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Fluorescence, photodestruction, photoionization and thermal degradation of *o*-phthalaldehyde/β-mercaptoethanol-labelled aliphatic α-oligopeptides

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Abstract

Photophysical and photochemical properties of *o*-phthalaldehyde/β-mercaptoethanol-labelled aliphatic α-peptides were investigated. It is found that α-peptide derivatives have lower fluorescence quantum yields, higher photodestruction quantum yields and lower yields for formation of solvated electrons as compared to amino acid and simple alkylamine derivatives in aqueous alkaline solution. These properties of the α-peptide derivatives sets narrow limits for their utilization in laser-based (high light intensity) detector systems. In contrast, the thermal stability of the peptide derivatives was found to be severalfold higher than for the parent amino acid derivatives. The differential rates of thermal derivative degradation could be utilized in a new approach towards selective determination of peptides.

1. Introduction

o-Phthalaldehyde/β-mercaptoethanol (OPA/βME) is well suited as a fluorogenic reagent for both pre- and post-separation labelling of amino acids in liquid chromatographic (LC) and capillary electrophoretic (CE) analyses [1–5]. The fluorescence intensity from OPA/βME-labelled α-peptides is weaker than for labelled amino acids [3,6], which in part is explained by the lower fluorescence quantum yields (Φ_f) of the α-peptide-containing chromophores [3]. However, even if corrected for differences in fluorescence quantum yields, the fluorescence signal obtained from α-peptide derivatives, also with conventional detectors (low light intensity), are much lower than expected. Thus, there must exist some process for depletion of ground state

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molecules that is favoured for peptide as compared to amino acid derivatives. The identity of this pathway has, hitherto, not been demonstrated. Further, it has not been established if laser-induced fluorescence (LIF) detection (high light intensity) will enable highly sensitive detection of α-peptide derivatives because the key photophysical and photochemical parameters required to optimize LIF detection [7] have not been fully characterized for these chromophores.

In the present study we determined photophysical and photochemical properties of OPA/βME α-peptide and amino acid derivatives that are of fundamental value for optimization of LIF detection. It is found that photodestruction quantum yields for α-peptide derivatives could be as high as 0.5. This, in combination with low fluorescence quantum yields, explains the weak fluorescence yielded even with low intensity excitation sources. Less than 0.2 photon/Ala peptide derivative and 13 photons/Ala derivative are expected at the most at high laser irradiances. At this level, a 80–90-fold detection selectivity for amino acid over peptide derivatives is yielded. As previously shown for certain OPA/βME-amino acid derivatives and cyclodeca[*a*]indene derivatives [8], also the derivatives in the present investigation were ionized upon excitation. The formation of solvated electrons correlated with the fluorescence lifetimes, hence α-peptide derivatives yielded fewer solvated electrons than did amino acid and alkylamine derivatives. We further describe two related ways to introduce selectivity over amino acids in peptide determinations. These approaches take advantage of the superior thermal stability in solution of the peptide derivatives. Degradation at basic pH is the most selective against amino acids, but is rather time consuming (several hours). In acidic solution, most amino acids could be degraded in less than 10 min, however, with a concomitant increased decay rate of peptide derivatives. Any fluorescent by-products could not be observed. This procedure could substantially aid in order to clean up samples from amino acids to more favourably detect peptides.

2. Experimental

2.1. Apparatus

Electrophoresis experiments in 780 mm × 50 μm I.D fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were performed using a laboratory-built apparatus based on a Bertan 2462 power supply (Bertan, Hickswill, NY, USA). The working potentials applied were unless otherwise noted 27 kV and currents were about 40 μA. Injections were done by raising the injection end of the capillary 6.5 cm above the negative end for 30 s. A 1-cm portion of the capillary polyimide layer was removed by a butane flame to serve as a detection window. A Shimadzu RF 530 (Kyoto, Japan) HPLC fluorescence detector fitted with a Scott UG11 filter on the excitation side was rebuilt for use with capillaries. The spectral power (340 nm) on the capillary detection window was estimated to be about 0.5 μW (18 nm bandwidth). Electropherograms were recorded using a Perkin-Elmer Model 561 strip chart recorder.

