# Optical Third Harmonic Generation Study of the Hydration of DNA Films

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ABSTRACT Optical third harmonic generation (THG) has been observed for the first time from DNA films. The THG signal is observed from NaDNA films exposed to relative humidities (RHs) between 0% and 98%. A strong enhancement (~5×) of the THG signal from NaDNA is observed at 84% RH; no enhancement is observed for RbDNA. The most likely mechanism for such an enhancement is an increased coherence length. A model calculation using estimates of the refractive indices at both the fundamental and third harmonic frequencies supports this interpretation. The observed THG signal has the same polarization as the incident (fundamental) light. For the A conformation, the THG signal polarized perpendicular to the helical axis is approximately twice as strong as the signal polarized parallel to the helical axis. No such anisotropy is observed for either the disordered conformation (below about 50% RH) or the B conformation (above 92% RH).

## INTRODUCTION

One of the central goals of biophysics is to attain a complete understanding of the physical properties of the DNA molecule, one of the most important molecules for life. Experimental studies have provided a wealth of information about DNA. Coupling these experimental results with theoretical considerations has led to substantial progress in deciphering the secrets of DNA. These works have dealt with the different structural conformations of DNA (Saenger, 1984), its vibrational (McCammon and Harvey, 1987; Lindsay et al., 1988; Lee et al., 1987; Powell et al., 1987; and Lavalle et al., 1991) and electronic properties (Weidlich et al., 1987 and Falk et al., 1963), energy transport along the molecule (Techera et al., 1989; Muto et al., 1988; and Krumhansl et al., 1985), and the interactions of DNA with other molecules (Beger and Prohofsky, 1991 and Lee et al., 1993).

Several theoretical efforts have investigated the possible nonlinear properties of DNA (Techera et al., 1989; Muto et al., 1988; and Krumhansl et al., 1985). Much attention has focused on the possible role of nonlinear wave equations (solitons) in energy transfer along the double helix and in conformation transitions. To date, no definitive experiments have shown that solitons are an integral part of the dynamics of DNA. An earlier report (Williamson et al., 1993) from this laboratory has described a nonlinear property of DNA: optical second harmonic generation (SHG). A weak SHG signal was found at a relative humidity (RH) of 92% when illuminated with 1.06 $\mu$m light from a pulsed Nd-YAG laser.

This paper presents our study of optical third harmonic generation (THG) by wet-spun films of DNA as a function of RH. The THG signal is found to be notably stronger than the SHG signal and to display interesting phenomena described below.

## EXPERIMENTAL

The wet-spinning process was used to produce highly oriented NaDNA films about 30 $\mu$m thick (Rupprecht, 1970 and Rupprecht and Forslind, 1970). These films were bathed in an ethanolic solution with about 0.02 M NaCl. X-ray diffraction patterns (Lindsay et al., 1988) of the NaDNA films indicate that such samples are highly crystalline. Films of RbDNA were prepared by first bathing the cylinder with spun NaDNA fibers for 2 weeks in ethanolic solution with about 0.45 M RbCl, to exchange the $Na^+$ counterion against $Rb^+$. Finally, the cylinder was bathed in an ethanolic solution with 0.02 M RbCl. High frequency Raman experiments (Weidlich et al., 1988) on such films reveal that the A-to-B transition takes place at 92%, and 90% RH for NaDNA and RbDNA, respectively.

These wet-spun films are not optically homogeneous. Structural inhomogeneities cause significant scattering when the film is illuminated with a HeNe laser beam. The half angle of the diffraction pattern is roughly 50°, implying that structural inhomogeneities in the film are of the order of 0.5 $\mu$m. This diffraction effect precludes accurate measurements of the efficiency of THG in DNA.

These films were mounted in sealed test tubes with an inner diameter of 1.6 cm. The water contents of the films were controlled by placing small amounts of saturated salt solutions in the bottom of the test tubes (Washburn, 1926). The samples were allowed to equilibrate for at least 3 days before each experiment. It was necessary to use such wide test tubes since glass can also generate THG. By using the appropriate focal length lenses with these wide tubes, the local power density at the glass was relatively low while the power density at the DNA film was very high. Consequently, essentially all of the detected THG signal comes from the DNA films. This was verified by translating the sample through the focus of the lens.

