Reaction of the Indole Group with Malondialdehyde: Application for the Derivatization of Tryptophan Residues in Peptides

Alexandra Foettinger,† Michael Melmer, Alexander Leitner, and Wolfgang Lindner*

Department of Analytical Chemistry and Food Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria.
Received January 2, 2007; Revised Manuscript Received June 14, 2007

A method for the selective modification of tryptophan residues based on the reaction of malondialdehyde with the indole nitrogen of the tryptophan side chain at acidic conditions is presented. The condensation reaction is quantitative and leads to a substituted acrolein moiety with a remaining reactive aldehyde group. As is shown, this group can be further converted to a hydrazone using hydrazide compounds, but if hydrazine or phenylhydrazine are used, release of the free indole group is observed upon cleavage of the substitution. Alternatively, secondary amines such as pyrrolidine may also act as cleavage reagents. This general reaction scheme has been adapted and optimized for the derivatization of tryptophan-containing peptides and small N-heterocyclic compounds. It serves as the basis of a reversible tagging scheme for Trp-peptides or molecules of interest carrying indole structures as it allows the specific attachment and removal of a reactive group that may be used for a variety of purposes such as affinity tagging.

INTRODUCTION

Chemical labeling strategies in the field of peptide and protein analysis play an increasingly important role not only for structure elucidation, but also for quantitative purposes. In this context, derivatization reactions are used to introduce stable isotopes that enable relative quantification or to enrich certain classes of peptides and proteins via affinity tags (*1*, *2*). Furthermore, labeling and cross-linking reactions can be used for protein conformation studies, especially in combination with mass spectrometry (*3*−*5*). Moreover, for the determination of residues contributing to active sites of, e.g., receptor proteins or enzymes, dedicated derivatization reactions can be applied (*6*, *7*).

Tryptophan is an interesting target for the development of chemical tagging strategies. It is a low abundance amino acid with 1.1% frequency (*8*), but approximately 90% of proteins contain at least one tryptophan residue in their sequence (*9*). There are several well-known methods for the derivatization of tryptophan residues in peptides and proteins, most of them targeting the C-2 or C-3 position of the indole ring (see Figure 1). 2-Hydroxy-5-nitrobenzylbromide, for example, finds broad application for the investigation of the role of Trp in active sites of enzymes (*6*, *7*) for the estimation of the tryptophan content in proteins (*10*, *11*) and has recently been used for the determination of the surface accessibility of Trp residues in proteins (*12*). Another frequently used group of reagents are sulfenyl halides. Compounds like 2-nitro- and 2,4-dinitrophenylsulfenylchloride reacting at the 2-position of the indole group can be used for studying active sites of enzymes (*13*). Applications in the field of proteomics, including relative quantification studies employing stable isotope-labeled analogues, were reported (*14*−*16*); *N*-bromosuccinimide is a derivatizing reagent used for similar applications (*17*). Another method, selectively targeting the indole nitrogen, uses rhodium carbenoids for the derivatization of tryptophan residues in proteins as recently reported by Antos et al. (*18*).

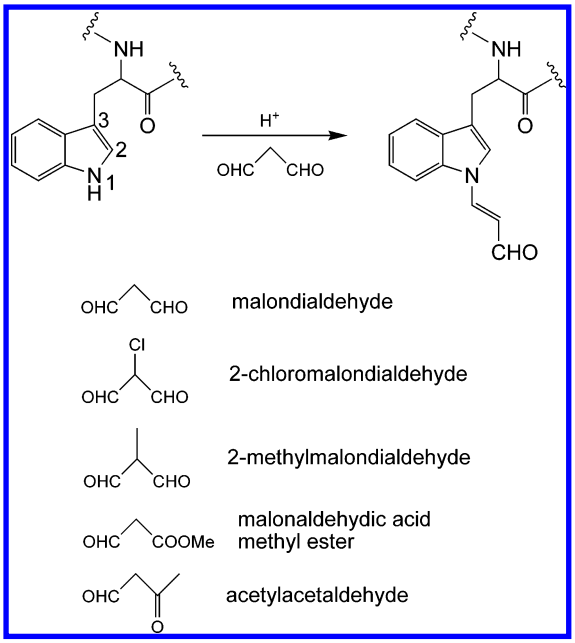

Figure 1. Reaction of the indole group of tryptophan in a peptide with malondialdehyde (MDA) and alternative reagents used in the study.

The principal reaction of the indole ring of the tryptophan side-chain with dicarbonyl compounds like acetylacetaldehyde or malondialdehyde (MDA) has already been briefly described in the 1960s by Teuber et al. (*19*, *20*) but was not followed up since then. The authors investigated the reaction on a synthetic scale for carbazole- and indole-type compounds in concentrated hydrochloric acid. Under these conditions, malondialdehyde also reacts with the guanidine group of arginine forming a pyrimidine (*21*) as reported by King and recently followed by work in our group (*22*).

