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Comparison between Size Exclusion Chromatography and Micro-Batch Analyses of Corn Starches in DMSO using Light Scattering Detector

The molecular structure of corn starches different in amylose content (waxy, normal, and high-amylose) was analyzed in 90% dimethyl sulfoxide (DMSO) solution by refractive index (RI) and multi-angle laser light scattering (MALLS) detectors. The starch sample solutions were measured either by medium-pressure size exclusion chromatography (MPSEC) or by the micro-batch mode. For waxy corn starch, the average molar mass (M_w) and radius of gyration (R_g) values were similar in both methods. However, for normal and high-amylose corn starches, M_w measured by the micro-batch mode was 2–4 times greater than that by the chromatographic method, although R_g values obtained from both methods were not very different. The M_w difference was the greater the higher the amylose content of starch.

Keywords: Corn starch; DMSO; Average molar mass; Radius of gyration

1 Introduction

Water is a common solvent for physical and chemical analysis of starch. However, native or amorphous starch, when in the dry state, usually requires excessive heating to be dissolved in neutral water. Furthermore, the starch chains dispersed readily aggregate when the solution is cooled or stored. Dimethyl sulfoxide (DMSO) is an alternative as starch solvent, in which the starch chain aggregation is minimized. To disperse starch in DMSO effectively, however, a small amount of water is often required to prevent the rapid swelling of starch granules or particles, because the surface gel layer could inhibit DMSO penetration into the granule [1]. DMSO has been also used as a mobile phase in size exclusion chromatography (SEC) for starch fractionation [2–6]. By using DMSO instead of water as an eluent, the chain aggregation and structural damage that could be induced by chromatography are effectively reduced.

A laser light-scattering analysis is one of the few methods available for the determination of absolute molar mass and shape of polymers. This method has often been used with high-performance size exclusion chromatography (HPSEC) and a refractive index (RI) detector for simultaneous analysis together with polymer fractionation. Alternatively, the light scattering detector can be used in micro-batch mode, in which the constituents in a solution are measured together. The light scattering analysis provides weight-average molar mass (M_w), radius of gyration (R_g), and the second virial coefficient (A_2) which characterizes the interactions between polymers and solvent [7].

When a HPSEC column is used, molecular degradation could occur due to the pressure, shear and friction prevailing in the column [8]. Thus, it was suggested that a medium- or low-pressure SEC column is desirable for the ideal fractionation of starch [9]. However, few studies have been reported on structural analysis of starch using a medium- or low-pressure column with multiangle laser light scattering (MALLS) detector. The chain degradation of starch by the shear or friction in the chromatographic mode may generate the discrepancy between the structural data from micro-batch and HPSEC modes [10].

In the present study, the molecular structure of corn starches containing different amylose contents was measured in both medium-pressure SEC and micro-batch modes using a MALLS detector and 90% DMSO as starch solvent and eluent, and the data from both modes were compared.

2 Materials and Methods

2.1 Materials

Waxy and normal corn starches were provided by Samyang Genex Co. (Seoul, Korea), and high-amylose corn starch was obtained from Cargill Inc. (Hammond, IN, USA). The starches were purified using DMSO and etha-

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nol. One gram of starch was dispersed in 90% DMSO (100 mL) by heating in a boiling water-bath for 1 h, and then magnetic-stirred at room temperature for 24 h. The starch was precipitated and washed with ethanol (300 mL), and then dried in a convection oven (30°C).

2.2 Solution preparation for SEC-MALLS

Dry and pure starch (10 mg, dry solids) was weighed in a glass vial and wetted with ethanol (20 μL), then 90% DMSO (5 mL) was added. The sample vial was capped, and then heated for 1 h in a boiling water bath with magnetic stirring. The starch solution was then mildly stirred (at 155 rpm) at room temperature for 8 h using a magnetic stirrer prior to SEC analysis.

2.3 Solubility

The starch sample solutions were filtered through a membrane filter (PTFE 5.0 μm, Millipore Corporation, Bedford, MA), and the starch concentrations before and after filtration were determined by the phenol-sulfuric acid colorimetric method [11]. Solubility was calculated under the assumption that the soluble starch solely passed the membrane filter.