A continuous mode-locked Nd-YAG laser ($\lambda_{fund}$ = 1.064 $\mu$m) with an acoustooptic Q switch was used to produce a train of approximately 25 100-ps pulses at a rate of 500 Hz. The output of the laser passed through a fixed polarizer to

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*Received for publication 6 July 1993 and in final form 29 September 1993.*

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0006-3495/93/12/2656/05 $2.00

control the polarization of the incident beam. The intensity of the laser beam was controlled by placing a rotatable polarizer before the fixed polarizer. To increase the THG efficiency the laser was focussed onto the DNA sample by a lens of 1 cm focal length. It is estimated that the focused irradiance was about ~6 GW/cm$^2$, which appeared to be below the damage threshold of these films. The helical axis of the DNA was oriented normal to the direction of the propagation of the incident light. Forward-scattered light passed through two filters: (1) a $CuSO_4$ solution which absorbed the IR component, and (2) a narrow bandpass filter centered at 353.5 nm (the THG wavelength was 354.7 nm). A third polarizer was placed in the beam of the forward-scattered light in order to determine the polarization state of the THG light. The signal was detected by a EMI 9635 photomultiplier tube (PMT). The output of the PMT was amplified and averaged (for at least 1000 laser pulses) by a boxcar integrator.

## RESULTS AND DISCUSSION

Narrow bandpass filters at 430 and 300 nm were used to confirm that the observed signal appeared only at the third harmonic wavelength. Additional experiments were performed to confirm that the intensity of the THG signal varied as the third power of the incident fundamental radiation, as shown in Fig. 1.

THG signal from NaDNA was observed from 0% to 98% RH, the range of the experiments, as shown in Fig. 2. The THG light was determined to have the same polarization as the incident fundamental light. For the A conformation of DNA (between ~50 and 92% RH for NaDNA), the THG signal was about twice as intense for light polarized perpendicular to the helical axis than for light polarized parallel to the helical axis. This anisotropy is reminescent of the results of the linear optical polarizabilites measured at 500 nm by Weidlich et al. (1987) This negative anisotropy is understood to be due to the delocalized $\pi$ electrons in the bases. That the THG signal has a similar anisotropy suggests that the $\pi$ electrons also play an important role in the nonlinear optical effects of third harmonic generation. For the B conformation (above 92% RH for NaDNA), the THG harmonic signal was not observed to have any anisotropy. This is surprising since the plane of the bases is almost exactly perpendicular to the helical axis in the B conformation, while in the A conformation the plane of the bases makes an angle of about 20° with respect to the helical axis. Based on this simple geometric observation, B-DNA would be expected to show a greater anisotropy in properties relating to the bases; the opposite is observed for THG. The observed isotropy for the B conformation suggests that the alignment of bases is not the only important parameter in third harmonic generation. X-ray diffraction patterns (Lindsay et al., 1988) show less order for B-DNA (consistent with the THG results). However, the linear polarizabilities study of Weidlich et al. (1987) shows similar anisotropies in both A- and B-DNA, arguing the difference in amount of order in A- and B-DNA does not affect the optical anisotropies.

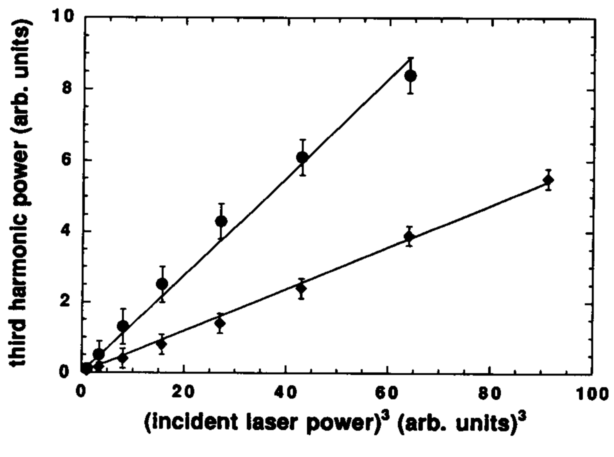

FIGURE 1 The intensity of the THG light from NaDNA as a function of the *cube* of the incident laser power for perpendicular (●) and parallel (♦) polarizations at 84% RH.

