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ACTIVE TRANSPORT OF GLYCINE AND POTASSIUM IN THE PHOTOSYNTHETIC BACTERIUM *CHROMATIUM VINOSUM*

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The photosynthetic bacterium *Chromatium vinosum* accumulates glycine in an energy-dependent reaction. Uptake is electrogenic but not driven by ΔpH. A K^+ gradient ($[K^+]_{out} > [K^+]_{in}$) is able to drive glycine uptake in *C. vinosum*, while gradients of other cations are ineffective. Among the amino acids tested, a glycine gradient ($[glycine]_{out} > [glycine]_{in}$) was unique in its ability to drive $^{42}K^+$ uptake.

Introduction

Active transport of glycine has been demonstrated in several nonphotosynthetic bacteria [1–4]. These glycine transport systems share several characteristics, including: (1) the utilization of the electrochemical proton gradient ($\Delta\bar{\mu}_{H^+}$) as the primary energy source; (2) net importation of positive charge into the cells during transport; (3) K_m values for glycine in the 2–30 μM range; and (4) competitive inhibition by D-alanine and L-alanine. Because the energetics of D-alanine, L-alanine and glycine transport in each of the previously characterized systems were similar for all three substrates, a hypothesis was formed that the substrates shared a common carrier [1–4].

The photosynthetic purple sulfur bacterium *Chromatium vinosum* has been shown to transport a variety of amino acids, sugars, dicarboxylic acids, and cations (see Ref. 5 for a recent review). All *C. vinosum* amino acid transport systems studied thus far show characteristics consistent with uptake via amino acid/H^+ or amino acid/Na^+ symports (see Refs, 6 and 7 for recent reviews of H^+ and Na^+ symport mechanisms). To date, glycine transport in this bacterium had not been investigated. Below, we report characteristics for glycine transport in *C. vinosum* that differ considerably from all previously described *C. vinosum* amino-acid transport systems and from glycine transport systems in nonphotosynthetic bacteria. Glycine uptake could be driven neither by ΔpH nor by a Na^+ gradient. Unexpectedly, a K^+ gradient, ($[K^+]_{out} > [K^+]_{in}$) was the only cation gradient capable of driving glycine uptake. These results are discussed in the context of a K^+/glycine symport mechanism.

Methods

C. vinosum, strain *D*, was grown on a malate-containing medium as previously described [8]. Bacteriochlorophyll (BChl) concentration was determined after extracting cells with 7 : 2 (v/v) acetone/methanol [9]. Cell protein was calculated using a value of 10 mg protein/μmol BChl (Davidson, V.L., Ph.D. Thesis, Texas Tech Univer-

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Abbreviations: BChl, Bacteriochlorophyll; $\Delta\bar{\mu}_{H^+}$, electrochemical proton gradient; $\Delta\psi$, membrane potential; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; DMSO, dimethylsulfoxide; Mops, 4-morpholinepropanesulfonic acid; TPP, tetraphenylphosphonium.

sity, 1982). Cells were washed twice after harvesting and resuspended in the buffer used for assays, either 50 mM potassium phosphate buffer or 50 mM Mops buffer adjusted to the desired pH with choline base. The $[K^+]$ in the Mops buffer was determined using a Perkin-Elmer Model 303 Atomic Absorbance Spectrometer.

Glycine transport was measured in the dark or at saturating light intensity using the membrane filter assay [10] or flow dialysis technique [11] described previously. Initial rates of uptake were taken at the 3-min point from time-course studies using the membrane filter assay. Samples were allowed to incubate for 10 min (except for samples containing arsenate, which were allowed to incubate for 30 min) under N_2 gas in the dark with any inhibitors, ionophores or uncouplers prior to assay. Concentrated solutions of the inhibitors, ionophores, or uncouplers were prepared in DMSO. Additions of these reagents to the reaction mixtures resulted in DMSO concentrations less than 2% (v/v). At this concentration, no effects of DMSO alone are noted. Additions of amino acids or cations during flow dialysis assays were less than 5% (v/v) of the final reaction mixture. Control experiments with identical volumes of H_2O indicated that no dilution artifacts were present. Radioactivity was measured using a Beckman Model LS 7000 scintillation counter and appropriate scintillation cocktails as described previously [11,12].

