Steric Effects of *N*-Acyl Group in *O*-Methacryloyl-*N*-Acyl Tyrosines on the Adhesiveness of Unetched Human Dentin

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We have prepared various *O*-methacryloyl-*N*-acyl tyrosines (MAATY) to reveal the relationship between molecular structure near carboxylic acid and adhesive strength of MAATY-HEMA type adhesive resin to unetched dentin. In this study, we attempted to change the steric hindrance effect without changing the HLB value, *i.e.*, introducing an iso-acyl group instead of *n*-acyl group into MAATY. *O*-methacryloyl-*N*-ethylbutyryl tyrosine (MIHTY) showed significantly lower adhesive strength when compared with *O*-methacryloyl-*N*-hexanoyl tyrosine even though both MAATY have the same HLB value. The possible explanation of the significantly different adhesive strength was that the 2-ethylbutyryl group in MIHTY was bulky, resulting in inhibition of the hydrogen bonding of the carboxylic group. The HLB value is independent of the steric effect of molecular structure, and thus the steric factor should be taken into consideration for the explanation of different adhesive strengths within the adhesive monomers having the same HLB value but different molecular structures.

Key words : Tyrosine derivative, Steric hindrance, HLB value

INTRODUCTION

To improve the adhesive strength of methacryloyl amino acid-HEMA type restorative resin to unetched dentin, and to understand the effects of molecular structure on the adhesive strength, we have prepared various kind of new methacryloyl amino acids and found that hydrogen bonding of the carboxylic group plays an important role in the bonding to teeth^{1–3)}. For example, when the carboxylic acid of methacryloyl amino acid is inactivated by ester the bond strength to unetched teeth becomes zero²⁾. Also, the structure of the neighboring carboxyl group is important to the bonding. We have found that *N*, *O*-dimethacryloyl tyrosine, a derivative of tyrosine, has the most favorable properties among the various methacryloyl amino acids derived from α -amino acids with respect to its adhesive characteristics to unetched human dentin^{1,2)}. Therefore, the introduction of various substituted groups into the amino group of tyrosine would help to elucidate the most favorable structure of neighboring carboxyl group.

In our previous study³⁾, we prepared five new *O*-methacryloyl-*N*-acyl tyrosines (MAATY) having *n*-acyl groups with one to seven methylene groups, and evaluated the adhesive tensile bond strength (TBS) of MAATY-HEMA type bonding resin to unetched dentin. The TBS value increased with an increase in the number of

introduced methylene groups, to reach a maximum value when the number of carbons in the acyl group was six. The TBS value was reduced when the number was eight. Although it became apparent that the number of methylene groups in the acyl group was important, the role of the methyl group has not been clarified. Kawaguchi *et al.*⁴⁾ reported that the hydrophile-lipophile balance (HLB) number^{5,6)} is important to obtain higher bond strength. Therefore, the effect of the methylene group on TBS value can be taken as a result of the HLB number. On the other hand, an increase of the number of methylene groups can be also taken as an increase of steric hindrance against the carboxylic group, which plays an important role in the bonding to dentin.

The role of the methylene group would be apparent by changing the steric hindrance effect without changing the HLB value, e.g., by introducing an iso-acyl group into MAATY. In other words, the steric effect of *n*- and iso-acyl groups is different even though their HLB value is the same.

In this study, therefore, three new MAATY with iso-acyl groups were prepared and the TBS value of MAATY-HEMA type restorative resin to unetched dentin was evaluated and compared with our previous results³⁾.