The quantum yields of photodestruction (Φ_D) were measured in an optical bench arrangement comprising a Xe arc lamp and a monochromator from Applied Photophysics (Leatherhead, UK) as described previously [8]. Azobenzene [9] calibrated against ferrioxalate [10] was used as an actinometer. Laser-flash photolyses were performed on continuously stirred and deoxygenated solutions using the 355 nm line (frequency-tripled 1064 nm line) of a Spectron Nd:YAG laser Model SL 803 G (Spectron Laser Systems, Rugby, UK). The pulse duration and energy were 7 ns (full width at half maximum) and 45 mJ, respectively. A commercially available laser-flash photolysis spectrometer from Applied Photophysics was used. Uncorrected fluorescence spectra were recorded on derivatized 10–50 μM standard solutions at pH 9.5 (2 min reaction time, at least 150-fold molar excess of reagent) using a Shimadzu RF5001 PC spectrofluorimeter. Corrected fluorescence spectra of the derivatives in 50 mM borate buffer (pH 9.5) were recorded using an Aminco SPF-500

spectrofluorimeter. Fluorescence quantum yields, corrected for differences in solvent refractive indices, were calculated using 9,10-diphenylanthracene in cyclohexane as a reference compound ($\Phi_f = 0.9$) [11]. UV absorption spectra were obtained on a Varian CARY 4 UV–Vis spectrophotometer (Varian, Australia). For reaction kinetics 160 μM standard solutions were derivatized using a 150-fold molar excess reagent. Molar absorptivities (corrected for a derivatized water blank) were obtained for derivatized 160–250 μM standards (2 min reaction time, 150-fold molar excess of reagent) against a distilled water blank. Thermal degradation kinetics were monitored using CE. Here, a derivatized sample was injected on the capillary after a controlled period of time as detailed below.

2.2. Standard solutions

Stock solutions were prepared in distilled deionized water with the occasional addition of HCl in order to properly dissolve the peptides. The standards were stored at −20°C. Ala, Gly, Gly_2 to Gly_5 and Ala_2 to Ala_5 were obtained from Sigma (St. Louis, MO, USA), and H–Ala_4–Glu_4–OH, Gly_6, Ala_6, Val–Val and Val–Ala from Bachem (Bubendorf, Switzerland). The combined amino acid standard AA-S-18 (Sigma) contains: Ala, NH_3, Arg, Asp, cystine, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr and Val in 0.1 M HCl. This was neutralized with an equal volume 0.1 M NaOH to yield a final concentration of 1.25 mM of each amino acid (except cystine, 0.625 mM).

2.3. Reagents and derivatization procedures

The borate buffer (ionic strength 54 mM, pH 9.6) used in the CE experiments was prepared by mixing 50 ml of 40 mM disodiumtetraborate (Merck, Darmstadt, Germany), 14.1 ml of 0.1 M NaOH and 35.9 ml of distilled deionized water. A borate buffer for use with reagents was prepared by dissolving boric acid in distilled deionized water to a final concentration of 0.8 M. The pH was adjusted to 9.5–10.5 with 5.0 M NaOH. The OPA/βME reagent solutions were prepared by dissolving 100–333 mg OPA (99%, Sigma) in 1 ml of MeOH and adding to this solution 100–333 μl of βME (99%). This was made up to 10 ml with the borate buffer. The hydrochloric acid (0.5 M) was glass distilled. βME (Sigma) was used as received. Derivatizations were performed in polypropylene vials washed in HNO_3 (1 M) and thereafter rinsed with distilled deionized water and ethanol. In the derivatization reactions, 1:1 to 1:8 reagent to standard volume ratios were typically used. In some experiments 0.5 M HCl was added to accelerate the degradation of amino acid derivatives. After a controlled period of time, as detailed in the proceeding text, the solution was injected on the capillary. In the laser-flash photolysis and photodegradation experiments, stoichiometric amounts of reactants were used. The completion of the derivatization reactions were then ascertained by absorption measurements at 340 nm.

3. Results and discussion

3.1. Derivative formation

In reactions of amino acids with OPA/βME, 1-alkylthio-2-alkylisoindoles are formed in less than 1 min, provided that the reagent is held at millimolar concentrations [2,3]. With many reagents, peptides requires lengthy reaction times and sometimes also heating. Here, however, the reaction was brought to completion in 2 min even with hexa- and octapeptides (Fig. 1). Such rapid reaction rates are favourable, in particular for post-separation derivatization schemes, where short reaction times are sought.

