

Bromination of Silk Fabrics

KEN-ICHI FURUHATA, MICHIKO KAWAMOTO

Department of Organic and Polymeric Materials, Faculty of Engineering, Tokyo Institute of Technology, 2-12-1 O-okayama, Meguro-ku, Tokyo, 152, Japan

Received 10 October 1996; accepted 22 November 1996

ABSTRACT: The bromination of silk fabrics was studied under heterogeneous conditions in *N,N*-dimethylformamide using three reagent systems, molecular bromine, methanesulfonyl bromide (MsBr), and *N*-bromosuccinimide (NBS)–triphenylphosphine (PPh₃) in combination with sodium bromide. Molecular bromine brominated tyrosine residues selectively and consecutively to 3-bromotyrosine and 3,5-dibromotyrosine residues. MsBr reacted only with serine residues to give 3-bromoalanine (Br-Ala) residues. The addition of sodium bromide to the NBS–PPh₃ system suppressed the bromination of tyrosine residues and slightly increased the yield of Br-Ala residues. © 1997 John Wiley & Sons, Inc. *J Appl Polym Sci* **64**: 2031–2036, 1997

Key words: bromination; silk fabric; 3-bromoalanine; bromotyrosines

INTRODUCTION

In the course of the studies on the chemical modification of protein fibers, we reported the halogenation of *Bombyx mori* silk fibroin under homogeneous¹ and heterogeneous^{2,3} conditions. One of the purposes of these studies is to convert serine (Ser) residues, abundant in *B. mori* silk fibroin (about 1.4 mmol/g), into 3-haloalanine (X-Ala) residues. The nucleophilic substitution of halogen atoms of X-Ala residues with amines^{1,2,4,5} and thiols^{3,5} were studied for the introduction of functional groups into silk. Chlorinated and consecutively amino group-enriched silk fabrics⁵ were used as supports for enzyme immobilization.⁶

In nucleophilic substitution reactions, bromine–carbon bonds are more reactive than chlorine–carbon bonds. We have shown^{7,8} that bromodeoxycellulose is more suitable than chlorodeoxycellulose for obtaining deoxymercaptocellulose derivatives by the reaction with thiols in the presence of a base. In the case of silk, 3-bromoalanine

(Br-Ala) residues formed from Ser residues were more labile than 3-chloroalanine (Cl-Ala) residues.³ They could be converted into 3-aminoalanine, 3-alkylaminoalanine, and *S*-substituted cysteine residues by the treatments with aqueous ammonia, alkylamines, and thiols, respectively.^{1–5} In our previous report on the bromination of silk fabrics under heterogeneous conditions,² *N*-bromosuccinimide (NBS) and triphenylphosphine (PPh₃) were used in *N,N*-dimethylformamide (DMF). This system is recommended for the replacement of primary hydroxyl groups with bromine.⁹ We found that the result of the reaction of silk fabrics with this system depended on the [PPh₃]/[NBS] molar ratio. The bromination of tyrosine (Tyr) residues to 3-bromotyrosine (Br-Tyr) and 3,5-dibromotyrosine (Br₂-Tyr) residues occurred along with the conversion of Ser residues to Br-Ala residues at [PPh₃]/[NBS] of 1.0 or lower. The bromination of Tyr residues prevailed at lower molar ratios. Only the conversion of Ser residues to Br-Ala residues was observed at [PPh₃]/[NBS] higher than 1.0 but the yield of Br-Ala was not high. It is therefore desirable to examine other reagents for the selective bromination of Ser or Tyr residues.

Correspondence to: K. Furuhashi.

© 1997 John Wiley & Sons, Inc. CCC 0021-8995/97/102031-06

This article describes the bromination of silk fabrics with three reagent systems in DMF. Molecular bromine brominates Tyr residues consecutively while methanesulfonyl bromide (MsBr) substitutes hydroxyl groups of Ser residues with bromine atoms. Amino acid residues other than those of Tyr or Ser are not modified with these reagents. The effect of the addition of sodium bromide to the NBS–PPh₃ system is also studied in an attempt to improve the selectivity for the formation of Br-Ala residues.