Membrane potential ($\Delta\psi$)-driven glycine uptake in the dark was demonstrated by using a K^+/valinomycin diffusion potential, as described in earlier reports [11,12]. Cells were washed twice with 50 mM Mops-choline buffer (pH 7.0) containing 100 mM KCl (final concentration). Cells were then resuspended in 50 mM Mops-choline buffer (pH 7.0) containing either 100 mM choline chloride or 100 mM potassium chloride (final concentration). [^{14}C]glycine was added to each sample, followed by 20 μM valinomycin to initiate transport. Samples were allowed to accumulate glycine for the designated time, then assayed as described previously [11,13]. DCCD was present at a concentration of 300 μM to prevent ATP-driven uptake [5].

CCCP, HOQNO, valinomycin and DCCD were obtained from Sigma. [^{14}C]Glycine (spec. act. 92.6 mCi/mmol) was purchased from ICN Chemical and Radioisotope Division. ^{42}KCl (carrier-free) was purchased from New England Nuclear.

Results

The time-course for glycine uptake by *C. vinosum* cells (see Fig. 1) illustrates that glycine is taken up to a considerably greater extent in the light than in the dark. Glycine uptake in *C. vinosum* exhibited a pH optimum at pH 7.5 and followed Michaelis-Menten kinetics. A Lineweaver-Burk plot of the initial rate of glycine uptake gave a K_m value of 39 μM and a maximum velocity of 14 mmol/min per mol BChl (1.4 nmol/min per mg protein). Identical K_m values were obtained for uptake in the light and in the dark. The K_m and V_{max} values were unaffected by the cation composition of the medium.

CCCP, an uncoupler [14] that eliminates the electrochemical gradient, $\Delta\bar{\mu}_{H^+}$, in *C. vinosum* cells [15] severely inhibits glycine uptake, both in the light and the dark. Furthermore, addition of CCCP

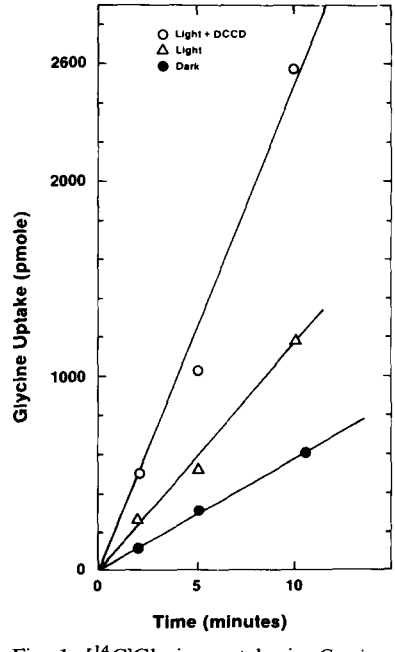

Fig. 1. [^{14}C]Glycine uptake in *C. vinosum* cells. Cells were prepared as described under Methods. Uptake was measured using the filter assay technique. [DCCD] = 300 μM, where present. [Glycine] = 25 μM. 50 mM potassium phosphate buffer (pH 7.5) was used. ○, Light + DCCD; △, light; ●, dark.

to cells which had previously been allowed to accumulate [^{14}C]glycine in the light for 5 min resulted in an efflux of 68% of the labeled material (data not shown). Presumably, the lack of complete glycine efflux after uncoupler addition results from some glycine metabolism during this 5-min period. These results suggest that glycine is transported by the cells in an energy-dependent fashion. HOQNO, an inhibitor of light-driven cyclic electron flow in *C. vinosum* [8,16], also inhibited glycine uptake in the light as shown in Table IA. These findings suggested that the energy source for light-driven glycine uptake might be the electrochemical proton gradient generated by cyclic electron flow. HOQNO did not inhibit glycine uptake in the dark. However, as shown in Table IB, glycine uptake in the dark was substantially inhibited when cells were incubated with arsenate or DCCD. Arsenate has been demonstrated to deplete *C. vinosum* cells of ATP [17], while DCCD is an inhibitor of the *C. vinosum* ATPase [18,19]. Arsenate or DCCD treatment eliminates ATP hydrolysis via the DCCD-sensitive ATPase as a means for generating $\Delta\bar{\mu}_{H^+}$ to provide energy for glycine uptake. When light-driven cyclic electron flow is available as an energy source, neither arsenate nor DCCD inhibited glycine uptake. In fact, as can be seen in Fig. 1, DCCD addition resulted in a significant stimulation of glycine uptake in the light.