We have investigated this reaction for the purpose of labeling arginine residues in peptides and observed a side reaction with tryptophan residues during optimization not observed by King.

* Correspondence to: Wolfgang Lindner, Department of Analytical Chemistry and Food Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria. Phone: +43 1 4277 52300. Fax: +43 1 3196312. E-mail: wolfgang.lindner@univie.ac.at.

† Current address: Baxter AG, Biomedical Research Center, Uferstrasse 15, 2304 Orth/Donau, Austria.

10.1021/bc070001h CCC: $37.00 © 2007 American Chemical Society
Published on Web 08/17/2007

Further studies have now revealed that the modification corresponds to a condensation product with the indole nitrogen as shown in Figure 1, in accordance with Teuber's previous work. Here, we report how this reaction scheme can be adapted for the selective derivatization of tryptophan residues in peptides and can be applied for the introduction of functional tags.

EXPERIMENTAL PROCEDURES

Materials. The derivatization reagents, 1,1,3,3-tetramethoxypropane (TMP) and 1,1,3,3-tetraethoxypropane (TEP), were from Aldrich (Steinheim, Germany). 1,1,3,3-tetraisopropoxypropane (*22*), 2-methylmalondialdehyde (*23*), and the sodium salt of MDA (*24*) were synthesized in-house.

All other chemicals and reagents were from Fluka (Buchs, Switzerland), Sigma (Deisenhofen, Germany), Aldrich, or Acros Organics (Geel, Belgium). All model peptides were acquired from Bachem and used as received (Weil am Rhein, Germany).

Instrumentation. Liquid chromatography−mass spectrometry was performed on an Agilent 1100 HPLC system coupled to an Agilent MSD Trap SL quadrupole ion trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with a standard electrospray source. Chromatographic separations were performed on a BetaBasic-18 column (150 × 4.6 mm, 3 μm, Thermo Electron Corporation, Runcorn, UK) equipped with a guard column containing the same material. The mobile phases for gradient elution were (A) 0.1% (v/v) formic acid in water and (B) 0.1% (v/v) formic acid in acetonitrile. The gradient was as follows: 0−5 min 5% B, 5−25 min 5−50% B, 25−30 min 50% B, 30−35 min 50−5% B, and 35−40 min 5% B. The flow rate was set to 0.7 mL min^{-1} and the column temperature to 25 °C. UV absorption was monitored at 230 nm using an Agilent 1100 variable wavelength detector.

For direct infusion experiments, samples were introduced with a syringe pump (kd scientific, Holliston, MA) at a flow rate of 5 μL min^{-1}.

Preparation and Characterization of the MDA Derivative of *N*-Acetyltryptophan (N-Ac-Trp) as Model/Reference Compound. Approximately 100 mg of N-Ac-Trp (400 μmol) was dissolved in 10 mL of 80% aqueous TFA and 800 μL of TMP (~10-fold molar excess) was added dropwise. The solution was stirred at room temperature for 1 h. Then, the solvent was evaporated in vacuo, and the product was taken up in ethyl acetate and purified by flash chromatography on silica with the mobile phase consisting of ethyl acetate, methanol, and acetic acid (40/1/1, v/v/v). Following removal of the solvents in vacuo, an intensively yellowish colored product was obtained in 72% yield.

^{1}H NMR (d_6-DMSO, 400 MHz): 9.49 (d, 1H), 8.55 (d, 1H), 8.09 (d, 1H), 7.90 (d, 1H), 7.77 (s, 1H), 7.62 (d, 1H), 7.35 (t, 1H), 7.26 (t, 1H), 6.4 (m, 1H), 4.56 (m, 1H), 3.18 (q, 1H), 3.08 (q, 1H), 1.83 (s, 3H) ppm.

^{13}C NMR (d_6-DMSO, 100 MHz): 191.95 (aldehyde C═O), 173.46 (carboxyl C═O), 169.58 (N-acetyl C═O), 145.84 (Ar CH), 136.45 (Ar), 130.26 (Ar), 124.31 (Ar CH), 123 (Ar CH), 122.92 (Ar CH), 119.84 (Ar CH), 119.68 (Ar), 112.58 (Ar CH), 111.09 (Ar CH), 52.76 (N-acetyl CH_3), 27.31 (CH_2), 22.81 (CH) ppm.

MS (ESI-quadrupole): $[M + H]^+ = 301.4$.

UV spectrum (methanol): 209, 272, 332 nm (ϵ 2.81 × 10^{-5}, 1.63 × 10^{-5}, 2.36 × 10^{-5} L mol^{-1} cm^{-1}).

Fluorescence spectrum (methanol): $\lambda_{exc.,max} = 230$ nm, $\lambda_{em.,max} = 380$ nm.

