT)]$_2$ (referred to as TAT triplex in this work) have suggested that it bind from the minor groove.[20]

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Interests in triple helical DNA increased following the discovery that short oligonucleotides can recognize specific sequences of duplex DNA[21,22] and that intermolecular triple helical DNA (H-DNA) can be formed in natural DNA.[23] Subsequent studies focused on biological and therapeutic applications such as inhibiting the transcription[24] or replication[25] of specific sequences, and preventing cellular proteins from binding to their designated target DNA[26] as well as on the design of artificial sequence specific nucleases.[27] The third strand is generally stabilized by addition of intercalators such as ethidium derivatives,[28] benzo[e]pyridoindoles,[29] and acridine derivatives.[30,31] An important motivation for studying drug-triplex interaction arises from interest in the structure motif of triplex DNA itself. In the triplex, the Hoogsteen paired purine strand lies in the major groove of the template duplex, running parallel to the pyrimidine strand of the duplex. Hence, binding of the third strand changes the binding mode or inhibit the binding of major groove interacting drugs.[32,33] Using this property, binding mode of [Ru(phen)$_2$L]$^{2+}$ complex (Figure 1) with TAT triplex was investigated by various spectroscopic methods, including normal absorption, circular and linear dichroism.[20] If the metal complex is located in the major groove, a significant alteration in the spectroscopic properties of the [Ru(phen)$_2$L]$^{2+}$-TAT complex was expected compared to that of the [Ru(phen)$_2$L]$^{2+}$-poly(dA)·poly(dT) complex because the major groove is blocked by the third strand in the triplex. However, the spectroscopic properties of the metal complex are similar in duplex and triplex, suggesting that expanded ligand intercalates between DNA base-pairs from the minor groove.

In this work, similar assumption was applied in order to investigate the binding mode of [Ru(phen)$_2$L]$^{2+}$ complex in poly(dG)·poly(dC)·poly(dC)$^+$ triplex (referred to as CGC$^+$

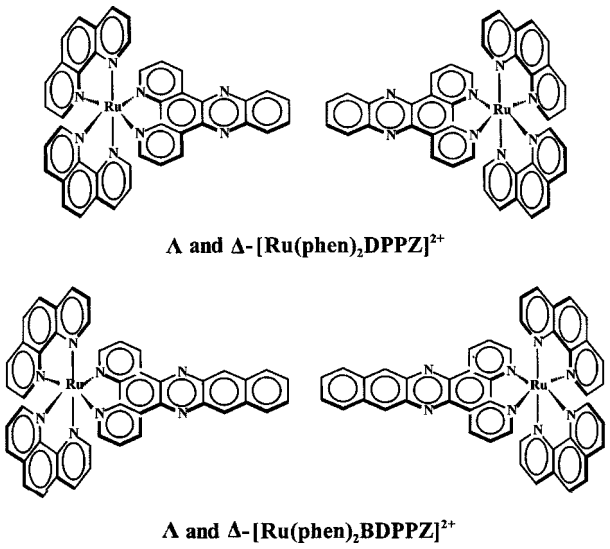

Figure 1. Structures of [Ru(II)(1,10-phenanthroline)$_2$L]$^{2+}$ complexes.

triplex in this work) at pH 5.0. In the CGC$^+$ triplex case, protonated poly(dC)$^+$ is located in the major groove of poly(dG)·poly(dC) duplex, thereby expected to alter the spectroscopic properties of the metal complexes if they locates in the major groove. It is also of interest that positively charged [Ru(phen)$_2$L]$^{2+}$ complex can form a complex with CGC$^+$ triplex which possesses a positive charge at every base triplet.

Experimental Section

Materials. All chemicals and solvents were of analytical grade and used without further purification. All polynucleotides were purchased from Pharmacia. Poly(dC) were dissolved in 5 mM phosphate buffer (pH = 7.0) containing 100 mM NaCl and were dialyzed five against 5 mM phosphate buffer (pH = 5.0). Poly(dG)·poly(dC), which is not soluble in neutral pH, was dissolved in 100 mM NaCl, 5 mM phosphate buffer at high pH (pH > 10.0) and was dialyzed five times against 5 mM phosphate buffer (pH = 5.0). Triple helical CGC$^+$ was prepared by simmering a molar mixture of poly(dG)·poly(dC) and poly(dC) at pH 5.0 for an hour followed by overnight annealing at room temperature. Formation of the triplex was confirmed by its characteristic CD spectrum. Concentration of the polynucleotide were determined spectrophotometrically using the molar extinction coefficients: 254 nm = 7,400 cm^{-1}M^{-1} for poly(dG)·poly(dC) and 274 nm = 6,800 cm^{-1}M^{-1} for poly(dC).