TABLE I

THE EFFECT OF UNCOUPLERS, IONOPHORES AND INHIBITORS ON GLYCINE UPTAKE IN *C. VINOSUM* CELLS

(A) Light-driven uptake and (B) dark uptake. Conditions for filter assays were as previously described under Methods. Control values were 16 mmol/min per mol BChl (light-driven uptake) and 6 mmol/min per mol BChl (dark uptake). 50 mM Mops-chlorine buffer (pH 7.5) was used. TPPBr, tetraphenylphosphonium bromide.

	Sample	% Control
A. Light		
1	30 μM [^{14}C]glycine	100
2	+20 μM CCCP	5
3	+300 μM DCCD	125
4	+150 μM HOQNO	40
5	+5 mM arsenate	113
6	+20 μM valinomycin	46
7	+100 μM TPPBr	36
B. Dark		
1	30 μM [^{14}C]glycine	100
2	+20 μM CCCP	4
3	+300 μM DCCD	17
4	+5 mM arsenate	42

Since $\Delta\bar{\mu}_{H^+}$ in *C. vinosum* has two components [15], $\Delta\psi$ (outside positive) and ΔpH (outside acidic), it seemed appropriate to identify the component(s) of $\Delta\bar{\mu}_{H^+}$ which contributed to the driving force for glycine transport. Under the conditions used for the glycine transport assays reported above (pH 7.5 in potassium phosphate buffer), *C. vinosum* cells maintain no pH gradient across the membrane [15], indicating that ΔpH is not an absolute requirement for glycine uptake. Fig. 2A shows that a HCl pulse sufficient to create a ΔpH of 1 pH unit ($pH_{in} > pH_{out}$) failed to drive glycine uptake, and indeed caused efflux of some ^{14}C-labeled material. Uptake of α-methylglucoside [10], aspartate [12] and leucine could readily be demonstrated in similar HCl pulse experiments. These results suggest strongly that ΔpH does not contribute to the driving force for glycine uptake, leaving $\Delta\psi$ as a remaining possible energy source for uptake. This hypothesis was supported by several findings. When $\Delta\psi$, outside positive, was generated using the potassium/valinomycin technique described in Methods, appreciable glycine uptake was seen (see Fig. 3, upper trace). Under these conditions, (dark plus DCCD), no energy source other than $\Delta\psi$ is available for transport. A control experiment in which little K^+ gradient should exist and thus little $\Delta\psi$ should be produced (Fig. 3, lower trace) resulted in significantly less glycine uptake. The cause of the glycine uptake in the control experiment ($\Delta\psi = 0$) is not known. However, it is clear that imposition of $\Delta\psi$ (outside positive) does result in considerably greater glycine uptake than in the control. The fact that glycine uptake is observed under these conditions suggests that glycine transport is an electrogenic process, with transport of glycine into the cell accompanied by import of positive charge. Further support for the involvement of the membrane potential in glycine uptake comes from the observation that agents such as valinomycin in the presence of K^+ [21] and the lipophilic tetraphenylphosphonium (TPP^+) cation [22] that diminish $\Delta\psi$, inhibit

