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Basic Matrices in the Analysis of Non-covalent Complexes by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry

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A number of potential matrix candidates were investigated with regard to the importance of the pH in the matrix-assisted laser desorption/ionization mass spectrometry (MALDI/MS) analysis of non-covalently bound protein complexes. The matrices examined were 2,5-dihydroxybenzoic acid (DHB), 4-hydroxy-α-cyanocinnamic acid (HCCA), 2-aminonicotinic acid (ANA), 4-nitroaniline (NA), 2-amino-4-methyl-5-nitropyridine (AMNP) and 3-hydroxypicolinic acid (HPA). In solution these matrix compounds permitted the preparation of MALDI samples at pH in the range 2–7. Among the matrices tested, complex formation, by specific non-covalent interactions, could only be observed when HPA (pH 3.8) was used as the matrix for the MALDI analysis. Under these conditions, specific non-covalent complex formation of recombinant streptavidin and glutathione-*S*-transferases were observed but not for human hemoglobin. The MALDI spectra obtained with the neutral compounds ANA (pH 4.4), NA (pH 6.4) and AMNP (pH 7.1) as matrices contain only peaks of the subunit with no signal of the non-covalent bound complexes present. Considering the results obtained in this study with basic and acidified matrix materials, there does not appear to be a strong correlation between the pH of the matrix solution and the utility of a matrix for the analysis of non-covalently bound complexes. **© 1998 John Wiley & Sons, Ltd.**

KEYWORDS: matrix-assisted laser desorption/ionization mass spectrometry; basic matrices; non-covalent interactions; complex formation

INTRODUCTION

Intermolecular non-covalent interactions are responsible for the aggregation of folded polypeptide chains into multimers, which determines a protein system's quaternary structure. The strengths of such intermolecular forces, including hydrogen bonding and hydrophobic and ionic interactions, can vary widely and are reflected by the dissociation constants (K_D) typically determined for a specific set of solution conditions. Changing the pH, solvent conditions and/or temperature will typically cause a protein in solution to denature and partially or completely unfold.

Although a few techniques exist for the direct observation of non-covalent macromolecular complexes, such as size exclusion chromatography, sedimentation equilibrium ultracentrifugation and non-denaturing gel electrophoresis, each has significant limitations and can only provide an approximate molecular mass of the complex. With the development of soft ionization techniques, such as electrospray ionization (ESI)[1,2] and matrix-assisted laser desorption/ionization (MALDI)[3,4] mass spectrometry (MS) has become an indispensable tool for accurate measurements of molecular masses in biochemistry and biomedical research involving structural analysis of proteins and peptides. Nevertheless, the detection of non-covalent complexes by MS methods is still a challenging task.

There have been several examples of the detection of non-covalently bound complexes by ESI-MS since the initial reports by Ganem *et al.*[5,6] Applied to aqueous solution conditions close to those of physiological interest, non-covalent complexes such as peptide–protein, protein–nucleic acid and protein–ligand, which form structurally specific associations in solution, have been preserved intact by the electrospray process (for reviews see Refs 7 and 8).

Whereas MALDI/MS has been successfully applied to determine the molecular mass and primary structure of biopolymers, non-covalently bound complexes almost always dissociate into individual components during the MALDI analysis. Accordingly, there are only a few examples where specific oligomeric complexes have been observed by this technique. The initial MALDI applications reported by Hillenkamp and co-workers included spectra of the undissociated tetrameric forms of glucose isomerase, catalase[9] and

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streptavidin[10] and the dimeric form of violet phophatase,[11] all obtained with nicotinic acid as matrix, at a desorption wavelength of 266 nm. The dimeric form of yeast alcohol dehydrogenase was observed with sinapinic acid (SA) as matrix at a desorption wavelength of 337 nm.[12] Later, the non-covalent association of membrane proteins was detected by MALDI/MS. The matrices were ferulic acid (355 nm) for the trimeric form of porin[13] and SA (337 nm) for the heptameric form of aerolysin.[14] When ferulic acid was used as the matrix, ions corresponding to complex formation were only observed for the first laser shot on a not yet irradiated sample spot.[13]