Analytical-Scale Derivatization Procedure for Trp-Containing Peptides. Typically, 50 μg of peptide was dissolved in 50 μL of aqueous acidic solution (hydrochloric acid, TFA, or acetic acid), and a 100-fold molar excess of TMP (0.9 μL) or TEP (1.2 μL) was added. After a reaction time of 1 h (at room temperature), the reaction mixture was diluted with water (1:10 for TFA and 1:100 for HCl conditions, respectively) and purified by solid-phase extraction on reversed-phase C_{18} material (100 mg sorbent, Phenomenex, Torrence, CA) to remove the acid and excess reagents. The elution solution was either used directly for infusion experiments or evaporated to dryness with nitrogen and redissolved in mobile phase for LC-MS analysis.

Formation of the Hydrazones. Typically, 50 μg of MDA-labeled peptide was dissolved in 200 μL ammonium acetate buffer (pH adjusted to 5 with acetic acid) containing 10 to 20 mM of hydrazide reagent (50−100 fold molar excess). Usually, the reaction proceeded for 1−2 h.

Cleavage Reaction Using Hydrazine Dihydrochloride. 50 μg of labeled peptide was incubated in 200 μL of a 20 mM aqueous hydrazine dihydrochloride solution with the pH adjusted to approximately 3 with acetic acid. The reaction mixture was incubated for 1 h at 50 °C to achieve complete cleavage.

Cleavage Reaction Using Pyrrolidine. 50 μg of labeled peptide was incubated in 200 μL of a water/acetonitrile mixture (50:50, v/v) containing 500 mM pyrrolidine. The reaction mixture was left for 1 h at room temperature to achieve complete cleavage.

Hazardous Substances Statement. Hydrazine dihydrochloride is a toxic and potentially carcinogenic reagent and should be handled with special care. Appropriate safety precautions should be taken when handling concentrated acids and bases.

RESULTS AND DISCUSSION

Reaction of the Indole Group of Tryptophan Residues with MDA−Reaction Conditions and Specificity. As mentioned in the Introduction, a side reaction with tryptophan residues was observed during MDA-labeling of arginine residues in peptides (*22*). To investigate this phenomenon further, dedicated studies were carried out using *N*-acetyltryptophan as a model compound. Using conditions comparable to the analytical-scale reaction for arginine labeling (12 M hydrochloric acid, room temperature), the N-Ac-Trp MDA-derivative was isolated in preparative scale (see Experimental Procedures for details). Characterization by ^{1}H and ^{13}C NMR as well as mass spectrometry allowed us to assign a structure like that shown in Figure 1, corresponding to a condensation product of MDA with the indole nitrogen in the form of an acrolein-type derivative.

To explore the possibility of applying this reaction for the modification of Trp-containing peptides, we studied whether lower concentrations of HCl or other organic acids can also be used for this reaction in order to avoid side reactions with arginine residues in peptides. Throughout this optimization procedure, N-Ac-Trp and the standard peptides PTHIK**W**GD, HPKRP**W**IL, L**W**MR, KG**W**K, and A**W** were used as model analytes. The reactivity was evaluated by means of mass spectrometry using an ion trap mass spectrometer with electrospray ionization either by direct infusion of the sample solution or by LC-MS.

First, we elucidated whether analyte-friendly conditions for a complete derivatization reaction can be achieved in reasonable time, avoiding degradation of the analytes and the occurrence of side reactions. Therefore, kinetic studies were performed with N-Ac-Trp at different concentrations of aqueous HCl (0.1, 1, 3, 6, and 12 M in water), TFA (20%, 50%, and 80% in water and 100%, v/v), and acetic acid (50% and 80% in water and 100%, v/v), and the reaction was followed by LC-MS and LC-UV. As a reagent, malondialdehyde may be applied in its commercially available acetal form (as tetramethoxy- or tetraethoxypropane) from which MDA is formed in situ during the reaction. Selected results illustrating the reaction kinetics in HCl