EXPERIMENTAL

Materials

Silk fabrics (standard adjacent fabric No. 2–1 conformable to JIS L0803, Nihon Kikaku Kyokai) were Soxhlet extracted with ethanol, washed with distilled water, air dried, and stored under reduced pressure over P₂O₅ in a desiccator. The observed serine and tyrosine contents were 1.390 and 0.622 mmol/g fabric, respectively. DMF was purified with a conventional procedure and stored over Linde Type 5A molecular sieves under nitrogen atmosphere. Commercial bromine was washed with concentrated sulfuric acid before use. MsBr was synthesized according to the method of Sieber.¹⁰ NBS and PPh₃ were recrystallized from distilled water and from ethanol, respectively, and stored under reduced pressure. Br-Tyr was synthesized according to the method of Zeynek.¹¹ Br₂-Tyr was a product of Tokyo Kasei Kogyo Co. The NMR analysis showed that it contained 5 mol % of Br-Tyr. 3-Propylaminoalanine was synthesized according to the method reported by Asquith and Carthew.¹² S-2-Aminoethylcysteine hydrochloride and 2-aminoadipic acid were purchased from Sigma Chemical Co. Commercial sodium bromide was dried under reduced pressure.

Bromination with Br₂

Pieces of the dried silk fabric (0.15 g) were immersed in DMF under nitrogen, kept for 18 h at room temperature, and 0.1M bromine in DMF was added. The molar ratio of bromine to Tyr residues in the sample was set to 30 and the liquor ratio was fixed to 200 in all experiments. The reaction flask was stoppered under nitrogen, set to an incubator and kept at 50°C for a predetermined period. The brominated samples were washed

with DMF for 2 h, with methanol for 24 h and dried under reduced pressure over P₂O₅ for 2 days.

Bromination with MsBr

A piece of the dried silk fabric (15 mg) was immersed in DMF under nitrogen, kept for 24 h at room temperature, and MsBr in DMF was added. The molar ratio of MsBr to Ser residues in the sample was set to 50 in all experiments and the liquor ratio was fixed to 200. The bromination was carried out under nitrogen at 60°C for 24 h in an incubator and the brominated samples were washed and dried as above.

Bromination with NBS–PPh₃ in the Presence of NaBr

Pieces of the dried silk fabric (0.25 g) were immersed in DMF under nitrogen and a calculated amount of sodium bromide (molar ratio to NBS, 0.25–1.0) was added. The mixture was kept at room temperature for 2 days, then cooled with ice water and NBS in DMF was added. The molar ratio of NBS to Ser residues in the sample was set to 9 in all experiments. A DMF solution of PPh₃ (equimolar to NBS) was then added and the liquor ratio was fixed to 40. The reaction flask was set to an incubator, held at room temperature for 18 h, and then at 50°C for 3 h. The brominated samples were washed and dried as above.

Treatment with Propylamine and 2-Aminoethanethiol

Brominated fabrics were treated at 45°C in an incubator with a 0.1M aqueous solution of propylamine for 4 h at the liquor ratio of 100, or with 0.1M alkaline solution of 2-aminoethanethiol (dissolved in 0.2 N NaOH) for 3 h at the liquor ratio of 200. The treated fabrics were washed with distilled water to neutral, then with methanol, and dried under reduced pressure over P₂O₅.