Dissociation into subunits is mainly due to the conditions in the matrix–analyte solution. The commonly used matrix compounds (at 337 nm) such as SA, 4-hydroxy-α-cyanocinnamic acid (HCCA) and 2,5-dihydroxybenzoic acid (DHB) are typically dissolved in an aqueous solution containing 30–70% acetonitrile and/or 0.1% trifluoroacetic acid (TFA). In the presence of an organic solvent[10] and under these highly acidic conditions (pH < 2–3), denaturation of protein structures and dissociation of specific interactions must generally be expected. Sample preparation avoiding acidic conditions has previously been carried out with 6-aza-2-thiothymine (ATT), providing a pH close to 7.0, for the observation of intact double-stranded oligonucleotides[15] and the specific complexes of enzyme RNAse S and leucine zipper dimer by MALDI/MS.[16] More recently, Cohen *et al.*[17] described the observation of specific non-covalent complexes of alcohol dehydrogenase, catalase and streptavidin, using the matrix 2,6-dihydroxyacetophenone (pH 4–5) in an organic solvent. Intact streptavidin tetramers were also observed with ferulic acid and other dihydroxyacetophenone derivatives. Similarly to Rosinke *et al.*,[13] ion signals corresponding the non-covalent complexes could only be obtained from the first shot at a given sample position.

Successful matrix desorption is only possible at pH values determined by the matrix compound itself or in a more acidic medium. When the pH of the matrix solution is increased above the p*K* of acid, the matrix molecules are transformed into anions, yielding the respective salts when dried, and matrix desorption is no longer possible.[18,19] In principle, matrices with neutral pH are required for possible desorption of intact non-covalent complexes and other molecular structures that are pH sensitive in their quaternary structure. A number of new matrices for MALDI/MS analysis that can be crystallized from aqueous solution at pH 2–8 have been investigated as possible matrices.[20] These matrices extend the utility of MALDI/MS to the analysis of acid-sensitive species. The goal of this study was to examine the potential of these more basic matrices and the importance of pH in the MALDI/MS analysis of non-covalently bound complexes. In addition to the two previously mentioned acidic matrices HCCA and DHB, the tested matrices included 3-hydroxypicolinic acid (HPA), 2-aminonicotinic acid (ANA), 4-nitroaniline (NA) and 2-amino-4-methyl-5-nitropyridine (AMNP): Protein that are known to form multimers were analyzed, i.e. recombinant streptavidin, human glutathione-*S*-transferase (GST) A1-1 and human hemoglobin.

EXPERIMENTAL

Materials

The matrices 2,5-dihydroxybenzoic acid (DHB), 2-aminonicotinic acid (ANA), 4-nitroaniline (NA), 2-amino-4-methyl-5-nitropyridine (AMNP) and 3-hydroxypicolinic acid (HPA) were obtained from Aldrich Chemie (Steinheim, Germany) and 4-hydroxy-α-cyanocinnamic acid (HCCA) from Sigma Chemical (St Louis, MO, USA). Human hemoglobin and α-chymotrysinogen A were purchased from Sigma Chemical and recombinant streptavidin from Boehringer Mannheim (Almere, The Netherlands). Human glutathione-*S*-transferases (GST) A1-1 were kindly provided by J. J. P. Bogaards (TNO Biological Toxicology Institute, Zeist, The Netherlands). Ultra-high-quality water, prepared using an Elgastate 4 (Elga, High Wycombe, Bucks, UK) was used for the matrix and sample solution preparations. Acids and organic solvents were of HPLC grade or better.

Matrix and sample preparation

The samples were all dissolved in water to yield protein at a concentration of 0.1 mg ml^{-1} for α-chymotrypsinogen A and GST A1-1 and 0.2 mg ml^{-1} for recombinant streptavidin and human hemoglobin. The matrix solutions were freshly prepared before use; HCCA was dissolved in 30% acetonitrile and all the others were prepared in water. The concentrations were 10 mg ml^{-1} for DHB, HCCA, ANA, NA and AMNP and 30 mg ml^{-1} for HPA. The pH measurements of the matrix solutions were performed at room temperature with a Model 5985-50 pH meter (pH Wand, Cole-Parmer Instruments, Chicago, IL, USA). An aliquot of the sample and the matrix solution was mixed directly on the target (1:1, v/v) and dried under a cold air stream before loading into the mass spectrometer.

