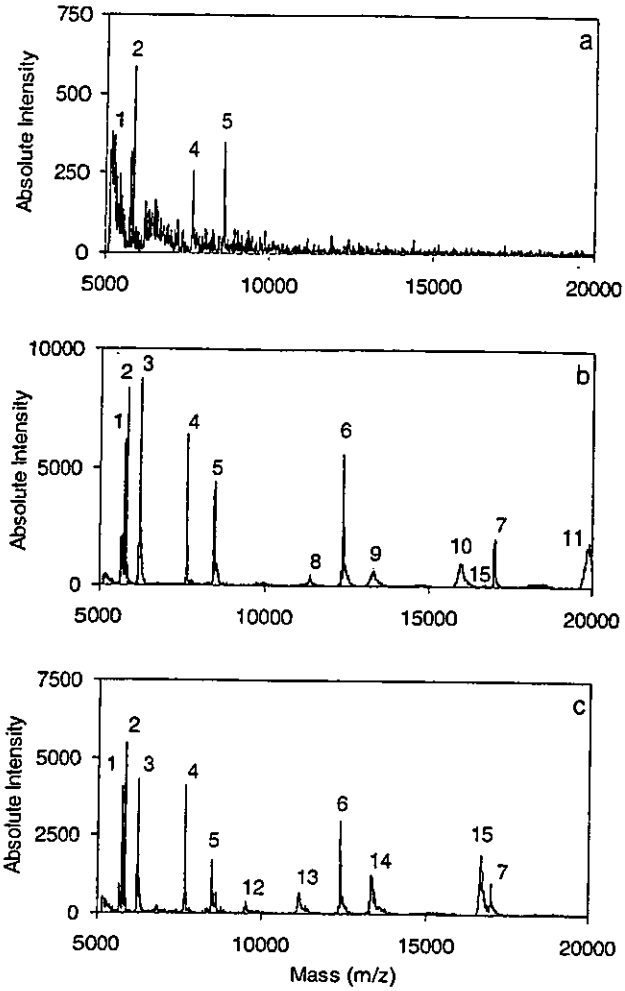

Figure 5. MALDI mass spectra of a mixture of peptides and proteins. The mixture of peptides and proteins (50 fmol/μL each) and the matrix solution were mixed together in equal volumes. The matrix solution was a 5:1 mixture of the CHCA solution with (a) deionized water; (b) Tf solution (0.10 μg/μL); and (c) BSA solution (0.10 μg/μL). Signal 1, [bovine insulin]$^{+}$ (5730 Da); 2, [human insulin]$^{+}$ (5808 Da); 3, [cytochrome C]$^{2+}$; 4, [IGF-I]$^{+}$ (7649 Da); 5, [apomyoglobin]$^{2+}$; 6, [cytochrome C]$^{+}$ (12 362 Da); 7, [apomyoglobin]$^{+}$ (16 952 Da); 8, [Tf]$^{7+}$; 9, [Tf]$^{6+}$; 10, [Tf]$^{5+}$; 11, [Tf]$^{4+}$; 12, [BSA]$^{7+}$; 13, [BSA]$^{6+}$; 14, [BSA]$^{5+}$; and 15, [BSA]$^{4+}$.

the effect should not be observed when using liquid matrices.[26,27]

The present results suggest that the enhancement brought about by either Tf or BSA could be applicable to the improvement of sensitivity in the detection of proteins by MALDI-TOFMS in general. However, when Tf or BSA was used as an enhancer in a MALDI-TOFMS system, signals from Tf and BSA were also detected, which sometimes interfered with the analysis of the target proteins. Therefore, neither Tf nor BSA appears to be the best possible enhancer. Further studies are currently underway in order to discover the best macromolecule as an enhancer.

CONCLUSIONS

We have demonstrated that the signal intensities of insulin and of several peptides and proteins were enhanced in CHCA premixed with Tf or other peptides or proteins. The characteristics of this type of enhancement are as follows: (1) Tf (80 kDa) and BSA (66 kDa) led to better signal enhancement than did small peptides and proteins (<20 kDa) or IgG (150 kDa); (2) the optimum S/N value was observed when the added amount of peptide or protein was within the range 0.26–0.62 pmol; and (3) the signals of peptides of high molecular weight (>3000 Da) were enhanced by the addition of Tf or BSA to CHCA, although the signals of small peptides (<2500 Da) were not enhanced. This type of enhancement may be useful for the improvement of protein analyses with MALDI-TOFMS.

Acknowledgements

This study was supported in part by a research grant on Human Genome, Tissue Engineering Food Biotechnology from the Ministry of Health, Labor and Welfare, Japan.