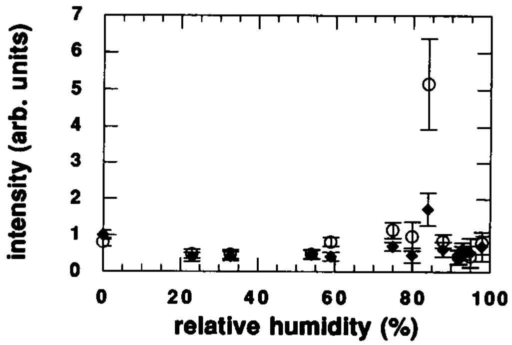

FIGURE 2 The intensity of third harmonic generation from NaDNA as a function of relative humidity for polarization perpendicular (○) and parallel (♦) to the helical axis.

Both THG and SHG were observed in these experiments. It was found that with the same incident laser power and the same detection system (except for a different interference filter) the THG was at least an order of magnitude stronger than the SHG signal. SHG might be expected to be stronger than THG since it involves the absorption of two photons rather than three. Perturbation theory usually finds that higher order processes are weaker. However, selection rules based on symmetry restrict SHG, as will now be discussed. Three photons are involved in SHG: two are absorbed and one is emitted. The states of a system with a center of inversion have definite parity (either even or odd). Since the parity of such a system changes for each absorption or emission of a photon under dipole selection rules, the parity of the initial and final states would be different for SHG, thereby forbidding SHG for a nondegenerate system with a center of inversion. In contrast, THG involves four photons: three are absorbed and one is emitted. This means that the parity of the initial and final states would be the same. Consequently, THG is possible for systems with a center of inversion. The restriction on SHG due to inversion symmetry can also exist for a solid sample composed of molecules without inversion symmetry if the sample has an effective inversion symmetry (on the scale of the volume illuminated by the laser) due to disorder in the sample. This situation has been found in rattail tendon (RTT) which is composed of equal numbers of up- and down-pointing collagen fibrils (Parry and Craig, 1977). The resulting inversion symmetry of RTT should preclude the possibility of SHG, in contrast to the experimental observation of SHG (Roth and Freund, 1981). Careful second harmonic microscopy experiments (Freund et al., 1986) revealed that no SHG was observed from most of the RTT sample. However, SHG was observed from polar filament-like structures within the RTT. Although no studies were done to determine the symmetry of these filament-like structures, they presumably do not have the overall inversion symmetry found in the rest of the sample. The DNA samples used in the current study are similar in structure since the DNA molecule itself does not have a center of symmetry but the helical direction of the DNA molecules in different crystallites is randomly pointing up or down. This greatly restricts the SHG from DNA, resulting in the fact that the THG signal is stronger than the SHG signal.

The most striking feature of Fig. 2 is the fact that the efficiency of THG by NaDNA displays a narrow peak at 84% RH. This peak is seen in both the perpendicular and parallel data, though it is more evident for the perpendicular polarization. The intensity of the third harmonic signal is related to $\chi^{(3)}$ (the third-order hyperpolarizability), $n_f$ and $n_{3f}$ (the refractive indices for the fundamental and third harmonic, respectively) by

$$I_{\mathrm{THG}} = \frac{A\,|\chi^{(3)}|^2}{(n_f - n_{3f})^2}. \tag{1}$$

where $A$ contains parameters which will not give rise to a peak in the THG signal as a function of RH. The physical origin of this peak in the THG signal is of great interest. Eq. 1 indicates that either the hyperpolarizability has a peak at 84% RH or that the condition of $n_f \approx n_{3f}$ (i.e., an increased coherence length) is attained at that RH.

It is unlikely that the hyperpolarizability has a peak of ~2.2 × at 84% RH. Changes in the hyperpolarizability would most likely be associated with a phase transition of DNA double helix. There is no such transition at that RH. The A to B transition occurs at 92% RH. Although the values 84% and 92% RH seem close, the water contents of NaDNA are significantly different at these two RHs: there are ~34 water molecules per base pair at 84% RH and ~47 water molecules per base pair at 92% RH (Lavalle et al., 1990).