Instrumentation

The mass spectrometer used was a Vision 2000 laser desorption reflectron time-of-flight instrument (Finnigan MAT, Bremen, Germany). Ions were formed using a pulsed nitrogen laser operating at 337 nm. The ions were accelerated to a potential of 6.5 kV in the ion source and post-accelerated by a conversion dynode in front of the detector to a potential of 20 kV. The effective drift length of the instrument was 1.7 m. Ions were detected by a secondary electron multiplier (SEM) and the signal was amplified and digitized by a high-speed transient recorder linked to a 486 personal computer. The laser irradiance was controlled by a variable attenuator and was kept just above the ion generation threshold. All spectra were obtained in positive-ion reflector mode; 20–30 single shot spectra were accumulated in order to obtain a good signal-to-noise ratio.

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RESULTS AND DISCUSSION

Matrices

Six matrix compounds (Fig. 1) permitting sample preparation at a pH in the range 2–7 were examined with regard to the importance of the pH in MALDI/MS analysis of non-covalently bound complexes. In addition to the commonly used acidic matrices HCCA and DHB, the matrices investigated include the more basic matrix HPA, first introduced for the MALDI/MS analysis of oligonucleotides,[21] and three less well known matrices, ANA, NA and AMNP, selected from a previously reported screening of a large number of basic matrices.[20] All the matrices were dissolved in water except for HCCA, which needs a proportion of organic solvent and was dissolved in 30% acetonitrile.

α-Chymotrypsinogen (reference conditions)

Together with the singly charged ion, multiply charged ions and cluster ions are generally observed in a MALDI mass spectrum. The singly charged ion (M^+) forms the base peak with the doubly charged ion (M^{2+}) and the dimer ion ($M_2{}^+$) at lower abundance.[22] The observation of oligomer cluster ions implies that non-specific non-covalent interactions can survive the desorption event. Therefore a primary consideration in the study of non-covalent associations is the determination of their specificity, i.e. establishing whether the species detected in the mass spectrometer are both structurally specific and derived from the species present in solution. α-Chymotrypsinogen A (25 656 Da), which is known to exist as a monomer only, was used to ensure that multimeric forms observed did not result from the desorption process under the conditions employed. No additional ion signals due to non-specific aggregation of the monomer were observed when α-chymotrypsinogen A was analyzed with any of the six matrices (results not shown).

Recombinant streptavidin

Recombinant streptavidin was chosen for the initial screening of the six matrices, because the tetrameric species in solution is relatively stable over a wide pH range and observation of this protein by MALDI/MS might therefore be less sensitive to the acidic conditions. Native streptavidin isolated from the bacterium *Streptomyces avidinii* exists in its active form as a non-covalent tetramer, which is comprised of four identical subunits each consisting of 159 amino acids. Recombinant streptavidin, which was used in these studies, contains amino acids 13–138 of the native streptavidin (mass 13 271 Da) giving a molecular mass of 53 084 Da for the tetrameric complex.

MALDI mass spectra of the recombinant streptavidin prepared with matrices of increasing pH are shown in Fig. 2(a)–(c). Figure 2(a) shows the MALDI mass spectrum of recombinant streptavidin obtained under standard MALDI/MS conditions, i.e. at pH 2.0 with DHB as matrix. As expected, the spectrum only shows the doubly (S^{2+}) and singly (S^+) charged ions, together with oligomer cluster ions up to the tetramer form ($2S^+$, $3S^+$ and $4S^+$) for the dissociated subunits. The singly charged ion at mass 13.3 kDa forms the base peak in the spectrum with the doubly charged ion and the multimeric ions at lower abundance. A comparable spectrum was obtained with HCCA (pH 2.7) as matrix.