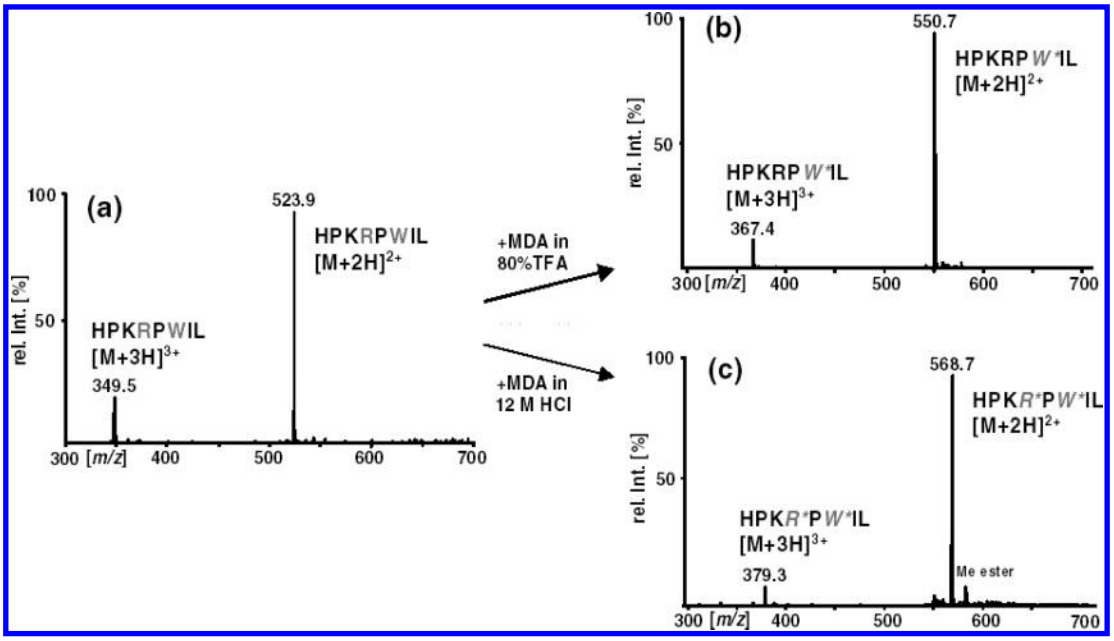

Figure 2. Mass spectra of the peptide HPKRPWIL (a) in the free form ($[M + 2H]^{2+} = 523.9$, $[M + 3H]^{3+} = 349.5$), (b) after MDA derivatization of Trp-residues in 80% TFA ($[M + 2H]^{2+} = 550.7$, $[M + 3H]^{3+} = 367.4$), and (c) after MDA derivatization of Trp and Arg residues in 12 M HCl ($[M + 2H]^{2+} = 568.7$, $[M + 3H]^{3+} = 379.3$). Modified residues are marked with asterisks.

and TFA solutions are shown as Supporting Information (Figures S1−S3).

Generally, the reaction proceeds in the presence of all three acids, but in acetic acid, even at a very high concentration, the reaction is not quantitative after 1 h (data not shown). When using smaller amounts of acid such as 1 M HCl or 20% TFA or less in aqueous solution, the formation of Schiff bases with amino groups that are usually present in amino acids and peptides (N-terminus, lysine) is observed and the yield of the desired reaction product is reduced. Consequently, we found that at least 3 M HCl or 50% of TFA in water are necessary to achieve complete indole group reaction within 1 h at room temperature and to avoid Schiff base formation. Using aqueous TFA (usually 80%), no side reactions such as Schiff base formation or conversion of the guanidine group of arginine (*22*) are observed. In contrast, when using HCl the reaction with arginine residues has to be considered, which always proceeds to some extent in the presence of HCl but completely only at 12 M HCl (*22*). An example of the derivatization reaction at different conditions is given in Figure 2 for the model peptide HPKRPWIL.

Additionally, ester formation with the alcohol released from the MDA−acetal is observed under hydrochloric acid conditions which could be minimized by using the isopropyl−acetal of MDA or the sodium salt of MDA (*24*) which are, however, not commercially available. Reaction yields (>98% for 80% TFA and ∼40% for 12 M HCl) were determined by using the synthetically prepared N-Ac-Trp MDA adduct as external standard for quantification by HPLC-UV, again favoring TFA conditions. Therefore, for all further experiments, 80% TFA was used unless otherwise stated.

Expansion of the Reaction Concept toward Other Analytes and Reagents. In order to elucidate the broader applicability of the MDA-based reaction, we tested various compounds at the optimized reaction conditions. These were, on one hand, various indole and tryptophan derivatives, but also other N-heterocyclic compounds. As already found by Teuber (*19*, *20*), carbazoles react in the same way as the indole group under the conditions we applied. Additionally, 3-chloroindazole and 3-indazolinone also reacted, whereas indazole itself did not react. Pyrrole was found to give the corresponding product at least partially. For pyrazole, benzotriazole, and benzimidazole derivatives, no reaction product was observed.

To explore the full potential of the reaction type outlined in Figure 1, reagents structurally related to malondialdehyde were also tested, and the reaction products were again evaluated by LC-MS. The 2-substituted derivatives of MDA, 2-chloromalondialdehyde and 2-methylmalondialdehyde, were found to react, but much more slowly than MDA itself. In TFA, almost no product was detected, and with hydrochloric acid as the medium even after a reaction time of 2 h, conversion was not complete for Trp−peptides, as could be judged from the underivatized analytes still present. Malonaldehydic acid methyl ester (applied as 3,3-dimethoxypropionic acid methyl ester), carrying one aldehyde and one ester group, does also react with the indole nitrogen in TFA as well as HCl. LC-MS experiments revealed that the reaction proceeds quantitatively with tryptophan and Trp-containing peptides, as no unmodified analytes were observed, but it turned out that the product is chromatographically not homogeneous (whereas the MDA derivative is). Another disadvantage of this reagent is that side products formed by the reagent itself were observed which interfere with the analysis of the derivatized peptides (e.g., as coeluting peaks during LC-MS analysis). Acetylacetaldehyde (used as the dimethylacetal) also reacts according to the general reaction scheme shown in Figure 1 as observed previously by Teuber et al. (*19*, *20*) but establishes the same problem concerning disturbing side products.