2.4 Size exclusion chromatography (SEC) mode

The mobile phase used for SEC was 90% DMSO (HPLC grade) containing LiBr (50 mM), that had been filtered through a 0.2 μm nylon 66 membrane filter (Supelco, Bellefonte, PA) and degassed. The SEC column (2.6 × 70 cm) contained Toyopearl HW 65 F resins (Tosoh Corp. Tokyo, Japan) of particle and pore sizes, 30–60 μm and 100 nm, respectively. The system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 1 mL loop (model 7072, Rheodyne, Cotati, CA), the SEC column, a MALLS detector (632.8 nm, DAWN DSP-F, Wyatt Technology, Santa Barbara, CA), and a RI detector (Optilab DSP, Wyatt Technology, Santa Barbara, CA). Flow rate was 0.8 mL/min, and the pump pressure was 275.9 kPa (40 psi). The starch solution was filtered through a 5.0 μm PTFE membrane filter (Millipore Corporation, Bedford, MA) and injected into the column. A specific refractive index increment value (d*n*/d*c*) was 0.074 mL/g [12].

2.5 Micro-batch mode

In the micro-batch mode, starch-DMSO solutions of different concentrations were prepared with the same treatment used for SEC mode. The starch concentrations in DMSO were in a range from 0.024 to 0.816 mg/mL. All M_w and R_g values were calculated form Berry plots of the scattered light intensity. The instrumental system was identical to the SEC mode.

3 Results and Discussion

To acquire reliable data, starch should be completely dissolved [5, 9]. *Millard* and co-workers [12] reported that the dissolution method affected the analytical data in size and shape for waxy maize starch. In this experiment, starch dissolution was done by following the optimized condition found in our previous study [9]: stirring for 8 h at room temperature after boiling for 1 h. The SEC recovery for all the three corn starches different in amylose content was more than 98% based on the RI responses, indicating that there was no detainment of starch on the filter or in the column.

Tab. 1 shows the comparison the data of molecular structure for the corn starches different in amylose content, determined by the SEC mode with MALLS detector. The M_w of waxy corn amylopectin was the highest, whereas that of high-amylose corn starch was lowest, indicating that the molar mass of amylopectin was reduced as the amylose content increased. *Klavons* and co-workers [13] analyzed the jet-cooked waxy corn starch in DMSO, and measured the M_w and R_g by light scattering to be 224×10^6 g/mol and 164 nm, respectively. *Yokoyama* and co-workers [4] heated waxy corn starch in

Tab. 1. Structural characteristics of corn starches of different amylose contents measured by a medium-pressure SEC mode.

Starches	Amylopectin			Amylose			Solubility [%]
	$M_w(\times 10^6)$	R_g [nm]	SV_g	$M_w(\times 10^6)$	R_g [nm]	SV_g	
Waxy	254±1.4	241±0.3	0.14	–	–	–	97.5±0.8
Normal	243±0.8	247±0.2	0.16	3.1±0.8	164±0.2	3.55	98.4±0.8
High-amylose	197±2.2	214±0.3	0.23	1.5±0.6	83±0.3	0.97	100±0.2

DMSO solution at 95°C for 15 min, and then cooled it at room temperature for 18 h with stirring. By light scatting analysis using SEC and MALLS detector, they obtained M_w and R_g of waxy corn starch, 215×10^6 g/mol and 252 nm, respectively. These values were somewhat lower than the values measured in the present experiment. This could result from the differences in the column and treatments for starch dissolution.

The specific volumes for gyration (SV_g), calculated under the assumption that the starch chains have a spherical gyration [14], were 0.14, 0.16, and 0.23 for waxy, normal, and high-amylose corn amylopectins, respectively. Although the R_g values were not much different among the amylopectins, different SV_g values indicated that the amylopectin structure in the three corn starches was not the same. In our previous study [15], the debranched chain length of amylopectins in waxy, normal and high amylose corn starches was analyzed. Waxy corn starch had the shortest B$\geq$2 chains (weight average chain length, CL_w, 144), whereas high-amylose corn starch contained the amylopectin with longest B$\geq$2 chains (CL_w 261, 12.1%). Moreover, distinctive differences were found in B1 chains. High-amylose corn starch contained longer B1 chains (CL_w 49.7) with greater proportion (28.5%), than did waxy corn starch (CL_w 40, 14.3%). Thus the greater chain length and proportion of the B chains in high amylose corn starch resulted in the relatively lower compactness of amylopectin.