The homochiral ruthenium complexes were synthesized by reported method.[8,19] Concentrations were determined using the molar extinction coefficients: 439 nm = 20,000 cm^{-1}M^{-1} for [Ru(phen)$_2$DPPZ]$^{2+}$, and 440 nm = 22,000 cm^{-1}M^{-1} for [Ru(phen)$_2$BDPPZ]$^{2+}$.

All spectroscopic measurements were performed at the mixing ratio, R, of [drug]/[base pair] or [drug]/[base triplet] of 0.1 and at an ambient temperature. The concentration of CGC$^+$ triplex was 30 μM base triplet in this work.

Absorption. Intercalation of a drug to polynucleotide manifest itself by a hypochromism, broadening of the envelop, and, usually, red-shift in the drug absorption region. These changes have been understood as π stacking interaction between nucleobases and aromatic part of the intercalating drugs. For groove binders, a large wavelength shift usually correlates with a ligand conformation change or drug-drug interaction. In our case, the absorption band corresponding to the $d \rightarrow \pi^*$ transition of metal to ligand charge transfer band (MLCT) as well as intraligand $\pi \rightarrow \pi^*$ transition are well separated from that of DNA. Change in the absorption band of the metal complexes in the MLCT region upon binding to DNA may be explained by (1) π to π stacking interaction, (2) ligand conformation change, and/or (3) drug-drug interaction because part of the metal complexes is expected to be intercalated and other part located in one of the groove. On the other hand, changes in absorption spectra in the intraligand absorption band may reflect pure π - π stacking between DNA bases and extended DPPZ or BDPPZ ligand. Absorption spectra were measured on a HP8452A diode array spectrophotometer equipped with a HP80890A peltier temperature controller.

Linear Dichroism and Reduced Linear Dichroism (LD and LD^r). LD is defined as the differential absorption of the light polarized parallel and perpendicular to some laboratory reference axis; in the case of flow LD, the parallel direction is the flow direction.[36-38] The measured LD spectrum is then divided by the isotropic absorption spectrum to give the reduced linear dichroism (LD^r). The angle of the drug's transition moments with respect to the DNA helix axis then can be calculated by assuming an effective angle of 86^o between the π - π^* transition moments of the nucleotide bases and the polynucleotide helix axis. Large ligands values 80^o-90^o are indicative of intercalation, whereas values range from 40^o to 50^o are consistent with the transition lying along the minor groove. The LD spectra of the flow-oriented sample were measured on a Jasco J-720, equipped with an Oxley prism to convert the circularly polarized light into linearly polarized light.[37]

Results

Absorption. Absorption spectra of both enantiomer of [Ru(phen)$_2$DPPZ]$^{2+}$ complex in the presence and absence of CGC$^+$ triplex are depicted in Figure 2. The absorption spectrum of the CGC$^+$ triplex was subtracted from that of the mixtures for easy of comparison. As previously reported for the TAT triplex,[20] the absorption spectra of Δ- and Λ- enantiomers in the presence of CGC$^+$ triplex are essentially the same with a strong phenanthroline peak in the 260-270 nm region, a broad MLCT band in the longer wavelength region (above 400 nm), and a band between 330 nm and 380 nm that corresponds to extended DPPZ ligand. In general, hypochromism and broadening of the envelop and red-shift in the drug absorption region can be observed. This effect is particularly pronounced for the DPPZ band, suggesting a strong π to π stacking interaction between DPPZ and nucleobases

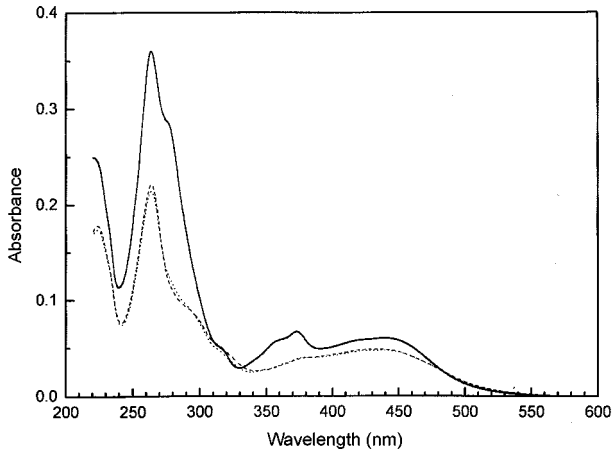

Figure 2. Absorption spectra of the Δ-(dashed curve) and Λ-(dotted curve) enantiomers of $[Ru(phen)_2DPPZ]^{2+}$ complexes bound to CGC^+ triplex. [metal complex] = 3 μM. [triplex] = 30 μM in base. That of DNA free metal complex is compared (solid curve).