Reagents having only keto groups, but no aldehyde functionalities such as acetylacetone and acetoacetateic acid methyl ester, did not show any reactivity toward the indole group. Using dichloroacetaldehyde (applied as the diethylacetal) as the reagent, no condensation product was observed, and monoaldehydes like propionaldehyde and butyraldehyde did not react in a defined way as well. Although formation of a double bond is possible in principle, this has not been observed, and a mixture of different products was observed by MS analysis. These were not characterized in detail, although evidence for cross-linking processes (i.e., aminal formation) was obtained from LC-MS/MS experiments.

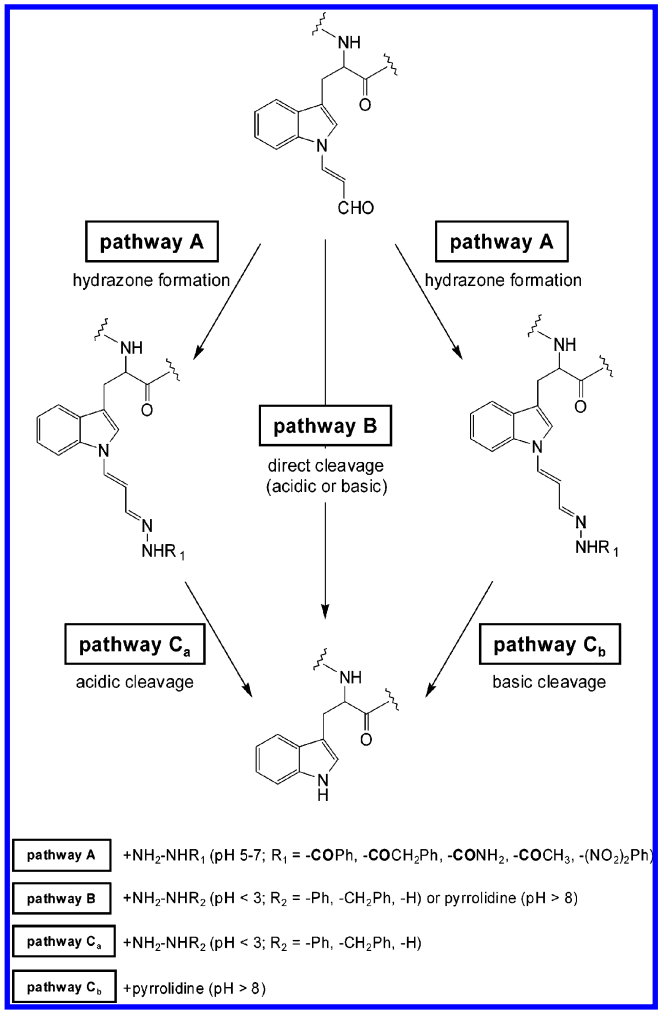

Figure 3. Reaction pathways of the tryptophan−MDA condensates (N-acroleinyl derivatives) that were evaluated in this study. For details, see text.