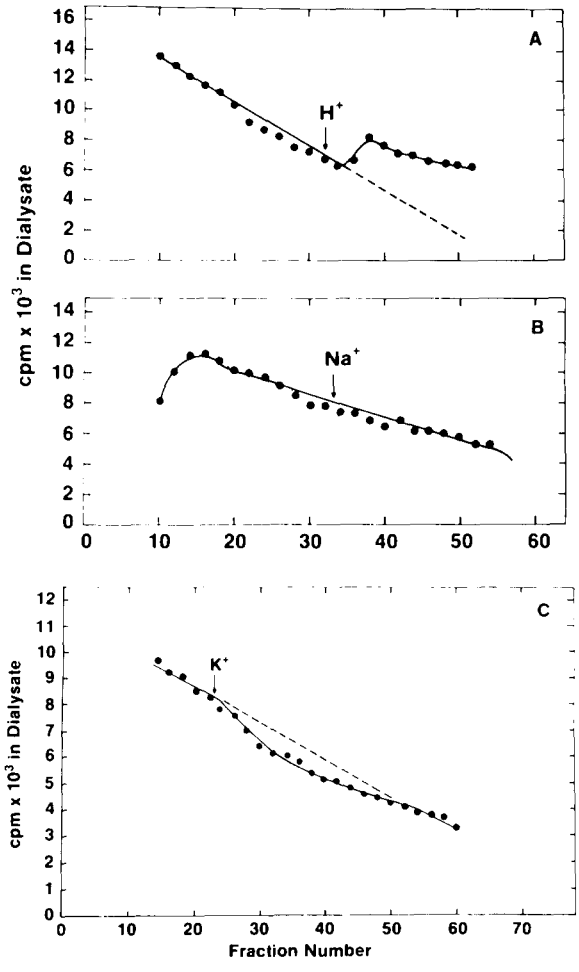

Fig. 2. (A) The effect of ΔpH on [^{14}C]glycine uptake in *C. vinosum*. Cells were prepared for flow dialysis as described in Methods. Where indicated, a 10 μl pulse of HCl was added. DCCD was present at a final concentration of 300 μM. [Glycine] = 30 μM. (B) The effect of a 25 mM NaCl pulse on [^{14}C]glycine uptake. Conditions were as in A except that CCCP was also present at a final concentration of 20 μM. (C) K^+-driven [^{14}C]glycine uptake in *C. vinosum* cells. Cells were prepared for flow dialysis, using 50 mM Mops-choline buffer (pH 7.5) as described in Methods. Where indicated, a 25 mM pulse of KCl was added. [DCCD] = 300 μM, [Glycine] = 30 μM, [CCCP] = 20 μM. The same behavior was noted if 50 mM potassium phosphate buffer was substituted for the Mops-choline buffer.

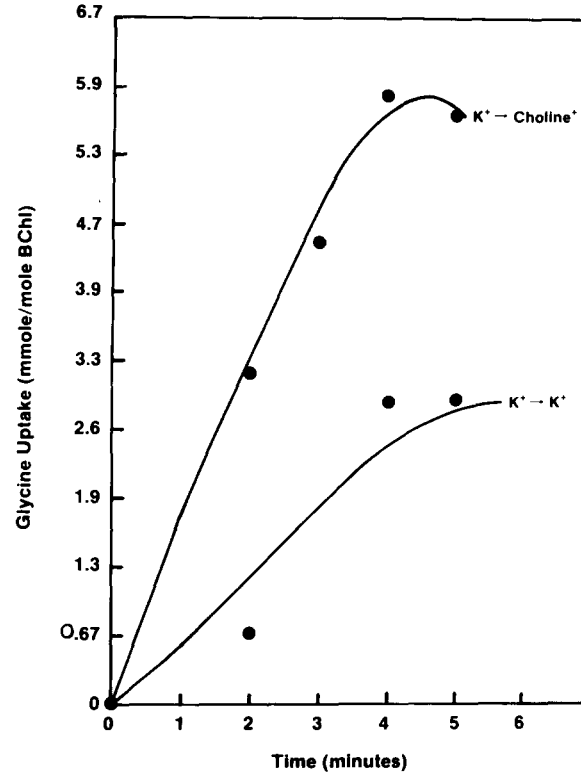

Fig. 3. [^{14}C]Glycine uptake driven by a K^+/valinomycin diffusion potential (outside positive). *C. vinosum* cells were prepared for filter assay as described previously. The procedure for generating the K^+/valinomycin diffusion potential is described in the Methods section. Uptake was measured in the dark. [Glycine] = 30 μM.

light-driven glycine uptake (See Table I).