REFERENCES

1. Roberts GD, Johnson WP, Burman S, Anumula KR, Carr SA. *Anal. Chem.* 1995; **67**: 3613.
2. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. *Nat. Biotechnol.* 1999; **17**: 994.
3. Münchbach M, Quadroni M, Miotto G, James P. *Anal. Chem.* 2000; **72**: 4047.
4. Yao X, Freas A, Ramirez J, Demirev PA, Fenselau C. *Anal. Chem.* 2001; **73**: 2836.
5. Cagney G, Emili A. *Nat. Biotechnol.* 2002; **20**: 163.
6. Vogt JA, Schroer K, Hözler K, Hunzinger C, Klemm M, Biefang-Arndt K, Schillo S, Cahill MA, Schlattenholz A, Matthies H, Stegmann W. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1273.
7. Kuyama H, Watanabe M, Toda C, Ando E, Tanaka K, Nishimura O. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1642.
8. Wu SL, Choudhary G, Ramström M, Bergquist J, Hancock WS. *J. Proteome Res.* 2003; **2**: 383.
9. Nakanishi T, Okamoto N, Tanaka K, Shimizu A. *Biol. Mass Spectrom.* 1994; **23**: 230.
10. Nelson RW, Krone JR, Bieber AL, Williams P. *Anal. Chem.* 1995; **67**: 1153.
11. Tubbs KA, Nedelkov D, Nelson RW. *Anal. Biochem.* 2001; **289**: 26.
12. Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. *Rapid Commun. Mass Spectrom.* 1988; **2**: 151.
13. Karas M, Hillenkamp F. *Anal. Chem.* 1988; **60**: 2299.
14. Beavis RC, Chait BT. *Anal. Chem.* 1990; **62**: 1836.
15. Strupat K, Karas M, Hillenkamp F. *Int. J. Mass Spectrom. Ion Processes* 1991; **111**: 89.
16. Beavis RC, Chaudhary T, Chait BT. *Org. Mass Spectrom.* 1992; **27**: 156.
17. Vorm O, Roepstorff P, Mann M. *Anal. Chem.* 1994; **66**: 3281.
18. Köstler C, Castoro JA, Wilkins CL. *J. Am. Chem. Soc.* 1992; **114**: 7572.
19. Billeci TM, Stults JT. *Anal. Chem.* 1993; **65**: 1709.
20. Karas M, Ehring H, Nordhoff E, Stahl B, Strupat K, Hillenkamp F, Grehl M, Krebs B. *Org. Mass Spectrom.* 1993; **28**: 1476.
21. Tsarbopoulos A, Karas M, Strupat K, Pramanik BN, Nagabhushan TL, Hillenkamp F. *Anal. Chem.* 1994; **66**: 2062.
22. Yuan X, Desiderio DM. *J. Mass Spectrom.* 2002; **37**: 512.
23. Botting CH. *Rapid Commun. Mass Spectrom.* 2003; **17**: 598.
24. Nelson RW, McLean MA. *Anal. Chem.* 1994; **66**: 1408.
25. Worrall TA, Cotter RJ, Woods AS. *Anal. Chem.* 1998; **70**: 750.
26. Carda-Broch S, Berthod A, Armstrong DW. *Rapid Commun. Mass Spectrom.* 2003; **17**: 553.
27. Turney K, Harrison WW. *Rapid Commun. Mass Spectrom.* 2004; **18**: 629.

15 Kovacs-Nolan J, Zhang JW, Hayakawa S, Mine Y: Immunochemical and structural analysis of pepsin-digested egg white ovomucoid. J Agric Food Chem 2000;48:6261–6266.
16 Besler M, Petersen A, Steinhart H, Paschke A: Identification of IgE-Binding Peptides Derived from Chemical and Enzymatic Cleavage of Ovomucoid (Gal d 1). Internet Symposium on Food Allergens 1999;1:1–12. http://www.food-allergens.de
17 Urisu A, Yamada K, Tokuda R, Ando H, Wada E, Kondo Y, Morita Y: Clinical significance of IgE-binding activity to enzymatic digests of ovomucoid in the diagnosis and the prediction of the outgrowing of egg white hypersensitivity. Int Arch Allergy Immunol 1999; 120:192–198.
18 Zacharius RM, Zell TE, Morrison JH, Woodlock JJ: Glycoprotein staining following electrophoresis on acrylamide gels. Anal Biochem 1969;30:148–152.
19 Mine Y, Zhang JW: Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Biochem Biophys Res Commun 2002; 292:1070–1074.
20 Beyer K, Ellman-Grunther L, Jarvinen KM, Wood RA, Hourihane J, Sampson HA: Measurement of peptide-specific IgE as an additional tool in identifying patients with clinical reactivity to peanuts. J Allergy Clin Immunol 2003;112:202–207.
21 Mine Y, Sasaki E, Zhang JW: Reduction of antigenicity and allergenicity of genetically modified egg white allergen, ovomucoid third domain. Biochem Biophys Res Commun 2003; 302:133–137.
22 Matsuda T, Gu J, Tsuruta K, Nakamura R: Immunoreactive glycopeptides separated from peptic hydrolysate of chicken egg white ovomucoid. J Food Sci 1985;50:592–594.
23 Cooke SK, Sampson HA: Allergenic properties of ovomucoid in man. J Immunol 1997;159: 2026–2032.
24 Kane PM, Holowka D, Baird B: Cross-linking of IgE receptor complexes by rigid bivalent antigens greater than 200 Å in length triggers cellular degranulation. J Cell Biol 1988;107: 969–980.