Analyses

For the analysis of constituent amino acids, samples (about 5 mg) were accurately weighed, put in 5 mL of 6 N HCl in a hydrolysis tube, degassed and hydrolyzed at 100°C for 24 h. A Shimadzu gas chromatograph–mass spectrometer LKB 9000 was used with a data analyzer Shimadzu GC-MSPAC 300 for the detection and structural anal-

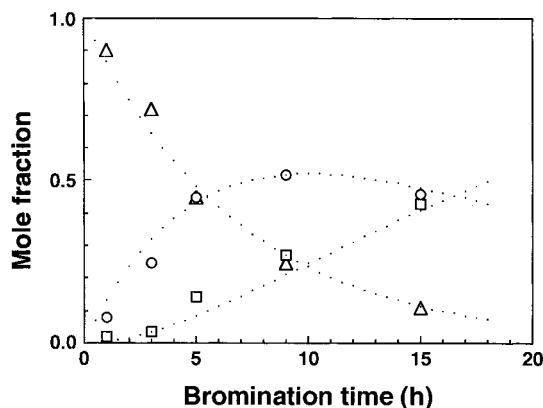


Figure 1 Mol fractions of tyrosine, 3-bromotyrosine and 3,5-dibromotyrosine in hydrolyzates as a function of bromination time: Δ , Tyr; \circ , Br-Tyr; \square , Br₂-Tyr. Silk fabrics were brominated with Br₂ in DMF at 50°C.

ysis of amino acids in the hydrolyzates.³ Amino acids were converted into volatile butyl ester of *N*(*O*)-trifluoroacetyl derivatives¹³ for the analysis. The stationary phase used was OV-17 coated on Chromosorb G HP (1.5 wt/wt %) and the column temperature was raised from 100°C at 6°C/min.

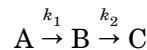
The concentrations of amino acids in the hydrolyzates were determined using an amino acid analyzer based on a Shimadzu high-performance liquid chromatograph LC4A with a data processor Shimadzu Chromatopac C-R3A. A lithium-type column ISC-07 was used and the detection was based on the fluorescent method using *o*-phthalaldehyde and *N*-acetylcysteine.¹⁴ The amino acid contents in treated samples were converted to the values in mmol/g of the dried original silk fabric. The total content of glycine and alanine was used for normalization.

RESULTS AND DISCUSSION

Bromination with Br₂

The amino acid analysis of the hydrolyzates of silk fabrics brominated with molecular bromine in a nonaqueous medium, DMF, shows that the bromination occurs at tyrosine residues to form Br-Tyr and Br₂-Tyr residues. Figure 1 shows the mol fractions of tyrosine and bromotyrosines as a function of reaction time. Br-Tyr is formed from the early stage of the reaction, and its fraction reaches a maximum and then decreases. The fraction of Br₂-Tyr is small at the early stages and

increases considerably at the later stages. This reaction profile indicates that the bromination of Tyr residues to Br-Tyr and Br₂-Tyr residues by molecular bromine in DMF is a consecutive reaction. Following equations¹⁵ express a consecutive reaction starting with species A in a homogeneous system;



$$[A]/[A]_0 = \exp(-k_1 t)$$

$$[B]/[A]_0 = k_1/(k_2 - k_1)$$

$$\times \{\exp(-k_1 t) - \exp(-k_2 t)\}$$

$$[C]/[A]_0 = 1 - \{k_2 \exp(-k_1 t)$$

$$- k_1 \exp(-k_2 t)\}/(k_2 - k_1)$$

where t is time and $[A]_0$ is the initial concentration of A. These equations are fitted to the data where A is Tyr, B is Br-Tyr, and C is Br₂-Tyr. Bromine is present in excess and the change in its concentration can be ignored. The mol fraction of B, $[B]/[A]_0$, reaches a maximum at t_{\max} and the following relations hold at $t = t_{\max}$;

$$k_1 \exp(-k_1 t_{\max}) = k_2 \exp(-k_2 t_{\max})$$

$$([B]/[A]_0)_{\max} = \exp(-k_2 t_{\max})$$

The probable values for k_1 and k_2 can be determined based on these relations. Dotted lines in Figure 1 are the theoretical curves calculated with the values of 0.145 and 0.065 h⁻¹ for k_1 and k_2 , respectively. The agreement between experimental and theoretical values is sufficiently good taking the heterogeneous nature of the present bromination into consideration. The lower value for k_2 as compared to k_1 is reasonable because bromine is an electron-withdrawing ortho-para director. The electrophilic substitution of hydrogen atom at position 5 with the second bromine atom will be suppressed by the presence of the first bromine atom at position 3. The values of apparent rate constants show that 44 h will be necessary to obtain Br₂-Tyr residues in 90% yield from Tyr residues under the reaction conditions adopted.