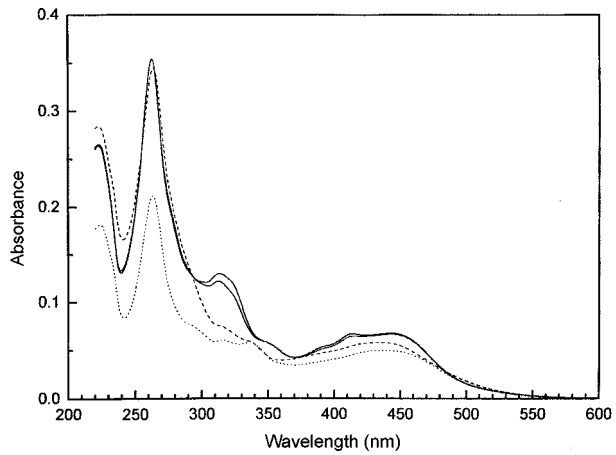

Figure 3. Absorption spectra of the Δ- and Λ-enantiomer of $[Ru(phen)_2BDPPZ]^{2+}$ complexes bound to CGC^+ triplex. Curve assignment for the enantiomers and the concentrations are the same as in Figure 1.

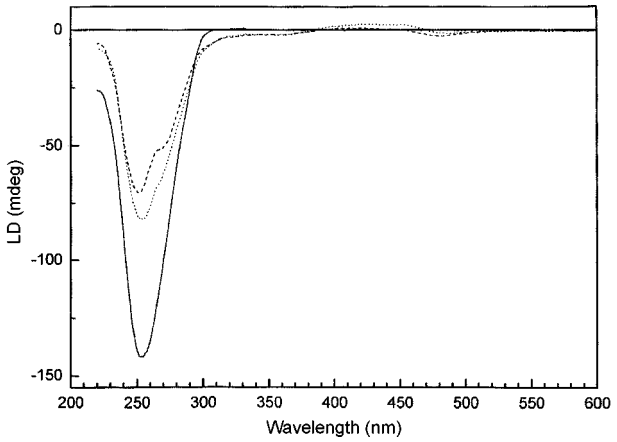

Figure 4. LD spectra of the Δ-(dashed curve) and Λ-enantiomers (dotted curve) of $[Ru(phen)_2DPPZ]^{2+}$ complexes bound to CGC^+ triplex. That of metal-free CGC^+ triplex is compared (solid curve). [metal complex] = 3 μM. [triplex] = 30 μM in base.

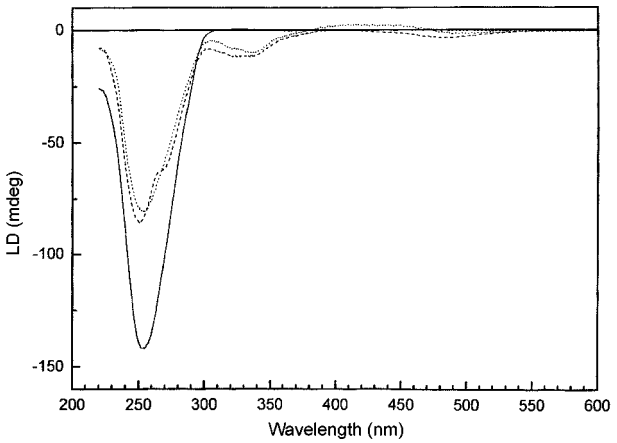

Figure 5. LD spectra of the Δ-(dashed curve) and Λ-(dotted curve) enantiomers of $[Ru(phen)_2BDPPZ]^{2+}$ complexes bound to CGC^+ triplex. [metal complex] = 3 μM. [triplex] = 30 μM in base.

of the triplex. Similar change in absorption spectra were observed for the $[Ru(phen)_2BDPPZ]^{2+}$ complex. Both enantiomers of the $[Ru(phen)_2DPPZ]^{2+}$ complex also exhibit strong hypochromism and red shift in the MLCT band and extended ligand absorption region, excepts for the DNA absorption region (~260 nm) of Λ-isomer (Figure 3).