In comparison, the ion pattern observed for recombinant streptavidin at pH 3.8 with HPA as matrix, shown in Fig. 2(b), differs from that of the dissociated subunits. The interpretation of the intensity distribution indicates the presence of a mixture of the intact non-covalent tetramer and the fully dissociated protein. The non-covalent tetramer at mass 54.0 kDa forms the base peak accompanied by the doubly (26.9 kDa) and triply (18.1 kDa) charged ions in addition to a

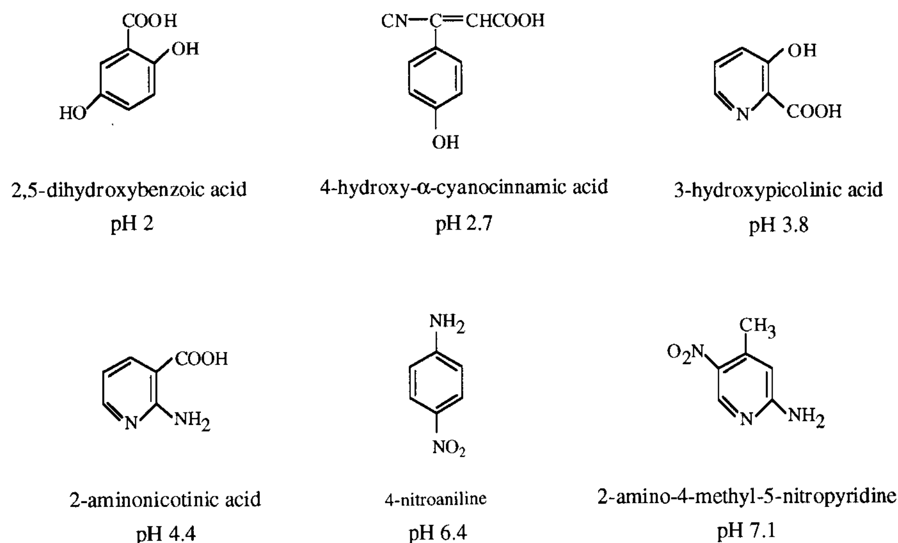

Figure 1. Structures of the six matrices investigated with regard to the importance of pH in MALDI analysis of non-covalently bound protein complexes.

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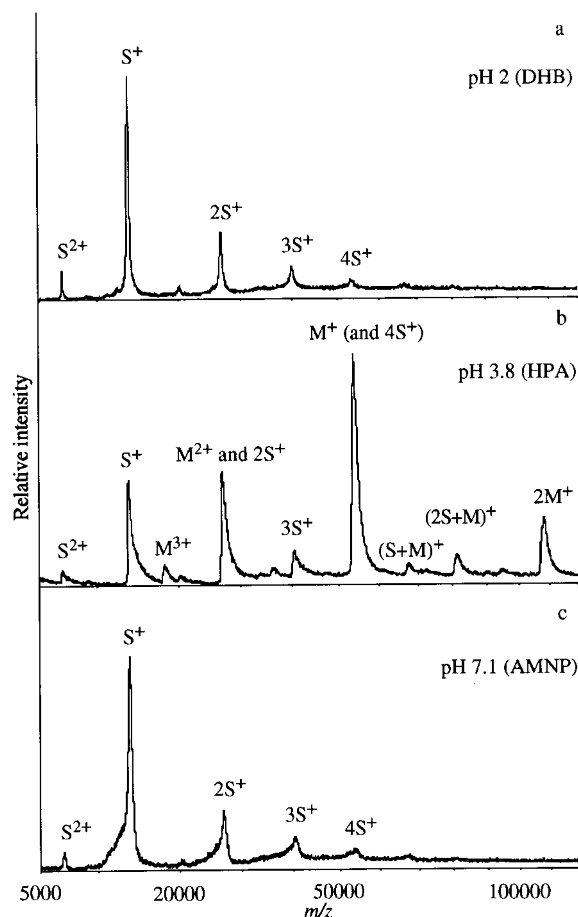

Figure 2. Comparison of MALDI mass spectra of recombinant streptavidin (0.2 mg ml^{-1}) obtained at different pH values with three different matrix compounds: (a) DHB in water (pH 2), (b) HPA in water (pH 3.8) and (c) AMNP in water (pH 7.1). All spectra were obtained with subsequent irradiation of the same sample area (sum of 20–30 laser shots). Peaks corresponding to the dissociated and undissociated subunits are denoted by S and M, respectively.

small amount of aggregation of the streptavidin tetramer into the octamer (i.e. the dimer of the tetrameric molecular complex) at mass 108.2 kDa. The relatively weak signal of the triply charged tetrameric molecular complex (M^{3+}) at mass 18.1 kDa suggests that the dimer cluster ion ($2S^+$) of the dissociated subunit (13.5 kDa) contributes significantly to the peak at mass 26.9 kDa, besides the doubly charged tetrameric complex (M^{2+}). The low abundance of the trimeric, pentameric and hexameric species suggests that the MALDI/MS data reflect the specific subunit association expected from solution behavior. In contrast to previous studies,[13,17] the observation of non-covalent complexes was not restricted to the first laser shot only. The spectrum shown in Fig. 2(b) was obtained under subsequent irradiation of the same sample area.