MATERIALS AND METHODS

Synthesis of MAATY

O-methacryloyl-*N*-butyryl tyrosine (MBTY), *O*-methacryloyl-*N*-valeryl tyrosine (MVTY), and *O*-methacryloyl-*N*-hexanoyl tyrosine (MHTY) with an *n*-acyl group were prepared according to the literature³⁾. *O*-methacryloyl-*N*-isobutyryl tyrosine (MIBTY), *O*-methacryloyl-*N*-isovaleryl tyrosine (MIVTY), and *O*-methacryloyl-*N*-(2-ethylbutyryl) tyrosine (MIHTY) with an iso-acyl group were also prepared accordingly³⁾. Each MAATY was re-crystallized three times from ethylacetate, filtered, and dried in vacuo at room temperature. Their molecular structure and purity was determined using elementary analysis (MT-5, Yanako, Kyoto, Japan), thin-layer chromatography (Iatroscan TH-10 TLC/FID analyzer, Iatron Laboratories, Inc., Tokyo, Japan), infrared spectroscopy (IR-810, Japan Spectroscopic Co., Ltd., Tokyo, Japan), and nuclear magnetic resonance spectroscopy (Varian VXR-500, Varian Instruments Ltd., CA, USA).

Index of steric hindrance

The chemical shift of MAATY was measured using ¹H-NMR and used as an index of steric hindrance. In brief, low molecular weight compounds with carboxylic groups stay as a stable dimer due to hydrogen bonding in nonpolar solvent, and thus exhibit carboxylic proton absorption at δ 10-13 ppm^{7,8)}. In contrast, a polar solvent dissociates the dimer and shifts the absorption position to lower magnetic fields⁸⁾. Therefore, the position of the chemical shift of the carboxylic proton can be used as an index of the degree of hydrogen bond formation.

Preparation of bonding agents

The bonding agent containing MAATY was composed of Liquids A and B. Liquid A: MAATY and HEMA as a comonomer (total monomer weight 1 g) were dissolved in 1 g of ethyl alcohol, and 10 mg of benzoyl peroxide (BPO) was also added as an initiator. Liquid B: 10 mg of the sodium salt of benzenesulfonic acid (BSANa) as an activator of polymerization was dissolved in 1 g of ethyl alcohol. An equal volume of Liquid A and B was mixed at the time of application onto the dentinal surface.

Preparation of specimens and the measurement of tensile bond strength

The labial surface of freshly extracted human permanent molars, stored in distilled water, was flattened with emery paper (JIS #600), and dried in air for 10 s. A polyethylene ring, 3.8 mm inside diameter, was fixed on the flattened dentin surface with doublesided tape. The bonding agent containing MAATY was applied onto the dentinal surface inside the polyethylene ring with a sponge pellet and dried in air for 15 s. Thereafter, a composite resin (Clearfil F-II, Kuraray, Okayama, Japan) was applied under its own weight. Specimens were stored in water at 37°C for 24 h.

Tensile bond strength was measured using a universal testing machine (Auto-graph DCS-2000, Shimadzu Corp., Kyoto, Japan) with a cross-head speed of 2 mm/min. Each experiment included 10 determinations. The data were evaluated by the analysis of variance and Scheffe's test for multiple comparisons between means at the $p=0.05$ level of significance.

RESULTS AND DISCUSSION

Elemental analyses and $^1\text{H-NMR}$ spectra of MAATY and their assignments are shown in Table 1 and Fig. 1, respectively. These results indicated that the MAATY obtained were of high purity.

The TBS values of MAATY-hydroxyethyl methacrylate (HEMA) bonding systems to unetched human dentin (#600) are shown in Table 2. The monomer feed of MAATY had an apparent influence on the TBS value. The optimal TBS value for each bonding system was observed when the system contained 15 mol% MAATY, and this is consistent with our previous results³⁾ except for the result of MIHTY that showed an optimal TBS value when the system contained 5 mol% MIHTY.