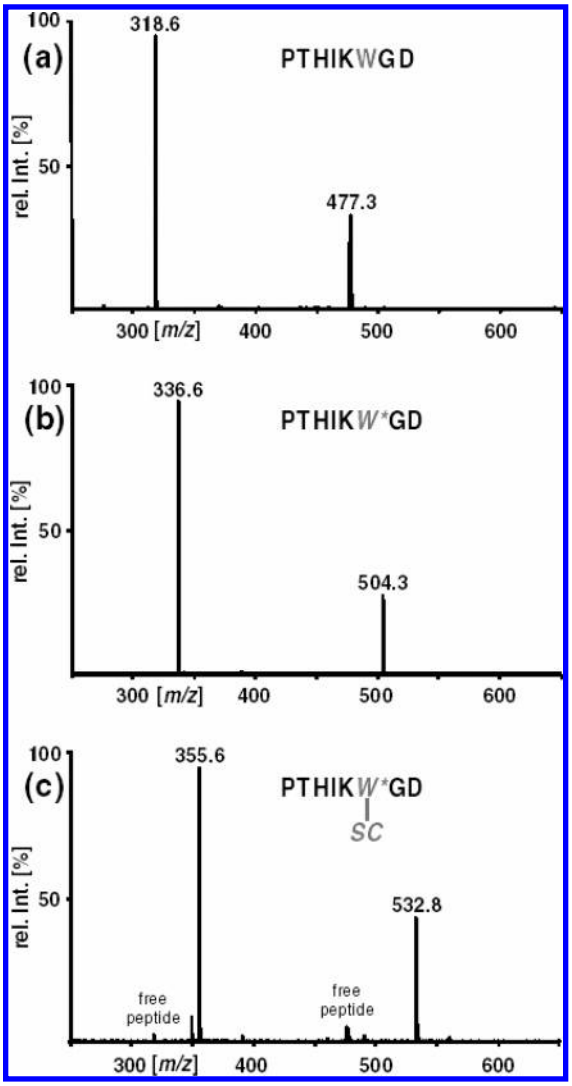

Figure 4. Mass spectra of the peptide PTHIKWGD (a) in the free form ($[M + 2H]^{2+} = 477.3$, $[M + 3H]^{3+} = 318.6$), (b) after reaction with MDA ($[M + 2H]^{2+} = 504.3$, $[M + 3H]^{3+} = 336.6$), and (c) after further reaction of the acroleinyl moiety (free aldehyde) to the semicarbazone ($[M + 2H]^{2+} = 532.8$, $[M + 3H]^{3+} = 355.6$).

Further Reactions of the α,β-Unsaturated Aldehyde Derivatives. The α,β-unsaturated aldehyde group resulting from the reaction of the tryptophan side chain with malondialdehyde, which de facto corresponds to a 3-substituted acrolein moiety, opens up the possibility for further reactions. Such carbonyl groups typically react with amino groups or hydrazines or carbazides to Schiff base-type products (see Figure 3). Dinitrophenylhydrazine, for instance, is a classical reagent used for the analysis of small aldehydes often applied in environmental chemistry (*25*), and also for studying protein oxidation (*26*).

Hydrazone formation (pathway A in Figure 3) was successfully tested with 2,4-dinitrophenylhydrazine and semicarbazide using the peptide PTHIKWGD as model analyte (see Figure 4). Using phenylhydrazine, however, we noticed the removal of the initially present acroleinyl label, resulting in the unlabeled indole moiety of the peptide being observed (reaction pathway B in Figure 3). Instability of the condensation product upon addition of excess phenylhydrazine was already noticed by Teuber in the 1960s (*19*). To investigate this in more detail, different hydrazines (phenylhydrazine, benzylhydrazine, hydrazine) and hydrazides (benzylhydrazide, acethydrazide, semicarbazide) were tested for their reactivity, and we observed that hydrazides, with a carbonyl group next to the hydrazine group, form stable adducts with the aldehyde (pathway A), while hydrazines lead to cleavage of the whole MDA adduct resulting in the free indole group (pathway B). 2,4-Dinitrophenylhydrazine seems to be an exception, but the explanation might be that the electron withdrawing nitro groups significantly influence the properties of the molecule. As a third option, we incubated a hydrazone-labeled peptide with hydrazine under acidic conditions, also resulting in the parent compound (see pathway C in Figure 3).

The conditions for both reactions, the hydrazone formation and the cleavage of the MDA derivative with hydrazine, were optimized concerning pH, buffer, temperature, and reagent excess followed by LC-MS analysis. Acetate and phosphate buffers were tested at different pH values. For hydrazone formation (pathway A), approximately 50-fold molar reagent excess at a pH of 5−7 was found to be the optimum. A slight increase in reaction speed can be achieved by increasing the temperature from room temperature to 50 °C. At acidic pH conditions, the hydrazones are unstable when the reagent excess is removed. In contrast, the hydrazone bond remains stable in alkaline milieu (pH > 9). The optimal results for the cleavage reaction (pathways B and C_a) were found using hydrazine (applied as the dihydrochloride salt) in ammonium acetate solution at a pH of approximately 3. Increasing the temperature during the cleavage step to 50 °C makes the reaction significantly faster. Using N-Ac-Trp as a model analyte, the individual yields of the three reaction steps (MDA adduct formation, conversion to a hydrazide using acethydrazide, cleavage using hydrazine) were evaluated under optimized conditions. Accord-

ing to LC-MS data, all reactions proceeded with yields in excess of 90%. Furthermore, these reaction conditions were found to be fully compatible with the integrity of a number of model peptides covering all proteinogenic amino acids, as no degradation products were observed in noticeable amounts.

The release of the free indole moiety upon addition of hydrazine is only observed at acidic or neutral pH. Incubation under alkaline conditions, for example, with hydrazine or phenylhydrazine in methanol, leads to formation of a stable hydrazone that can be detected by HPLC-MS, but upon acidification, the whole label is released, leading to the free indole moiety. This very specific cleavage reaction is only possible for the malondialdehyde adduct but not for products resulting from the reaction with the other reagents such as malonaldehydic acid methyl ester (see Figure 1). This led us to the assumption that cleavage of the N-linked acroleinyl substituent only occurs after intermediate formation of the hydrazone, which seems to activate the cleavage reaction. When this process cannot be promoted, the cleavage reaction according to Figure 3 (pathways B and C_a) does not occur.