When recombinant streptavidin was prepared with one of the more neutral compounds ANA (pH 4.4), NA (pH 6.4) or AMNP (pH 7.1) as matrix, surprising only an ion signal corresponding to the subunit and no signal of the intact tetrameric complex were observed. For comparison, the MALDI spectrum of recombinant streptavidin at pH 7.1 with AMNP as matrix is shown in Fig. 2(c); the mass of the subunit was determined to be 13.4 kDa.

The ion signals were significantly broadened under more neutral pH conditions with either HPA, ANA, NA or AMNP as matrix as compared with the ion signals generated when the acidic DHB or HCCA matrix was used. Similar results were obtained by Fitzgerald *et al.*[20] when screening potential basic matrices for MALDI/MS analysis and the loss of mass resolution was explained by the formation of multiple matrix adducts. This accounts for the less accurate molecular mass determination with the more basic matrices.

Since dissociation into subunits is expected to occur with acidification and/or addition of an organic solvent, the stability of the tetrameric complex of recombinant streptavidin was further examined, preparing the HPA matrix with 0.1% TFA (pH 2) or with an increasing amount of acetonitrile (0–100%). Figure 3(a) and (b) show the spectra obtained for recombinant streptavidin with HPA dissolved in 0.1% TFA and 100% acetonitrile, respectively. Contrary to what was expected, the spectra obtained show no further dissociation into subunits than with HPA in water as matrix [Fig. 2(b)]. Dissociation into subunits was only found to be enhanced and completed using a higher concentration of the strong acid TFA (1–10%) as matrix solution, giving a pH below 1.

Human glutathione-*S*-transferase A1-1

Glutathione-*S*-transferase (GST) A1-1 belongs to the GST alpha family and is a dimeric liver enzyme com-

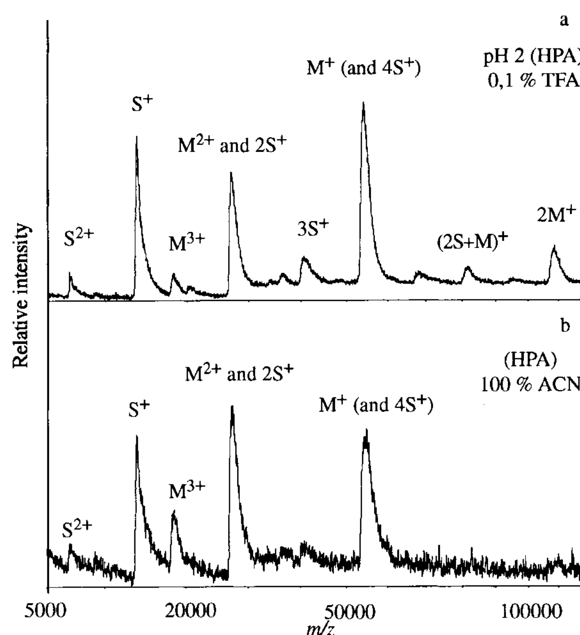

Figure 3. MALDI mass spectra of recombinant streptavidin (0.2 mg ml^{-1}) obtained from two HPA matrix solutions: (a) 0.1% TFA and (b) 100% acetonitrile. All spectra were obtained with subsequent irradiation of the same sample area (sum of 20–30 laser shots). Peaks corresponding to the dissociated and undissociated subunits are denoted by S and M, respectively.

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posed of two identical subunits (A1-1) with an average molecular mass of 25 499 Da.