2-Amino adipic acid (A-Adp) from lysine (Lys) residues was found along with cysteic acid and Br₂-Tyr in the hydrolyzate of a wool sample obtained in the Allwörden reaction in bromine-water.¹⁶ In the present bromination of silk by molec-

Table I Halogenation of Silk Fabrics with Methanesulfonyl Halide

	Original Silk	Halogenated with	
		MsBr	MsCl ^a
Ser ^b	1.390	1.214	0.827
AE-Cys ^b	—	0.102	0.421
X (%) ^c	—	1.54	2.56
Δ Ser ^b			
Observed	—	0.176	0.563
Calculated ^d	—	0.195	0.732

Silk fabrics were treated with methanesulfonyl halide in DMF at 60°C for 24 h at the liquor ratio of 200. [MsX]/[Ser] was 50. AET treatments were carried out at 45°C for 4 h.

^a One of the data shown in Figure 1 of ref. 5.

^b Amount in mmol/g silk.

^c Halogen content before AET treatment.

^d Equal to the amount of X-Ala residues calculated with the assumption that all the halogen atoms are contained in X-Ala residues.

ular bromine in DMF, however, A-Adp was not found in the hydrolyzates.

Bromination with MsBr

MsBr is considered to form a complex of iminium ester type with DMF as in the case of methanesulfonyl chloride (MsCl),¹⁷ and was used for the regioselective substitution of primary hydroxyl groups in saccharides with bromine.¹⁸ Table I compares the result of the bromination with MsBr to that of the chlorination with MsCl under heterogeneous conditions in DMF.⁵ The halogenated samples were treated with 2-aminoethanethiol (AET) in 0.2 N NaOH to convert X-Ala residues into *S*-2-aminoethylcysteine (AE-Cys) residues.³ The amino group of AET does not participate in the substitution because the nucleophilicity of the sulfide ion is much higher than that of the nitrogen atom in the amino group.

Ser residues react with MsBr as expected while the contents of other amino acids in the sample remain practically unchanged after the bromination. The decrease in Ser content (Δ Ser) is 0.176 mmol/g silk and large values of Δ Ser could not be achieved. The amount of AE-Cys residues or additional amino groups introduced into the sample is more than twice as large as that of Lys residues originally present in the silk fibroin (found, 0.040 mmol/g). The selectivity for AE-Cys based on Δ Ser are 58 and 75% for brominated

and chlorinated silk fabrics, respectively. This difference can be explained based on an idea that under alkaline conditions Br-Ala residues are more easily converted into dehydroalanine (De-Ala) residues than Cl-Ala residues.³ De-Ala residues will be converted into pyruvic acid during acid hydrolysis.

As can be seen from the table, the substitution of hydroxyl groups with bromine atoms is much slower than that with chlorine atoms judging from the Δ Ser values. The important step in the substitution is the nucleophilic attack of a halide ion onto the carbon linked to the ester oxygen of the intermediate iminium ester.¹⁷ The reason for the slower substitution in the bromination can be explained in terms of the lower nucleophilicity of bromide ion (formed from MsBr) as compared to chloride ion in polar aprotic solvents such as DMF.¹⁹ We reported previously¹ that MsBr reacted only with Ser residues in silk fibroin to give Br-Ala residues under homogeneous conditions in LiBr-DMF. However, the extent of bromination attained was much smaller than that of chlorination with MsCl under otherwise same conditions in LiCl-DMF. This was also ascribed to the lower nucleophilicity of bromide ion as compared with chloride ion. In the present heterogeneous bromination, no extra bromide ions are present and the reaction is slower than that under homogeneous conditions in LiBr-DMF.¹