Weidlich et al. (1987) measured the linear optical polarizabilities of Li- and NaDNA at 500 nm. They found that the polarizabilities of either Li- or NaDNA in the B or C conformations were about 75 Å$^3$ for the parallel polarization and about 87 Å$^3$ for the perpendicular polarization. For NaDNA in the A conformation, the polarizabilities were about 60 Å$^3$ for the parallel polarization and about 70 Å$^3$ for the perpendicular polarization. These changes of about 20% show that the first order polarizabilities have only a weak dependence on the counterion. It would be reasonable to assume that the third order hyperpolarizabilities would have a similar weak dependence on the counterion. To test this hypothesis, THG was measured from RbDNA for a range of humidities near 84% RH, as shown in Fig. 3. No peak was observed in the data for RbDNA in this range of humidities. This lack of a peak supports the conjecture that the hyperpolarizabilities are not responsible for the peak in the efficiency of the THG.

The lack of a peak in the THG for RbDNA is consistent with the hypothesis that the peak for NaDNA is due to an increased coherence length (i.e., a minimum in the difference between $n_f$ and $n_{3f}$). Weidlich et al. (1987) found that, at a given RH, the refractive indices of DNA depended on the identity of the counterion. This dependence is due to the fact that the volume of the unit cell depends on the counterion since the counterion changes the intermolecular separation. RbDNA would be expected to have different refractive indices than NaDNA. Although $n_f - n_{3f}$ was at a minimum at 84% RH for NaDNA, such a minimum would not be expected at the same RH for RbDNA. Hence, there would be no peak in the THG signal, as observed.

A number of changes accompany the A to B transition (at 92% RH for NaDNA), which involves significant local movement of the atoms of DNA: the helical pitch changes and the bases move with respect to the helical axis. The measurements of the linear optical polarizabilities of Weidlich et al. (1987) showed ~35% increase of the polarizabilities at the A to B transition. It would not be surprising if the third order hyperpolarizabilities were to change appreciably at this transition. However, no increase is noted in the THG efficiency from NaDNA at the A to B transition (92% RH), implying no changes in the hyperpolarizabilities. The fact that the hyperpolarizabilities do not change at the A to B transition makes it even less likely that they change at 84% RH.

Phase matching occurs when the refractive indices of the fundamental and harmonic light waves are equal. As can be seen from Eq. 1, a maximum in the observed THG signal may be due to a minimum in the differences between the refractive indices of the fundamental and third harmonic light waves, corresponding to an increased coherence length, $L_{\mathrm{coh}} \equiv \lambda / |n_f - n_{3f}|$ (which gives a measure of the distance required for the fundamental and third harmonic waves to become 180°

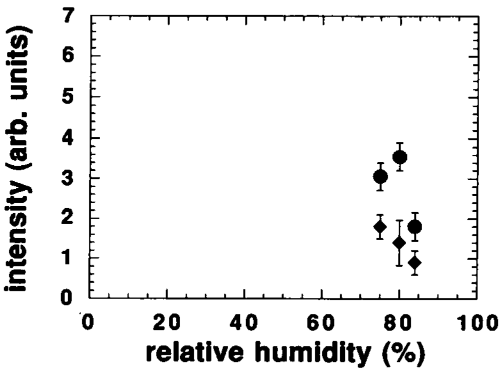

FIGURE 3 The intensity of third harmonic generation from RbDNA as a function of relative humidity for polarization perpendicular (●) and parallel (◆) to the helical axis.

out of phase). In order to determine whether the peak in the THG of NaDNA at 84% RH is due to an increased coherence length, one needs independent measurements of the refractive indices at both the fundamental and third harmonic wavelengths (for both polarizations). No such measurements have been reported. The only reported measurements (Weidlich et al., 1987) of the refractive indices of DNA films as a function of RH were performed on Li- and NaDNA wet-spun films for both parallel and perpendicular polarizations at 500 nm. As mentioned in the Experimental section, these wet-spun films are not of perfect optical quality. The surfaces of the films are rough due to the fact that a thin fibril of DNA is deposited on a rotating cylinder. A thin film is created by moving the fibril back and forth over a certain length along the cylinder (Rupprecht, 1970). The resulting rough surface precludes measurements which depend on an optically flat surface. Weidlich et al. (1987) exploited the rough surface of the wet-spun films by using an index-matching technique. They immersed their hydrated films into fluids of various refractive indices and viewed the immersed film with a microscope (with an interference filter and polarizer) in order to determine which fluid had the same refractive index as the film. Such measurements are not possible for the wavelengths of concern in this THG experiment: the fundamental is in the near IR (at 1.064 $\mu$m) and the third harmonic is in the UV (at 355 nm).