MALDI mass spectra of GST A1-1 using DHB, HPA and AMNP as matrices are shown in Fig. 4(a), (b) and (c), respectively. Ions corresponding to the dimer (mass 51.3 kDa) and the monomer (mass 25.6 kDa) could be obtained with equal abundance when HPA was used as the matrix [Fig. 4(b)]. The doubly and triply charged ions together with the multimeric ions appeared at lower abundance. The relatively weak signal of the tetrameric ion (i.e. the dimer of the dimer) at mass 102.9 kDa, suggests that in addition to the trimeric complex ($3S^+$ at 77.0 kDa), cluster formation between the mono- and dimeric species (SM^+) contributes significantly to the peak at mass 77.0 kDa. As for recombinant streptavidin, dissociation into the subunits could only be enhanced and completed by adding a higher concentration of a strong acid such as TFA to the matrix solution.

When the sample was prepared using either the more acidic DHB (pH 2) or the more basic AMNP (pH 7.1) as matrix, the mass spectra [Fig. 4(a) and (c)] showed peaks indicative of the dissociated monomer only. The mass of the subunit was determined to be 25.5 and 26.3 kDa using DHB and AMNP as the matrix, respectively.

Human hemoglobin

In contrast to the identical subunits composing recombinant streptavidin and GSH transferases A1-1, human hemoglobin is a more complex non-covalent tetrameric protein consisting of two identical α-subunits (141 amino acids, mass 15 125 Da) and two identical β-subunits (146 amino acids, mass 15 865 Da) in addition to the heme moiety (616 Da) non-covalently bound to each chain.

When hemoglobin was prepared with either of the three matrices DHB, HPA or AMNP, only ion signals related to the individual α- and β-subunits were obtained [Fig. 5(a), (b) and (c), respectively]. With the more acidic matrix DHB [Fig. 5(a)] the three cluster ions obtained just above 30 kDa could be assigned to the homo dimers 2α and 2β and the hetero dimer $\alpha\beta$, whereas these signals were unresolved when HPA [Fig. 5(b)] or AMNP [Fig. 5(c)] was used as the matrix. In contrast to the results obtained for recombinant streptavidin and GSH transferases A1-1, it was not possible to

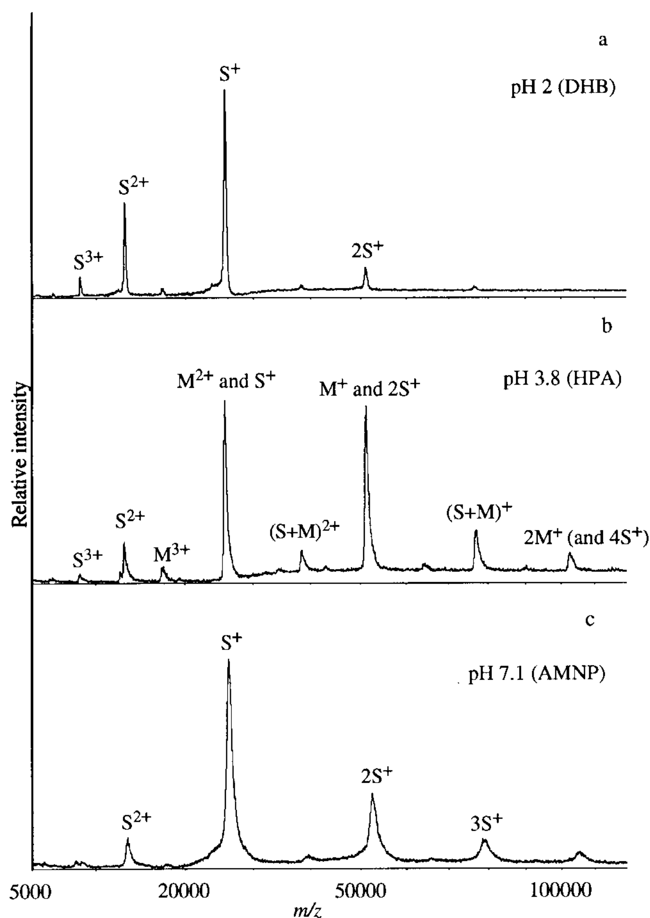

Figure 4. Comparison of MALDI mass spectra of glutathione-*S*-transferase obtained at different pH values with three different matrix compounds: (a) DHB in water (pH 2), (b) HPA in water (pH 3.8) and (c) AMNP in water (pH 7.1). All spectra were obtained with subsequent irradiation of the same sample area (sum of 20–30 laser shots). Peaks corresponding to the dissociated and undissociated subunits are denoted by S and M, respectively.