Linear Dichroism and Reduced Linear Dichroism (LD and LD^r). LD spectra of the metal complexes associated with CGC^+ triplex are depicted in Figure 4 for both the Δ and Λ isomers of $[Ru(phen)_2DPPZ]^{2+}$-CGC^+ complex, and in Figure 5 for $[Ru(phen)_2BDPPZ]^{2+}$-CGC^+ complex. The LD signal in the DNA absorption region for all complexes was negative as expected for our set up: DNA base transitions are aligned normal to flow direction (DNA helix axis). A strong negative LD band was followed in the extended ligand absorption region. A heterogeneous LD signal in the MLCT band was also apparent. At a glance, it is clear that the extended ligand (DPPZ and BDPPZ) of the metal complexes is not aligned along any groove of the B DNA: if it were situated in the groove, a positive LD would be expected. The magnitude in the DNA absorption region was decreased upon binding of the metal complex, suggesting a dissociation of double helix near binding site or bending of the DNA stem.

The LD^r spectrum of both the Δ- and Λ- $[Ru(phen)_2DPPZ]^{2+}$ complex bound to CGC^+ triplex is depicted in Figure 6 and 7. Some positive contribution in the DNA absorption region (270-290 nm) is apparent (Figures 6 and 7, thick curve), which was not observed for double stranded DNA as well as TAT triplex, indicating the cytosine base in the CGC^+ triplex is strongly tilted. The LD^r spectra for both complexes are heterogeneous, indicating that several electric transition moments of the metal complex with different directions or several binding geometries of the metal complex are related for these complexes. The latter possibility may be ruled out from the fact that the shape of the LD^r spectrum is invariant of the mixing ratio (data not shown). The shape of the LD^r

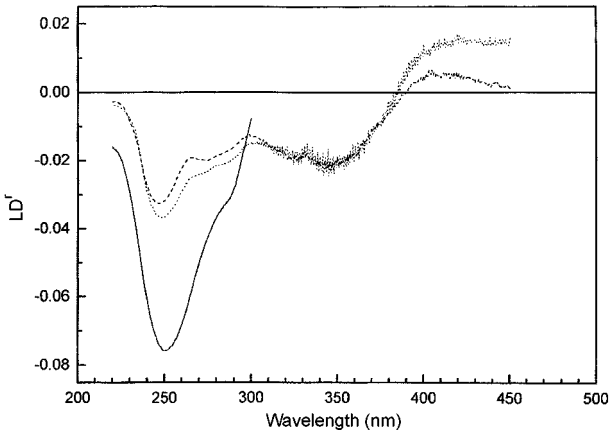

Figure 6. LD^r spectrum of the *Δ*-(dashed curve) and *Λ*-(dotted curve) enantiomers of [Ru(phen)$_2$DPPZ]$^{2+}$ complexes bound to CGC$^+$ triplex. Solid curve denotes the LD^r spectrum of metal complex free CGC$^+$ triplex. The samples are the same as in Figure 4.

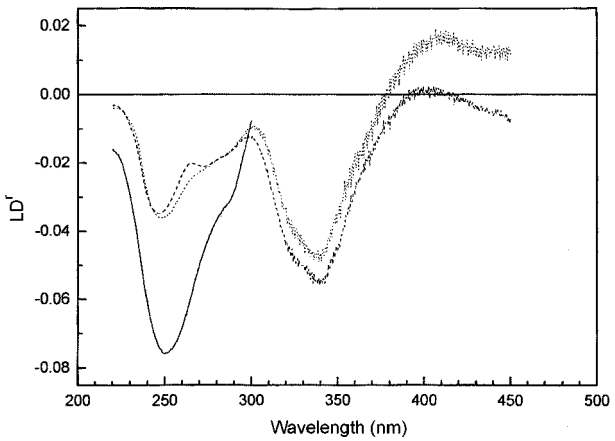

Figure 7. LD^r spectrum of the *Δ*-(dashed curve) and *Λ*-(dotted curve) enantiomers of [Ru(phen)$_2$BDPPZ]$^{2+}$ complexes bound to CGC$^+$ triplex. Solid curve denotes the LD^r spectrum of metal complex free CGC$^+$ triplex. The samples are the same as in Figure 5.

spectra of the [Ru(phen)$_2$L]$^{2+}$-CGC$^+$ triplex are essentially the same with those complexes with TAT triplex.[20]