Estimates of the dependence of the refractive indices at these two wavelengths (355 nm and 1.064 $\mu$m) as a function of RH can be obtained. The refractive indices at 355 nm can be estimated by using the fact that the UV absorption has been measured. This absorption can be described by a complex refractive index $n^* = n + ik$ where $k$ is related to the absorption coefficient $\alpha$ by $\alpha = 4\pi k/\lambda$. The real and imaginary parts of the complex dielectric function $\epsilon^* = \epsilon_1 + i\,\epsilon_2$ are related to the $n$ and $k$ via $\epsilon_1 = n^2 - k^2$ and $\epsilon_2 = 2\,nk$. A simple quantum mechanical analysis (Wooten, 1972) yields

$$\epsilon^* = 1 + \frac{4\pi Ne^2}{m}\sum_j \frac{f_{j0}}{\omega_{j0}^2 - \omega^2 - i\Gamma\omega}, \tag{2}$$

where $f_{j0}$ is the oscillator strength of the transition between the initial state 0 and the final state $j$. As is well-known from linear response theory, the real and imaginary parts of the dielectric function are related by a Kramers-Kronig relation due to causality.

The UV absorption of native DNA is dominated by a band centered at 260 nm (Saenger, 1984). This UV absorption is due to electronic transitions involving the bases (Cantor and Schimmel, 1980). The wavelength of the third harmonic (355 nm) is in the "tail" of this absorption band. Consequently, this band is expected to dominate the optical properties at 355 nm. This assumption permits a truncation of the summation in Eq. 2 to include only this transition. This transition has been studied in oriented NaDNA films as function of RH by Falk et al. (1963). Eq. 2 will permit the determination of the *changes* in refractive indices as a function of RH from the absorption data. The other allowed transitions will influence the optical properties at 355 nm, but their influence is diminished since their energies are substantially different. These bands would shift the overall value for the optical constants, though, implying that our calculations of the refractive indices are only accurate to within a constant factor.

The absorption data of Falk et al. (1963) was used with Eq. 2 to determine the parameters $4\pi Ne^2 f_{10}/m$, $\omega_{10}$, and $\Gamma$ of $\epsilon_1$ and $\epsilon_2$. These were then used to evaluate the real part of the refractive index at 355 nm. One uncertainty in this analysis stems from the fact that the thickness of the very thin films of NaDNA had a large uncertainty. The thickness of the films prepared by Falk et al. (1963) was quoted as being between 1 and 5 $\mu$m. This introduces an uncertainty in the magnitude of $k$. This uncertainty is not serious since our concern is with the changes in the optical properties of the film as the water content is changed. A series of calculations were performed with different assumed thicknesses of the films of Falk et al. (1963). These calculations showed that the *dependence* of the refractive indices on RH did not substantially change over the allowed range of thicknesses.

DNA has no absorptions between the 260 nm band in the UV and the 3-$\mu$m band in the IR due to hydrogen stretching motions. The incident laser beam is at 1.064 $\mu$m, which is well-removed from any absorptions. Thus, the optical properties at 1.064 $\mu$m are primarily due to the effects of the transitions at 3 $\mu$m and 260 nm. Simple modeling using Eq. 2 to obtain the real part of the refractive index at 1.064 $\mu$m is not reliable in this region between absorptions. Consequently, a different method is used to estimate the dependence of the refractive indices at 1.064 $\mu$m on the RH. Recall that Weidlich et al. (1987) measured the refractive indices at 0.500 $\mu$m, which is also in the frequency region between the electronic and vibrational absorptions. Although these data are somewhat removed from the desired wavelength of 1.064 $\mu$m, it is reasonable to assume that the refractive indices at the two wavelengths will *change* in the same manner with respect to RH since both wavelengths are in the regions *between* the allowed transitions. Using this assumption we now have all of the information needed to evaluate the possible role of increased coherence length in the observed THG. As our numbers for the two refractive indices (at 355 nm and 1.064 $\mu$m) are both uncertain to within a constant, there are two adjustable parameters in our fits: the constant $A\,|\chi^{(3)}|^2$ of Eq. 1 and the difference between the refractive indices *at a single RH*. Fixing these two parameters (*at a single RH*) constrains the theory. It should be noted that the presence of these two adjustable parameters precludes making any definitive statements about the role of the increased coherence length in the peak of the observed THG signal. A definitive statement could be made only if the refractive indices were actually measured at 1064 and 355 nm as a function of RH.