The demonstration that glycine uptake in *C. vinosum* is probably electrogenic suggested that glycine (which is approximately electroneutral over the pH range in which its uptake is observed) is cotransported with some cation. The data presented above for glycine uptake show considerable similarity to previously characterized *C. vinosum* amino-acid transport systems which are driven by Na^+ gradients across the bacterial plasma membrane ($[Na^+]_{out} > [Na^+]_{in}$) [11–13]. For this reason, a series of flow dialysis experiments was performed to determine whether Na^+, Li^+ (which act as Na^+ analogs in some transport systems [7,23,24]), or any other cations were cotransported with glycine. Fig. 2B shows the results of one such experiment. A 25 mM NaCl pulse capable of driving uptake of several other amino acids in *C. vinosum* [11–13] in the absence of other energy sources failed to drive glycine uptake. A LiCl pulse also failed to produce glycine uptake. The lack of Na^+ gradient-driven glycine transport would seem to eliminate a Na^+/glycine symport mechanism

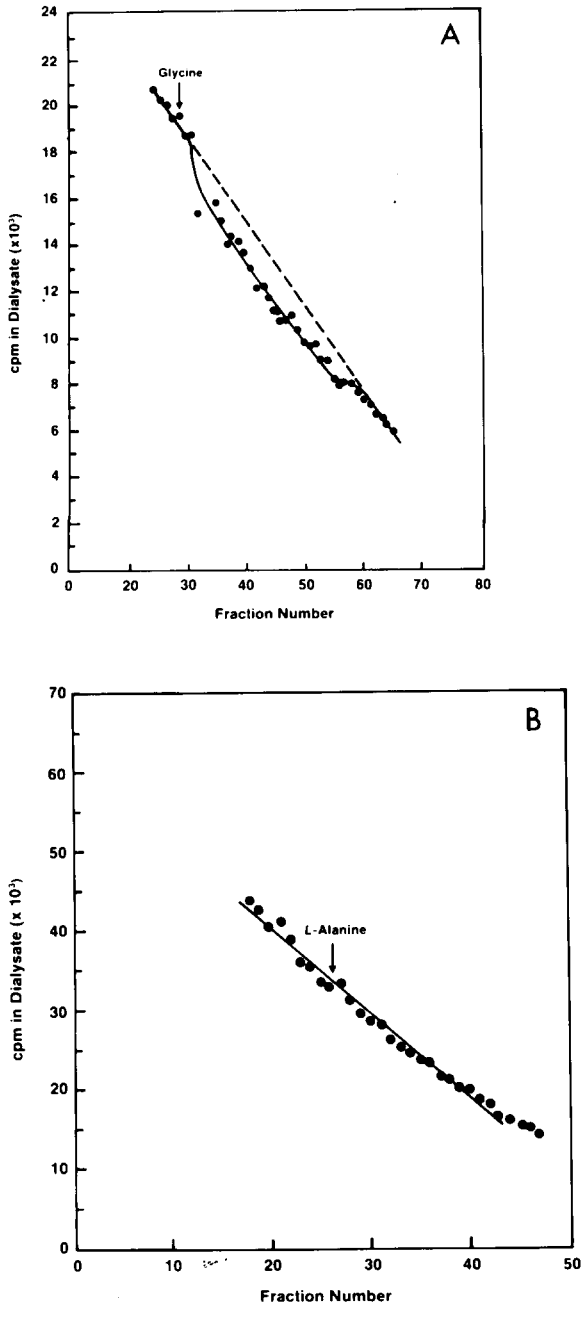

Fig. 4. (A) $^{42}K^+$ uptake driven by a 50 μM glycine pulse. Cells were prepared for flow dialysis, using 50 mM Mops-choline buffer (pH 7.5). Cells were incubated with 300 μM DCCD and 20 μM CCCP prior to addition of the KCl. Spec. act. of ^{42}KCl at the time of this trace was approx. 125 mCi/mmol and [KCl] = 3.0 mM. (B) The effect of 50 μM L-alanine on ^{42}KCl uptake. Conditions were as in (A), except that L-alanine replaced glycine.

for glycine transport in *C. vinosum*. In contrast, K^+ pulses (which had no effect on previously characterized *C. vinosum* amino-acid transport systems [11–13,20]) were able to drive glycine uptake (Fig. 2C) in the absence of any other energy source (DCCD was present to block glycine uptake in the dark driven by ATP hydrolysis and CCCP was present to eliminate any $\Delta\psi$ and ΔpH). K^+ pulse-dependent glycine uptake (in the presence of DCCD and CCCP) was also demonstrated using the membrane filter assay (data not shown). Pulses of other cations (Tl^+, Rb^+) used as K^+ analogs in earlier *C. vinosum* transport experiments [17,25] did not produce any glycine uptake. RbCl had no effect at all on glycine transport and a TlCl pulse caused efflux of some ^{14}C-labeled material, similar to that produced by an HCl pulse shown in Fig. 2A.