Fig. 2 summarizes the tensile bond strengths of MAATY (15 mol%)-HEMA bonding systems as a function of carbon number in the *N*-acyl group and its configura-

Table 1 Elementary analyses of *O*-methacryloyl-*N*-acyl tyrosine (MAATY)

MAATY		C%	H%	N%
<i>O</i> -methacryloyl- <i>N</i> -isobutyryl tyrosine (MIBTY)	Obs'd:	63.75	6.63	4.36
	(Calc'd):	(63.94)	(6.63)	(4.39)
<i>O</i> -methacryloyl- <i>N</i> -isovaleryl tyrosine (MIVTY)	Obs'd:	64.72	6.69	4.22
	(Calc'd):	(65.05)	(6.67)	(4.21)
<i>O</i> -methacryloyl- <i>N</i> -(2-ethylbutyryl) tyrosine (MIHTY)	Obs'd:	65.22	7.31	4.09
	(Calc'd):	(65.69)	(7.25)	(4.03)

Fig. 1 Proton NMR spectra of MAATY at 500 MHz in DMSO-d₆ at room temperature.

tion. The chemical shift position of carboxylic proton in MAATY is also shown. Basically, all MAATY-HEMA bonding systems showed the same TBS value except for MIHTY, which showed a significantly reduced TBS value. As discussed in our previous work³, the optimal tensile bond strength of MAATY-HEMA bonding systems to unetched dentin showed a maximum value when the HLB value of MAATY was

Table 2 Effect of the molar ratio of MAATY in the MAATY-HEMA system on the adhesive strength to the unetched human dentin (#600)

Molar ratio of MAATY	Tensile bond strength (MPa)		
	MAATY		
	MIBTY	MIVTY	MIHTY
0	0.92 (0.61)	0.92 (0.61)	0.92 (0.61)
0.05	1.51 (1.13)	2.76 (1.38)	3.17 (1.30)
0.10	3.61 (1.30)	3.58 (0.94)	2.82 (1.01)
0.15	4.25 (0.96)	4.48 (1.07)	2.53 (0.71)
0.20	3.16 (0.64)	3.50 (1.09)	2.52 (0.46)

n=10, SD in parentheses

Means connected by bars were not significantly different.

Fig. 2 Influence of carbon number in *N*-acyl group and its configuration on the tensile bond strength of MAATY (15 mol%)-HEMA bonding systems and chemical shift position of carboxylic proton in MAATY. The same letter near the symbols of mean bond strength shows that there is no significant difference at the level of $p=0.05$.

■ : *n*-acyl group (Our previous work³⁾)

□ : iso-acyl group

around 7.5 in the same manner as Kawaguchi *et al.*⁴⁾. Since the HLB values of MBTY and MIBTY, MVTY and MIVTY, MHTY and MIHTY are 8.2, 7.9 and 7.5, respectively, it is reasonable that we achieved similar TBS values except for MIHTY. Although we feel that the HLB value is an important factor in determining adhesive strength, the HLB value was independent of the steric hindrance effect, and thus did not give a different HLB value for MAATY with *n*- and iso-acyl groups when the carbon number

was the same. Although many factors must be involved in the resulting adhesive strength, we compared chemical shifts within MAATY that have the same carbon number in the acyl group to evaluate the difference of configuration in acyl group in this study. As can be seen in Fig. 2, MAATY with iso-acyl groups have smaller chemical shifts, *i.e.*, larger steric effects when compared with those of MAATY that contain n-acyl groups. The difference of steric hindrance effect between iso- and n-acyl groups was large when the acyl group contained six carbon whereas the difference of chemical shift was smaller when the acyl group contained fewer carbons. When the results of steric hindrance effect was taken into consideration for the TBS value, we can explain the reduced TBS value of MIHTY when compared with that of MHTY. In the other words, MIHTY showed a reduced TBS value when compared with MHTY since MIHTY received a larger steric hindrance effect than MHTY. Unfortunately, we could not find a difference of TBS value between MBTY and MIBTY, where the number of carbons is four, or between MVTY and MIVTY, where the number of carbon is five, since the difference in steric hindrance effect is small.

In conclusion, the different TBS values of MAATY-HEMA type retorative resin where MAATY has the same HLB value can be explained by combining the steric hindrance factors, *i.e.*, the configuration of acyl groups.

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