Only few studies were reported on M_w and R_g of amylose by SEC and MALLS detector using DMSO as eluent. *Radosta* and co-workers [5] measured synthetic amylose in DMSO, and reported its M_w and R_g to be 1.3–2.3×10^5 g/mol and 19–36 nm, respectively. These values were much smaller than ours, indicating that the amyloses tested might not be same. The amylose conformation in DMSO, based on intrinsic viscosity and molecular weight, may vary from a random coil [16] to a helical structure [17, 18]. *Cheetham* and *Tao* [19] reported that amylose in 100% DMSO existed in tight helices, but with addition of water, the interaction between amylose and DMSO was reduced progressively, leading to the conformational transition to loose helices. However, we obtained much greater SV_g value of normal corn amylose in DMSO (3.55) than that in water (1.0, from a separate experiment). This suggests that the hydrodynamic volume of normal corn amylose is larger in DMSO than in water. As a starch solvent, DMSO allows the amylose chains fully extended.

The SV_g value of amylose in high-amylose corn starch was much smaller than that in normal corn amylose, suggesting that the former configured with more compactness (Tab. 1). This result was opposite to the case for amylopectin. *Han* and co-workers [15] compared the length and proportion of debranched amylose chains between high-amylose and normal corn starches. The relative content of the long amylose chains in debranched normal corn starch was 20.3%, whereas that in debranched high amylose corn starch was 35.9%. These percentages were much lower than the actual amylose contents in both starches (approximately 28 and 70% for normal and high-amylose corn starch, respectively). Thus, the amylose in both starches contained the short chains that could elute with amylopection chains in the chromatograms of debranched starches. Therefore, because the difference in the contents of the long amylose chains and total amylose (35.9 vs. 70%) was much greater for high amylose corn starch than that for normal corn starch (20.3 vs. 28%), high amylose corn starch contained more short amylose chains than did normal corn starch. *Takeda* and co-workers [20] also reported that amylose in amylomaize (high amylose corn) was comprised of branched molecules with the inner chains, shorter than the inner chains in normal corn amylose. The larger proportion of the short amylose chains, therefore, allows the amylose in high amylose corn starch to form more compact structure than normal corn amylose, as indicated by the SV_g values measured in this experiment.

Fig. 1 shows the RI chromatograms of the three corn starches tested in 90% DMSO. Waxy corn starch showed a single elution peak at the void volume of the column. Normal corn starch showed a bimodal distribution with the amylose content of 28.2%. But for high-amylose corn starch, the tail part of the amylose peak overlapped with the salt peak, because the minimum size limit of the column was in the M_w range of amylose. There was a clean separation between amylopectin and amylose fractions in the chromatogram.

Fig. 2 shows the Berry plot of the square root of $K \cdot c/R_\Theta$ vs. sin (θ/2) for waxy corn starch, where K was the optical constant and R_θ was the scattering intensity at scattering angle θ. The plot displayed a good theoretical agreement of the scattering intensity for the M_w calculation. Normal and high amylose corn starches also displayed the similar plots with good theoretical agreements.

Tab. 2 shows the data from both micro-batch and SEC chromatography modes. The M_w (R_g) of waxy corn starch by chromatography and micro-batch modes were 254×10^6 g/mol (241 nm), and 274×10^6 g/mol (255 nm), respectively. The values from the batch mode were slightly greater than those from the chromatographic analysis. Several researchers have measured molecular structure of starch by micro-batch and SEC modes for comparison [5, 10, 13]. *Radosta* and co-workers [5] measured synthetic amylose by batch and SEC modes,