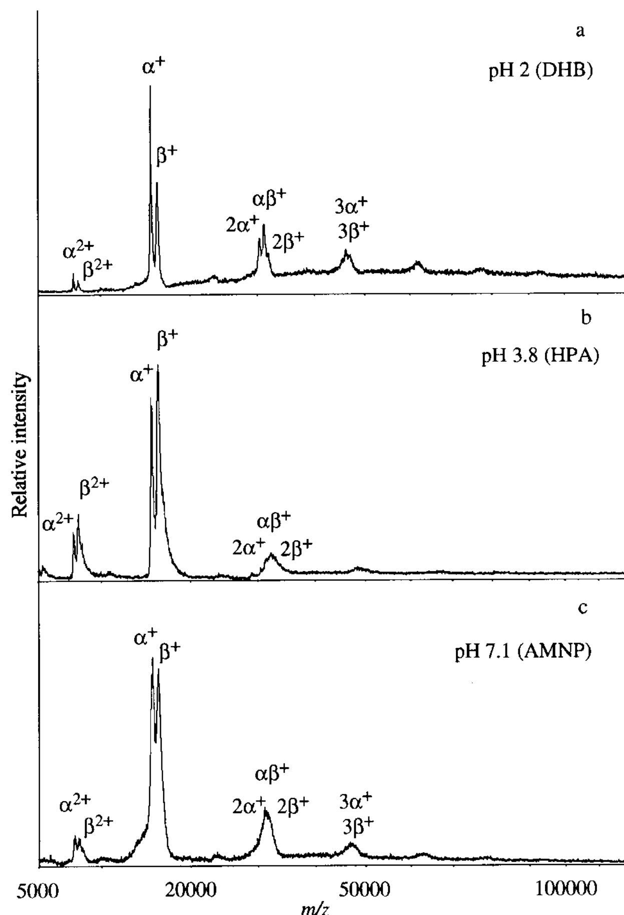

Figure 5. Comparison of MALDI mass spectra of human hemoglobin obtained at different pH values with three different matrix compounds: (a) DHB in water (pH 2), (b) HPA in water (pH 3.8) and (c) AMNP in water (pH 7.1). All spectra were obtained with subsequent irradiation of the same sample area (sum of 20–30 laser shots). Peaks corresponding to the dissociated α- and β-chains of human hemoglobin are denoted by α and β, respectively.

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maintain the physiologically significant tetrameric forms of human hemoglobin during MALDI/MS analysis with HPA as matrix [Fig. 5(b)]. Previous studies have shown that hemoglobin fully dissociates in solution into predominantly α/β dimers at pH 3.8.[11] Increasing the HPA matrix solution pH from 3.8 to 7, aimed at preventing the dissociation into the α- and β-subunits, revealed no significant change in the ion signal pattern (results not shown). Since dissociation of the non-covalent structure can occur not only in solution or during the transition from solution to solid phase, but also upon desorption/ionization after laser irradiation, photodissociation could be the reason for the unsuccessful detection of the $\alpha_2\beta_2$ complex.

All ions detected represent species without the heme group (apohemoglobin). As found with the quadrupled Nd:YAG laser at 266 nm with nicotinic acid as matrix, the dissociation of the heme group from the protein has been induced by the laser irradiation upon the strong deposition of energy.[11]

CONCLUSION

The ability to detect non-covalent complex ions in MALDI/MS was highly dependent on the choice of the matrix. Among the six matrices tested, specific non-covalent interactions could only be observed with HPA. In the case of recombinant streptavidin and GST A1-1, the non-covalent forces holding the subunits together were sufficiently strong to maintain the physiologically significant complex forms under the conditions employed with HPA as matrix. For human hemoglobin it was not possible to maintain the tetrameric complex with any of the matrices tested.

In contrast to previous studies,[13,17] the 'first laser shot phenomenon' was not observed for either streptavid or GST A1-1. The spectra were obtained with subsequent irradiation of the same sample area, indicating that the intact complexes are incorporated equally well at any level within the HPA matrix crystal.