If the K^+ gradient-driven glycine uptake results from a K^+/glycine cotransport (symport) system, one might expect that a glycine gradient ($[glycine]_{out} > [glycine]_{in}$) could drive $^{42}K^+$ uptake. The flow dialysis experiment shown in Fig. 4A, demonstrates that a glycine pulse was indeed able to drive $^{42}K^+$ into the cell in absence of any other energy source (CCCP and DCCD were present). Similar glycine-dependent $^{42}K^+$ uptake was observed using the membrane filter assay. Neither L-alanine (Fig. 4B), L-leucine, nor the glucose analog, α-methylglucoside was capable of driving similar $^{42}K^+$ uptake. These results support the idea that K^+ transport is specifically linked to glycine cotransport and not to the transport of other substrates. When 20 μM valinomycin was added to the reaction mixture prior to addition of glycine so that no K^+ gradient could be formed [21], no $^{42}K^+$ uptake was observed after a glycine pulse.

Discussion

The results presented above indicate that the photosynthetic purple sulfur bacterium *C. vinosum* transports glycine in an energy-dependent reaction. Energy for glycine uptake can be provided by the $\Delta\psi$ (outside positive) maintained by *C. vinosum* cells using ATP hydrolysis via a DCCD-sensitive ATPase or light-dependent cyclic electron flow as an energy source [15].

Elimination of $\Delta\psi$ by high concentrations of the

lipophilic TPP^+ cation, under conditions where TPP^+ should not affect other cations gradients, supports the idea that $\Delta\psi$ is a major contributor to the energy for glycine uptake. Further support for the involvement of $\Delta\psi$ in glycine uptake is provided by the ability of CCCP to virtually abolish glycine uptake. While CCCP and TPP^+ both abolish, $\Delta\psi$, the more pronounced inhibition by CCCP on glycine uptake may perhaps be attributed to the ability of CCCP (and not TPP^+) to abolish light-driven K^+ efflux via the *C. vinosum* K^+/H^+ antiport [25]. Inhibition of such K^+ efflux would increase $[K^+]_{in}$ which, in turn, would make coupled K^+/glycine uptake less favorable.

The transport mechanism seems to involve a K^+/glycine symport, as evidenced by the ability of an artificially generated K^+ gradient to drive glycine uptake in the absence of any other energy source and by the ability of glycine gradients to drive $^{42}K^+$ uptake. The specificity of the glycine transporter for K^+ is also supported by the inability of other cations, including the K^+ analogs (Rb^+ and Tl^+), to drive glycine uptake. The results presented above represent the first evidence for a possible bacterial K^+-amino acid cotransport system. In addition, it is possible that in *C. vinosum*, some K^+-independent glycine uptake exists. Other work in our laboratory has shown that glycine acts as a competitive inhibitor for Na^+-dependent D-alanine and L-alanine transport (unpublished data). If glycine is indeed also transported to some extent by these alanine/Na^+ symports, the inability of valinomycin (which should abolish $\Delta\psi$ and K^+ gradients [15]) to completely inhibit glycine transport in *C. vinosum* may be more easily understood.

Although a K^+/glycine symport may explain the observations presented above, it is somewhat perplexing that the kinetic parameters for glycine uptake are not affected by the addition of K^+ to the reaction mixtures. Although the choline-Mops buffers used to prepare the reaction mixtures for this study contain less than 3 μM K^+, analysis of the reaction mixtures showed K^+ concentrations in the 200–300 μM range. Presumably, this K^+ is due to carry-over from the growth medium and/or loss of K^+ from the cells during the course of the assay. If the K_m for K^+ of the putative K^+/glycine symport is significantly less than 200 μM, then no effect of additional K^+ would be expected during light-driven glycine uptake.

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