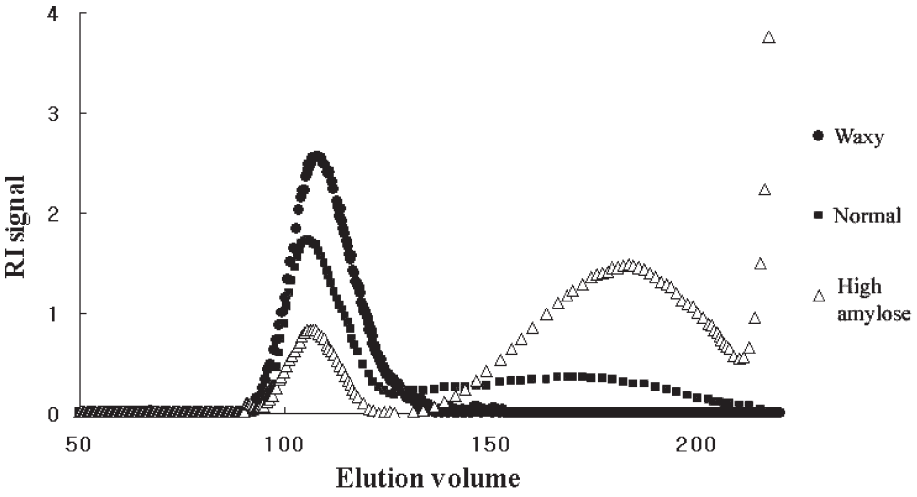

Fig. 1. RI chromatogram of corn starches of different amylose contents in 90% DMSO.

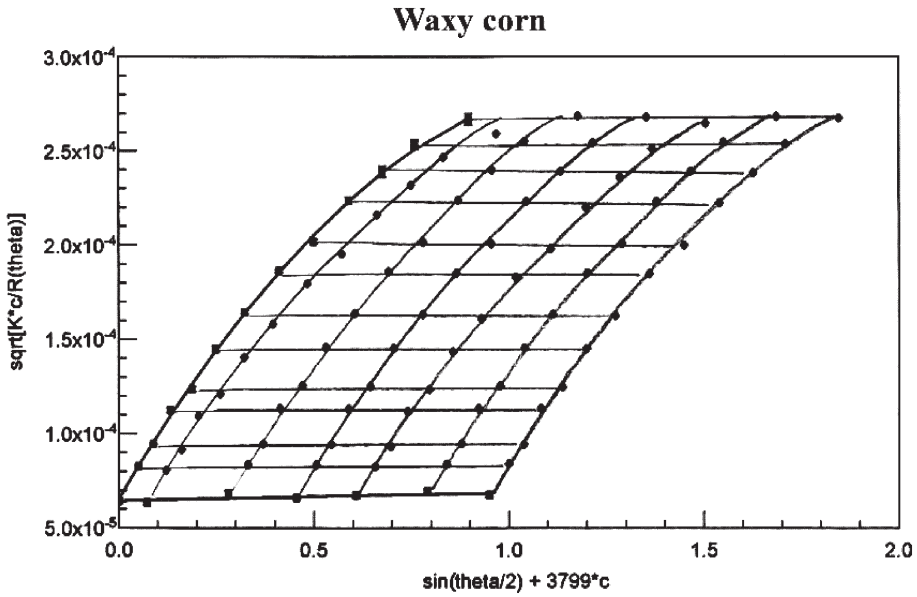

Fig. 2. Berry plot of light scattering intensity for waxy corn starch in 90% DMSO, measured in micro-batch mode.

Tab. 2. Comparison in M_w and R_g values between SEC and micro-batch modes.

Starch	SEC (Total)		Micro-batch		
	M_w ($\times$ 10^6)	R_g [nm]	M_w ($\times$ 10^6)	R_g [nm]	A_2 ($\times$ 10^{-6})
Waxy	254±1.4	241±0.3	274±2.5	255±1.3	1.14
Normal	127±0.9	219±0.2	208±1.9	246±1.2	1.51
High-amylose	28±1.2	173±0.4	128±3.3	194±0.8	2.35

and reported that the values of molecular parameters such as M_w and R_g were in good agreement by two modes. However, *You* et al. [10] found that M_w of debranched wheat amylopectin was higher in micro-batch analysis than in high-pressure SEC mode. They hypothesized that the macromolecules could be degraded by the shear while the polymers passing through the pressurized column. *Barth* and *Carlin* [8] also reported the possibility of the shear-induced depolymerization when a pressurized column was used. However, the medium-pressure

column used in this experiment would induce almost no shear degradation. Due to this, the difference in M_w and R_g of waxy corn starch, between SEC and micro-batch modes, was minor.