Our model calculation proceeds as follows: At each RH, the measured $\alpha$ is used to calculate $k$ ($= \alpha\lambda/4\pi$). The parameters $4\pi Ne^2 f_{10}/m$, $\omega_{10}$, and $\Gamma$ are determined by using $k = \{1/2[(\epsilon_1{}^2 + \epsilon_2{}^2)^{1/2} - \epsilon_1]\}^{1/2}$ where $\epsilon_1$ and $\epsilon_2$ are given by Eq. 2 (truncated to include only the dominant 260-nm band). It should be noted that only $4\pi Ne^2 f_{10}/m$ changes as a function of RH; both $\omega_{10}$ and $\Gamma$ are independent of RH. The values of $4\pi Ne^2 f_{10}/m$, $\omega_{10}$, and $\Gamma$ are used to calculate $n$ via $n = \{1/2[(\epsilon_1^2 + \epsilon_2^2)^{1/2} + \epsilon_1]\}^{1/2}$. Repeating this process at each RH yields the dependence of $n_{3f}$ on RH. The refractive indices at 1.064 $\mu$m, $n_f$, are assumed to have the same dependence on RH as the measured refractive indices at 0.500 $\mu$m (Weidlich et al., 1987). That is, the refractive indices at 1.064 $\mu$m are assumed to be the same as at 0.500 $\mu$m except for an overall shift. Fig. 4 shows the intensity of the THG light with perpendicular polarization along with the results of Eq. 2 after adjusting the two parameters: $A\,|\chi^{(3)}|^2$ and the difference between $n_{3f}$ and $n_f$ *at a single RH*. This figure shows very good qualitative agreement between the experimental results and this simple theoretical model. Though the lack of actual experimental data for the refractive indices at 1064 and 355 nm precludes a definitive statement about the role of an increased coherence length in the THG generation, the fact that this simple theoretical model provides a reasonable fit to the experimental data supports this interpretation.

## SUMMARY

Optical third harmonic generation (THG) has been observed for the first time from films of Na- and RbDNA. The THG signal is observed from films exposed to relative humidities (RHs) from 0 to 98%. A strong enhancement (~5×) of the THG signal from NaDNA is observed at 84% RH; no enhancement is observed for RbDNA. The most likely mechanism for such an enhancement is an increased coherence length. Estimates of the refractive indices at both the fundamental and third harmonic frequencies support this interpretation. The observed THG signal has the same polarization as the incident fundamental. For the A conformation, the THG signal polarized perpendicular to the helical axis is approximately twice as strong as the signal polarized parallel to the helical axis. No such anisotropy is observed for either the disordered conformation (below about 50% RH) or the B conformation (above 92% RH).

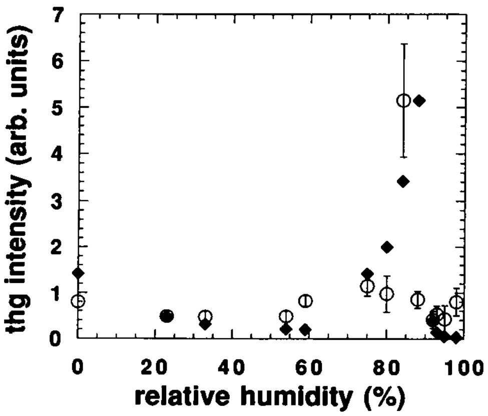

FIGURE 4 The intensity of third harmonic generation from NaDNA as a function of relative humidity with polarization perpendicular to the helical axis. The open circles (○) are the experimental data and the filled diamonds (◆) are the results of the model calculation.

This work has been supported in part by the Office of Naval Research (N00014-91-J1475) and the Swedish Medical Research Council.

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