The transition moments of the [Ru(phen)$_2$L]$^{2+}$ complex have been studied by Lincoln *et al.*[19] and can be summarized as the strong, broad absorption band centred around 440 nm due to $d \rightarrow \pi^*$ metal to ligand charge transfer transition, the absorption around 260 nm due to $\pi \rightarrow \pi^*$ transition, and an intraligand $\pi \rightarrow \pi^*$ transition polarized along the C_2 symmetry long axis of the complex centred at 350 nm for the [Ru(phen)$_2$DPPZ]$^{2+}$ complex and 310 nm for the [Ru(phen)$_2$-BDPPZ]$^{2+}$ complex. In 300 nm-380 nm region, a strong negative LD^r peak whose magnitude is as large as (or larger than) that in the DNA absorption region is observed for both [Ru(phen)$_2$DPPZ]$^{2+}$ and [Ru(phen)$_2$BDPPZ]$^{2+}$ complex. This result indicates that the extended ligand of the metal complex is perpendicular to the DNA helix axis. In the MLCT band region, the shapes of the LD^r spectra for each metal complex with CGC$^+$ triplex are similar to those with TAT triplex, indicating similar binding geometries with CGC$^+$ and TAT triplex.

Discussion

In the presence of CGC$^+$ triplex, the magnitude and shape of the absorption bands of the [Ru(phen)$_2$L]$^{2+}$ complexes are altered noticeably compared to those of DNA-free metal complexes. For the [Ru(phen)$_2$DPPZ]$^{2+}$ complex, the spectral changes for two enantiomers differ only slightly from each other. On the other hand, the extent of changes in absorption spectrum of *Λ* isomer of the [Ru(phen)$_2$-BDPPZ]$^{2+}$ complex is significantly small. Nevertheless, the nature of the absorption change for all metal complex upon binding to CGC$^+$ triplex is the same, indicating that the environment of the CGC$^+$ triplex bound metal complexes and their interactions with DNA base are essentially the same.

The comparable (or higher) LD^r magnitude and negative sign of the intra-ligand $\pi \rightarrow \pi^*$ transition region in the CGC$^+$ triplex and the DNA absorption region strongly indicates that the plane of the extended ligand lies almost parallel to the DNA base plane (and perpendicular to the DNA helix axis). Together with a strong hypochromism that is specific for the $\pi \rightarrow \pi^*$ transition of the extended ligand, intercalation of this ligand between the DNA base is indicative. The shape of LD^r of the metal complex bound to CGC$^+$ triplex is quite similar to those bound to TAT triplex.[20] There are abundant evidences indicate the extended ligand intercalates between double stranded DNAs: viscometry of DNA with [Ru(phen)$_2$DPPZ]$^{2+}$ complex showing elongation of DNA upon metal complex binding,[18] and unwinding of supercoiled DNA,[39] and strong hypochromism in the absorption spectrum particularly in the intra ligand band.[8,19,20] Since the spectral properties of the metal complexes bound to CGC$^+$ triplex are so similar to those bound to DNA, poly[d(A-T)$_2$] and TAT triplex,[19,20] it is conclusive that the binding modes are similar for all DNAs. Considering that the spectroscopic properties of any of the metal complex enantiomers were similar for binding to CGC$^+$ or TAT triplex DNA, it is indicative that the third strand, which occupies the major groove of the template duplex, has little effect on the binding geometries and hence supports the hypothesis that the metal complexes all bind from the minor groove with DPPZ and BDPPZ ligands intercalated.

The negative LD^r magnitude of CGC$^+$ triplex decreases upon binding of the metal complexes. This may partially due to the positive LD^r signal of the cytosine base in the third strand or of the bound metal complex. The LD^r magnitude can also be reduced by increasing DNA flexibility which is originated, for instance, local denaturation or static bending of the DNA stem upon binding of the drugs. The reducing in the LD^r magnitude of the triplex DNA is observed not only for intercalators such as acridine but also for groove binders such as DAPI and Hoechst.[30] Therefore, it is not possible to draw any conclusion about binding geometry from such a decreasing. Finally, it is note worthy that the cytosine bases in the third strand are positively charged at pH 5.0, which is

the prerequisite of the triplex formation. It is therefore, surprising that the metal complex that carries two positive charges can form a complex with such a positively charged sequence. The main force that drives this complex formation is the stacking interaction between the DNA bases and extended ligand and the electrostatic interaction between the positive charge of the metal complex and the negative charge of the DNA phosphate. The positive charges of the metal complex may be compensated by the negative charges of the phosphate group via close contact.

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