Normal and high-amylose corn starches, however, showed significant differences in M_w and R_g values between the two analysis modes. In normal corn starch, the total M_w (amylopectin plus amylose) was 127×10^6 g/mol in SEC mode and 208×10^6 g/mol in micro-batch mode. In high-amylose corn starch, the total M_w was 28×10^6, and 128×10^6 g/mol, respectively, in SEC and in micro-batch modes. In both cases, the values from micro-batch mode were twice or four times greater than those from SEC mode, and the difference was greater when the amylose content of the starch was higher. In the micro-batch mode, both starch chains were measured together in a single batch, whereas in the chromatography mode, both were analyzed individually after being separated. So it was expected that amylopectin chains giving much greater scattering intensity could dominate when all starch chains were measured in batch mode. This could result in the higher M_w than the real average value, as suggested by *You* et al. [10]. But it was noteworthy that the difference in the radius of gyration (R_g) was not as significant as that in M_w (Tab. 2). Because amylose chains had a relatively higher specific volume than did amylopectin chains, the difference in R_g between amylopectin and amylose was much less than that in M_w (Tab. 1). Therefore, the effect of dominating response of amylopectin was much less on the average R_g value than that on the average M_w.

The A_2 value, the second viral coefficient, measured by the batch mode is a measure of polymer-polymer, polymer-solvent and solvent-solvent interactions. Therefore, a greater A_2 indicates more interactions between polymer and solvent. *Nakanishi* and co-workers [21] reported that as the molar mass of a polymer increased, A_2 value decreased. When comparing the three corn starches, the A_2 values of waxy, normal and high-amylose corn starches were 1.14, 1.51 and 2.35 $\mu mol \cdot mL/g^2$, respectively. Although the differences were small, they clearly showed a tendency of A_2 increase with rising amylose content. *Radosta* and co-workers [5] measured A_2 value of synthetic amylose in DMSO as $0.87–1.14 \times 10^{-3}$ $mol \cdot mL/g^2$. This value is relatively larger than the values in the present experiment. This suggests that amylose itself has a much greater virial coefficient than does amylopectin. The larger specific volume resulting from the chain linearity might allow the amylose to interact with solvent more readily.

Although a laser light scattering detector is an instrument for the determination of molar mass for macromolecules in solution, difficulties in interpreting light scattering results for large amylopectin polymers have been reported in recent investigations [22, 23]. Also, light scattering measurement could often give a wrong value, as solutions can be easily contaminated with dust [24]. However, in an organic system such as DMSO, the contamination with dust is much less than in an aqueous system. Moreover, proper and complete dissolution of the starch is the prerequisite to give accurate M_w and R_g values. As *Han* and *Lim* [9] emphasized, maximum solubilization with minimum starch degradation should be achieved prior to measurement, in order to obtain accurate data. Thus, with proper and careful sample treatment and optimized analytical conditions, the experimental error can be minimized.

4 Conclusion

In comparison between the two analytical modes, a sample of single composition, like waxy starches, molecular characterization could result in identical data either from medium pressure SEC or from micro-batch mode. However, in a sample solution which contains two or more components, significantly different in size and shape, like amylose and amylopectin, fractionation of the components is highly recommended to obtain accurate data.

References

[1] O.-J. Peng, A. S. Perlin: Observations on N.M.R. spectra of starches in dimethyl sulfoxide, iodine-complexing, and solvation in water-dimethyl sulfoxide. *Carbohydr. Res.* **1987**, *160*, 57–72.

[2] S. Kobayashi, S. J. Schwartz, D. R. Lineback: Rapid analysis of starch, amylose and amylopectin by high-performance size-exclusion chromatography. *J. Chromatogr.* **1985**, *319*, 205–214.

[3] J.-Y. Chuang, R. J. Sydor: High performance size exclusion chromatography of starch with dimethyl sulfoxide as the mobile phase. *J. Appl. Polym. Sci.* **1987**, *34*, 1739–1748.

[4] W. Yokoyama, J. J. Renner-Nantz, C. F. Shoemaker: Starch molecular mass and size by size-exclusion chromatography in DMSO-LiBr coupled with multiple angle laser light scattering. *Cereal Chem.* **1998**, *75*, 530–535.