Bromination with NBS-PPh₃ in the Presence of NaBr

NBS and PPh₃ form a 1 : 1 addition compound that reacts with an alcohol to form a triphenylphosphonium ester bromide and succinimide.²⁰ The nucleophilic attack of bromide ion onto the carbon linked to the ester oxygen yields a brominated compound and triphenylphosphine oxide. If bromide ions are present in excess in the system, they will participate in the nucleophilic substitution and may facilitate the conversion of Ser residues to Br-Ala residues.²¹ We, therefore, studied the effect of the addition of bromide ion using NaBr. Silk fibers and fabrics dissolve in LiBr-DMF¹⁴ but apparently no change was observed for silk fabrics when kept in NaBr-DMF at the reaction temperature of 50°C.

Table II shows the results of the bromination of silk fabrics with NBS-PPh₃ in the presence of NaBr where NBS and PPh₃ were used in equimolar amounts. The direct quantitation of Br-Ala by

Table II Bromination of Silk Fabrics with NBS-PPh₃ in the Presence of NaBr

	Original Silk	[NaBr]/[NBS]			
		0.0	0.25	0.5	1.0
Ser ^a	1.390	1.064	1.040	1.024	1.034
ΔSer ^b	—	0.326	0.350	0.366	0.356
PrA-Ala ^c	—	0.151	0.150	0.158	0.162
Tyr ^a	0.622	0.330	0.304	0.355	0.470
ΔTyr ^d	—	0.292	0.318	0.267	0.152
Br-Tyr ^e	—	0.074	0.044	0.044	0.025
Br ₂ -Tyr ^f	—	0.190	0.206	0.157	0.058

Samples were brominated at 60°C for 24 h at [NBS] : [PPh₃] : [Ser] molar ratio of 9 : 9 : 1 and at the liquor ratio of 40. Propylamine treatments were carried out at 45°C for 4 h.

^a Amount in mmol/g silk.

^b Decrease in Ser in mmol/g silk.

^c 3-Propylaminoalanine in mmol/g silk.

^d Decrease in Tyr in mmol/g silk.

^e 3-Bromotyrosine in mmol/g silk.

^f 3,5-Dibromotyrosine in mmol/g silk.

amino acid analysis is practically impossible because Br-Ala decomposes quickly under acid hydrolysis conditions.³ The brominated samples were treated with propylamine to convert labile Br-Ala residues to 3-propylaminoalanine (PrA-Ala) residues for the estimation of the amounts of Br-Ala residues in the brominated samples.

The amount of PrA-Ala residues increases slightly but steadily with the increase in the [NaBr]/[NBS] molar ratio. The selectivity for PrA-Ala residues based on ΔSer is 43–50%, which is lower than that for AE-Cys residues (58%) shown in Table I. This can be ascribed to the lower nucleophilicity of amino nitrogen as compared with sulfide ion. The low efficiency of the conversion of Br-Ala residues to PrA-Ala residues can be ascribed, at least in part, to the ease of the dehydrobromination of Br-Ala residues to De-Ala residues during the propylamine treatment. The oxidation of Ser residues by free NBS present in the reaction system might be another reason. NBS itself is an oxidizing reagent. It oxidizes α-amino acids to aldehydes, ketones, and so on, brominates 3 and 5 positions of Tyr derivatives and preferentially cleaves tyrosyl-peptide bonds in proteins.²²

The decrease in Tyr content (ΔTyr) shows a tendency different from that of ΔSer. It decreases as [NaBr]/[NBS] increases from 0.25 to 1.0. The decreases in the formation of Br-Tyr and Br₂-Tyr are more remarkable. The selectivity for the brominated tyrosines based on ΔTyr exceeds 90% in

the absence of NaBr, while the difference between the total amount of brominated tyrosines and ΔTyr becomes large and almost constant (0.07 mmol/g silk) for the reactions in the presence of NaBr. In the present study, however, no compound was detected with the formation of which the difference between the total amount of brominated tyrosines and ΔTyr could be explained. Contrary to the expectation, the addition of NaBr has little effect in facilitating the formation of Br-Ala residues while it suppresses the bromination of Tyr residues.