As an alternative route for the removal of the MDA adduct, we investigated an amine-mediated nucleophilic cleavage. A similar strategy was employed by Villarasa and co-workers (*27*) for the cleavage of N-substituted nucleosides resulting from the reaction with methyl propynoate (other bases may be used as well (*28*, *29*)). Several primary and secondary amines were studied, including ammonium hydroxide, diethylamine, pyrrolidine, and piperidine. Removal of the acroleinyl group from the indole nitrogen was possible with all reagents to some extent, but pyrrolidine consistently gave the best results in terms of reaction speed and clean reaction. Even at room temperature, quantitative cleavage was observed in 1 h upon incubation with 500 mM pyrrolidine in aqueous or hydroorganic medium (water/acetonitrile, 50:50, v/v). Pyrrolidine-mediated cleavage is possible both directly from the MDA adduct (pathways B) and via the hydrazone (pathway C_b).

Conclusions and Outlook; Potential Applications of the Reversible MDA Tag on Tryptophan Residues. In this contribution, we have described the adaptation and optimization of the condensation reaction of malondialdehyde (MDA) with the indole nitrogen of tryptophan residues in peptides under strongly acidic conditions. The resulting acroleinyl label at the indole nitrogen can be used for further reactions, e.g., Schiff base formation, as we have shown for the formation of hydrazides. Cleavage of these MDA labels to the free indole group was found to be possible with reagents such as hydrazine (at acidic conditions) and pyrrolidine (at alkaline conditions).

This highly chemoselective cascade of reactions, including the cleavage of the MDA "tag" resulting in the release of the free intact indole group, opens up the possibility for various highly interesting applications. For instance, we have now developed a solid-phase capture strategy for tryptophan-containing peptides involving a solid support carrying hydrazide groups (*30*). This protocol can be used for the selective enrichment of other analytes of interest that carry indole moieties from complex samples such as biofluids. Furthermore, labels for specific fluorescence detection or isotope labels and so forth could be introduced via the acroleinyl group, always with the possibility of complete cleavage of the label at a later stage, resulting in the unlabeled, intact starting analytes. This could be important for further MS analysis, because high molecular mass labels or tags may be problematic when LC-MS and MS/MS analysis is performed if they show decreased sensitivity or unfavorable fragmentation properties (*31*). This has also been observed for some MDA-labeled Trp−peptides that show dominant cleavage of water during MS/MS, but can be overcome by release of the free indole group before MS analysis.

ACKNOWLEDGMENT

The authors would like to thank the FWF (Austrian Science Fund, project #P15482) for financial support.

Supporting Information Available: LC-MS and LC-UV chromatograms illustrating the reaction kinetics of N-Ac-Trp with MDA in 1, 3, and 6 M HCl as well as 80% TFA (Figures S1 and S2, respectively); overview of reaction kinetics (% yield in dependence of reaction time) for the MS and UV assays (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Leitner, A., and Lindner, W. (2004) Current chemical tagging strategies for proteome analysis by mass spectrometry. *J. Chromatogr., B 813*, 1−26.

(2) Leitner, A., and Lindner, W. (2006) Chemistry meets proteomics: The use of chemical tagging reactions for mass spectrometry-based proteomics. *Proteomics 6*, 5418−5434.

(3) Ethier, M., Lambert, J.-P., Vasilescu, J., and Figeys, D. (2006) Analysis of protein interaction networks using mass spectrometry compatible techniques. *Anal. Chim. Acta 564*, 10−18.

(4) Sinz, A. (2003) Chemical crosslinking and mass spectrometry for mapping three-dimensional structures of proteins and protein complexes. *J. Mass Spectrom. 38*, 1225−1237.

(5) Winston, R. L., and Fitzgerald, M. C. (1997) Mass spectrometry as a readout of protein structure and function. *Mass Spectrom. Rev. 16*, 165−179.

(6) Wink, M. R., Buffon, A., Bonan, C. D., Valenzuela, M. A., Sarkis, J. J. F., and Battastini, A. M. O. (1999) Effect of protein-modifying reagents on ecto-apyrase from rat brain. *Int. J. Biochem. Cell Biol. 32*, 105−113.

(7) Bell, J. E., Castellino, F. J., Trayer, I. P., and Hill, R. L. (1975) Modification of bovine alpha-lactalbumin with N-bromosuccinimide and 2-hydroxy-5-nitrobenzylbromide. *J. Biol. Chem. 250*, 7579−7585.

(8) Gilis, D., Massar, S., Cerf, N. J., and Rooman, M. (2001) Optimality of the genetic code with respect to protein stability and amino-acid frequencies. *Genome Biol. 2*, research0049.1-0049.12. (DOI:10.1186/gb-2001-2-11-research0049).

(9) Gevaert, K., Van Damme, P., Martens, L., and Vandekerckhove, J. (2005) Diagonal reverse-phase chromatography applications in peptide-centric proteomics: Ahead of catalogue-omics? *Anal. Biochem. 345*, 18−29.

(10) Horton, H. R., and Koshland, D. E., Jr. (1965) A highly reactive colored reagent with selectivity for the tryptophan residue in proteins. 2-Hydroxy-5-nitrobenzyl bromide. *J. Am. Chem. Soc. 87*, 1126−1132.