Although the more acidic pH of the commonly used DHB and HCCA matrix solutions may be responsible for the dissociation of the proteins into subunits, unexpectedly dissociation of recombinant streptavidin and GST A1-1 complexes was not induced on lowering the pH of the HPA matrix solution. In addition, when the samples were prepared with more basic matrices ANA (pH 4.4), NA (pH 6.4) and AMNP (pH 7.1), only ion signals corresponding to the individual subunits appeared in the MALDI spectra. Considering these results, there does not appear to be a strong correlation between the matrix solution pH and the utility of a matrix for the analysis of non-covalently bound complexes, indicating that the matrix itself appears to be a key factor in the MALDI process. In addition, the unsuccessful detection of the hemoglobin $\alpha_2\beta_2$ complex indicates that the stability of non-covalent complexes might depend upon the deposition of the energy induced by the laser irradiation. Elucidation of matrix chemistry and desorption mechanism(s) will be of crucial importance for further developments in this field.

REFERENCES

1. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science* **246**, 64 (1989).
2. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.* **9**, 37 (1990).
3. M. Karas and F. Hillenkamp, *Anal. Chem.* **60**, 2299 (1988).
4. M. Karas, D. Bachmann, U. Bahr and F. Hillenkamp, *Int. J. Mass Spectrom. Ion Processes* **78**, 53 (1987).
5. B. Ganem, Y. Li and J. D. Henion, *J. Am. Chem. Soc.* **113**, 6294 (1991).
6. B. Ganem, Y. Li and J. D. Henion, *J. Am. Chem. Soc.* **113**, 7818 (1991).
7. J. A. Loo, *Bioconj. Chem.* **6**, 644 (1995).
8. M. Przybylski, J. Kast, M. O. Glocker, E. Dürr, H. R. Bosshard, S. Nock and M. Sprinzl, *Toxicol. Lett.* **82/83**, 567 (1995).
9. M. Karas, U. Bahr, A. Ingendoh and F. Hillenkamp, *Angew. Chem., Int. Ed. Engl.* **28**, 760 (1989).
10. M. Karas, U. Bahr, A. Ingendoh, E. Nordhoff, B. Stahl, K. Strupat and F. Hillenkamp, *Anal. Chim. Acta* **241**, 175 (1990).
11. F. Hillenkamp, M. Karas, A. Ingendoh and B. Stahl, A. L. Burlingame and J. A. McCloskey, p. 49. Elsevier, Amsterdam (1990).
12. M. J.-F. Suter, W. T. Moore, T. B. Farmer, J. S. Cottrell and R. M. Caprioli, R. H. Angeletti, p. 447. Academic Press, New York (1992).
13. B. Rosinke, K. Strupat, F. Hillenkamp, J. Rosenbusch, N. Dencher, U. Krüger and H.-J. Galla, *J. Mass Spectrom.* **30**, 1462 (1995).
14. M. Moniatte, F. G. v. d. Goot, J. T. Buckley, F. Pattus and A. v. Dorsselaer, *FEBS Lett.* **384**, 269 (1996).
15. P. Lecchi and L. K. Pannell, *J. Am. Soc. Mass Spectrom.* **6**, 972 (1995).
16. M. O. Glocker, S. H. J. Bauer, J. Kast, J. Volz and M. Przybylski, *J. Mass Spectrom.* **31**, 1221 (1996).
17. L. R. H. Cohen, K. Strupat and F. Hillenkamp, *J. Am. Soc. Mass Spectrom.* **8**, 1046 (1997).
18. D. Dogruel, R. W. Nelson and P. Williams, *Rapid Commun. Mass Spectrom.* **10**, 801 (1996).
19. H. Ehring, M. Karas and F. Hillenkamp, *Org. Mass Spectrom.* **27**, 472 (1992).
20. M. C. Fitzgerald, G. R. Parr and L. M. Smith, *Anal. Chem.* **65**, 3204 (1993).
21. K. J. Wu, A. Steding and C. H. Becker, *Rapid Commun. Mass Spectrom.* **7**, 142 (1992).
22. F. Hillenkamp and M. Karas, *Methods Enzymol.* 00, 280 (1990).

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