In conclusion, we have succeeded in selective bromination of Tyr residues with molecular bromine in DMF. This reaction is expected to be useful for some purposes such as to give silk fabrics nonflammability and resistance against entomic or microbial attack. The efficient conversion of Ser residues to Br-Ala residues could not be achieved with the reagent systems examined. This can be ascribed to the low nucleophilicity of bromide ion.

REFERENCES

1. K. Furuhashi, A. Okada, H. Niwa, and M. Sakamoto, *J. Seric. Sci. Jpn.*, **64**, 302 (1995).
2. M. Sakamoto, F. Masuko, M. Nishimoto, Y. Kumeno, K. Igarashi, and K. Furuhashi, *Proc. 7th Intern. Wool Text. Res. Conf. Tokyo*, **4**, 431 (1985).
3. M. Sakamoto, Y. Kumeno, S. Deno, T. Yamauchi,

- M. Nishimoto, K. Furuhashi, and F. Masuko, *Textile Res. J.*, **58**, 689 (1988).
4. M. Sakamoto, F. Masuko, and S. Nakanishi, *Textile Res. J.*, **56**, 429 (1986).
5. K. Furuhashi, S. Deno, T. Yamauchi, and M. Sakamoto, *J. Seric. Sci. Jpn.*, **65**, 319 (1996).
6. K. Furuhashi, S. Deno, and M. Sakamoto, *J. Seric. Sci. Jpn.*, **65**, 396 (1996).
7. N. Aoki, K. Koganei, H.-S. Chang, K. Furuhashi, and M. Sakamoto, *Carbohydr. Polym.*, **27**, 13 (1995).
8. N. Aoki, K. Furuhashi, Y. Saegusa, S. Nakamura, and M. Sakamoto, *J. Appl. Polym. Sci.*, **61**, 1173 (1996).
9. S. Hanessian, M. M. Ponpipom, and P. Lavallee, *Carbohydr. Res.*, **24**, 45 (1972).
10. G. Sieber, *Liebigs Ann. Chem.*, **631**, 180 (1960).
11. R. Zynek, *Z. Physiol. Chem.*, **144**, 246 (1925).
12. R. S. Asquith and P. Carthew, *Tetrahedron*, **28**, 4769 (1972).
13. F. E. Kaiser, C. W. Gehrke, R. W. Zumwalt, and K. C. Kuo, *J. Chromatogr.*, **94**, 113 (1974).
14. K. Furuhashi, A. Okada, Y. Chen, Y. Y. Xu, and M. Sakamoto, *J. Seric. Sci. Jpn.*, **63**, 315 (1994).
15. K. J. Laidler, *Chemical Kinetics*, 2nd ed., McGraw Hill, New York, 1965.
16. A. J. Ultee, *J. Text. Inst.*, **64**, 547 (1973).
17. J. D. Albright, E. Benz, A. E. Lanzilotti, and L. Goldman, *Chem. Commun.*, **1965**, 413.
18. K. Takeo, T. Sumimoto, and T. Kuge, *Stärke*, **26**, 111 (1974).
19. A. J. Parker, *Chem. Rev.*, **69**, 1 (1969).
20. G. Hodosi, B. Podányi, and J. Kuzmann, *Carbohydr. Res.*, **230**, 327 (1992).
21. I. I. Ziderman, *Textile Res. J.*, **51**, 777 (1981).
22. R. Filler, *Chem. Rev.*, **63**, 21 (1963).