(11) Barman, T. E., and Koshland, D. E., Jr. (1967) A colorimetric procedure for the quantitative determination of tryptophan residues in proteins. *J. Biol. Chem. 242*, 5771−5776.

(12) Strohalm, M., Santrucek, J., Hynek, R., and Kodicek, M. (2004) Analysis of tryptophan surface accessibility in proteins by MALDI-TOF mass spectrometry. *Biochem. Biophys. Res. Commun. 323*, 1134−1138.

(13) Scoffone, E., Fontana, A., and Rocchi, R. (1968) Sulfenyl halides as modifying reagents for polypeptides and proteins. I. Modification of tryptophan residues. *Biochemistry 7*, 971−979.

(14) Kuyama, H., Watanabe, M., Toda, C., Ando, E., Tanaka, K., and Nishimura, O. (2003) An approach to quantitative proteome analysis by labeling tryptophane residues. *Rapid Commun. Mass Spectrom. 17*, 1642−1650.

(15) Ou, K., et al. (2006) Quantitative profiling of drug-associated proteomic alterations by combined 2-nitrobenzenesulfenyl chloride (NBS) isotope labeling and 2DE/MS identification. *J. Proteome Res. 5*, 2194−2206.

(16) Li, C., Gawandi, V., Protos, A., Phillips, R. S., and Amster, I. J. (2006) A matrix-assisted laser desorption/ionization compatible reagent for tagging tryptophen residues. *Eur. J. Mass Spectrom. 12*, 213−221.

(17) Spande, T. F., Green, N. M., and Witkop, B. (1966) The reactivity toward N-bromosuccinimide of tryptophan in enzymes, zymogens, and inhibited enzymes. *Biochemistry 5*, 1926−1933.

(18) Antos, J. M., and Francis, M. B. (2004) Selective tryptophan modification with rhodium carbenoids in aqueous solution. *J. Am. Chem. Soc. 126*, 10256−10257.

(19) Teuber, H. J., Cornelius, D., and Pfaff, H. (1963) N-Substitution of indoles with hydroxymethylene ketones. *Chem. Ber. 96*, 2617−2631.

(20) Teuber, H. J., Glosauer, O., and Hochmuth, U. (1964) Syntheses of heterocycles with hydroxymethylene ketones. IV. A new condensation product from tryptamine and acetoacetaldehyde. *Chem. Ber. 97*, 557−562.

(21) King, T. P. (1966) Selective chemical modification of arginyl residues. *Biochemistry 5*, 3454−3459.

(22) Foettinger, A., Leitner, A., and Lindner, W. (2006) Derivatisation of arginine residues with malondialdehyde for the analysis of peptides and protein digests by LC-ESI-MS/MS. *J. Mass Spectrom. 41*, 623−632.

(23) Arnold, Z., and Sorm, F. (1958) Synthetical reactions of dimethylformamide I. General synthesis of β-dialdehydes. *Coll. Czech. Chem. Commun. 23*, 452−461.

(24) Slatter, D. A., Murray, M., and Bailey, A. J. (1998) Formation of a dihydropyridine derivative as a potential cross-link derived from malondialdehyde in physiological systems. *FEBS Lett. 421*, 180−184.

(25) Vogel, M., Buldt, A., and Karst, U. (2000) Hydrazine reagents as derivatizing agents in environmental analysis-a critical review. *Fresenius J. Anal. Chem. 366*, 781−791.

(26) Levine, R. L., Williams, J. A., Stadtman, E. R., and Shacter, E. (1994) Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol. 233*, 346−357.

(27) Faja, M., Ariza, X., Gálvez, C., and Villarasa, J. (1995) Reaction of uridines and thymidines with methyl propynoate. A new N-3 protecting group. *Tetrahedron Lett. 36*, 3261−3264.

(28) Sun, L.-P., and Dai, W.-M. (2006) An engineered linker capable of promoting on-resin reactions for microwave-assisted solid-phase organic synthesis. *Angew. Chem., Int. Ed. 45*, 7255−*7258.*

(29) Dai, W.-M., Guo, D.-S., and Sun, L.-P. Chemistry of aminophenols. Part 1: Remarkable additive effect on Sonogashira cross-coupling of 2-carboxamidoaryl triflates and application to novel synthesis of indoles. *Tetrahedron Lett. 42*, 5275−5278.

(30) Foettinger, A., Leitner, A., and Lindner, W. (2007) Selective enrichment of tryptophan-containing peptides from protein digests employing a reversible derivatization with malondialdehyde and solid-phase capture on hydrazide beads. *J. Proteome Res.*, in press, DOI:10.1021/pr0702767.

(31) Borisov, O. V., Goshe, M. B., Conrads, T. P., Rakov, V. S., Veenstra, T. D., and Smith, R. D. (2002) Low-energy collision-induced dissociation fragmentation analysis of cysteinyl-modified peptides. *Anal. Chem. 74*, 2284−2292.

BC070001H