[5] S. Radosta, M. Haberer, W. Vorwerg: Molecular characteristics of amylose and starch in dimethyl sulfoxide. *Biomacromolecules* **2001**, *2*, 970–978.

[6] W. Vorwerg, S. Radosta, E. Leibnitz: Study of a preparative-scale process for the production of amylose. *Carbohydr. Polym.* **2002**, *47*, 181–189.

[7] S. Girod, P. Baldet-Dupy, H. Maillols, J.-M. Devoisselle: On-line direct determination of the second viral coefficient of a natural polysaccharide using size-exclusion chromatography and multi-angle laser light scattering. *J. Chromatogr. A* **2001**, *943*, 147–152.

[8] H. G. Barth, F. J. Carlin: A review of polymer shear degradation in size-exclusion chromatography. *J. Liquid Chromatogr.* **1984**, *7*, 1717–1738.

[9] J.-A. Han, S.-T. Lim: Structural changes of corn starches by heating and stirring in DMSO measured by SEC-MALLS-RI system. *Carbohydr. Polym.* **2004**, *55*, 265–272.

[10] S. You, M. Fiedorowicz, S.-T. Lim: Molecular characterization of wheat amylopectins by multiangle laser light scattering analysis. *Cereal Chem.* **1999**, *76*, 116–121.

[11] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith: Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

[12] M. M. Millard, F. R. Dintzis, J. L. Willett, J. A. Klavons: Light-scattering molecular weights and intrinsic viscosities of processed waxy maize starches in 90% dimethyl sulfoxide and H_2O. *Cereal Chem.* **1997**, *74*, 687–691.

[13] J. A. Klavons, F. R. Dintzis, M. M. Millard: Hydrodynamic chromatography of waxy maize starch. *Cereal Chem.* **1997**, *74*, 832–836.

[14] S. You, S.-T. Lim: Molecular characterization of corn starch using an aqueous HPSEC-MALLS-RI system under various dissolution and analytical conditions. *Cereal Chem.* **2000**, *77*, 303–308.

[15] J.-A. Han, J. N. BeMiller, B. Hamaker, S.-T. Lim: Structural changes of debranched corn starch by aqueous heating and stirring. *Cereal Chem.* **2003**, *80*, 323–328.

[16] W. Banks, C. T. Greenwood: The hydrodynamic behavior of native amylose in good solvent. *Carbohydr. Res.* **1968**, *7*, 414–420.

[17] J. M. G. Cowie: Studies on amylose and its derivatives. Part 1. Molecular size and configuration of amylose molecule in various solvents. *Macromol. Chem*. **1961**, *42*, 230–247.

[18] M. Fujii, K. Honda, H. Fujita: Dilute solution of amylose in dimethylsulfoxide. *Biopolymers* **1973**, *12*, 1177–1195.

[19] N. W. H. Cheetham, L. Tao: Amylose conformational transitions in binary DMSO/water mixture. *Carbohydr. Polym.* **1998**, *35*, 287–295.

[20] C. Takeda, Y. Takeda, S. Hizukuri: Structure of amylomaize amylose. *Cereal Chem.* **1989**, *66*, 22–25.

[21] Y. Nakanishi, T. Norisuye, A. Teramoto: Conformation of amylose in dimethyl sulfoxide. *Macromolecules* **1993**, *26*, 4220–4225.

[22] T. Aberle, W. Burchard, W. Vorwerg, S. Radosta: Conformational contributions of amylose and amylopectin to the structural properties of starches from various sources. *Starch/Stärke* **1994**, *46*, 329–335.

[23] R. Hanselmann, M. Ehart, H. M. Widmer: Sedimentation field flow fractionation combined with multi-angle laser light scattering applied for characterization of starch polymers. *Starch/Stärke* **1995**, *46*, 345–349.

[24] M. M. Millard, W. J. Wolf, F. R. Dintzis, J. L. Willett: The hydrodynamic characterization of waxy maize amylopectin in 90% dimethyl sulfoxide-water by analytical ultracentrifugation, dynamic and static light scattering. *Carbohydr. Polym.* **1999**, *39*. 315–320.

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