3.2. Photophysical and photochemical properties

Qualitatively, OPA/βME-amino acid and -peptide derivative fluorescence spectra were similar to each other. The maxima of the excitation spectra and the maxima of the emission spectra were centered around 336 and 450 nm,

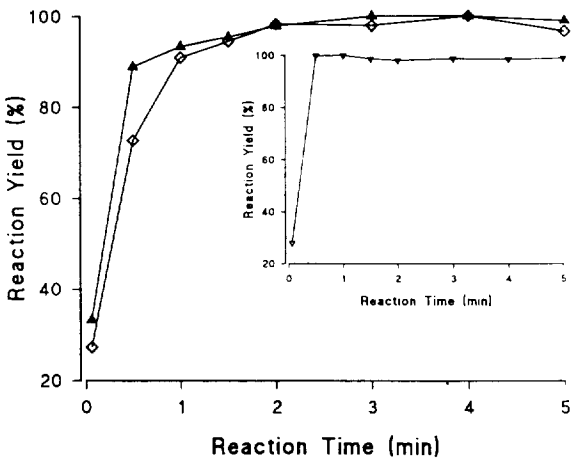

Fig. 1. Influence on reaction time for formation of OPA/βME-peptide derivatives. Gly_6 (◇), Ala_6 (▲) and inset: Ala_4–Glu_4. The reactions were followed by UV absorption measurements at 340 nm.

respectively. For all derivatives molar absorptivities were in the range $6000 \pm 300\ M^{-1}\ cm^{-1}$ at an absorption wavelength of 336 nm. The fluorescence quantum yields, however, depend significantly on the structure of the analyte (Table 1). Our results for the Ala, Gly_2, Gly_3 and Ala_3 derivatives are in agreement with those of Chen et al. [3]. Within the respective series, the largest decrement in fluorescence quantum yield occurs between the amino acid and the dipeptide, whereas further extension of the peptide backbone has little influence. It has been shown previously that OPA/βME-labelled peptides and amino acid amides have, in addition to lower fluorescence quantum yields, significantly shorter excited state lifetimes as compared to amino acid derivatives [3]. The peptide derivatives thus have an increased rate of some non-radiative process.

We previously showed that certain OPA/βME-amino acid and cyclodeca[*a*]indene derivatives undergo facile photochemical decomposition with quantum yields, Φ_D, in the range 0.01–0.14 [8]. Here, we determined the Φ_D values for the *n*-propylamine, Ala and the peptide derivatives in the Ala series (Table 1). With Φ_D values as high as 0.5, α-peptide derivatives are the most photolabile of OPA/βME derivatives determined so far. It is interesting to note that photodestruction is the major pathway and considerably more important than fluorescence for deexcitation of α-peptide derivatives while the

Table 1
Fluorescence quantum yields, fluorescence lifetimes, photodestruction quantum yields and photodestruction lifetimes of OPA/βME derivatives

Amine	Φ_f [a]	τ_f/ns [b]	Φ_D [c]	τ_d/ns
n-Propyl-NH_2	0.52	–	0.023	–
Ala	0.40	18.4	0.031	593.5
Ala_2	0.085	–	0.49	–
Ala_3	0.061	4.0	0.45	8.9
Ala_4	0.057	–	0.41	–
Ala_5	0.055	–	0.39	–
Ala_6	0.050	–	0.34	–
Gly_2	0.173	7.3		
Gly_3	0.094	6.7		
Gly_4	0.088			
Gly_5	0.086			
Gly_6	0.079			
Val–Val	0.050			
Val–Ala	0.063			

Φ_f = fluorescence quantum yield; τ_f = fluorescence lifetime; Φ_D = photodestruction quantum yield; τ_d = photodestruction lifetime.
[a] Corrected fluorescence spectra were recorded with the derivatives dissolved in a 50 m*M* borate buffer (pH 9.5).
[b] Data taken from Chen et al. [3].
[c] The Φ_D values were obtained for derivatives dissolved in a 50 m*M* borate buffer (pH 8.8).

reverse is true for amino acid and alkylamine derivatives.

Apart from fluorescence and photodestruction, photoionization is an important route for OPA/βME-amino acid and cyclodeca[*a*]indene-peptide derivatives following excitation [8]. Pulsed-laser excitation ($\lambda = 355$ nm) of the derivatives in the present study also yielded spectra characteristic for solvated electrons [12] (Fig. 2). The yield of the solvated electrons increased linearly with the laser intensity in the range 5–45 mJ, indicating a one-photon process as is usually the case for photoejection of electrons from aromatic chromophores in water solution [13]. However, bi-photonic processes might be important at higher light intensities. Decay curves for solvated electrons in equimolar Ala and Ala_4 derivative solutions, respectively, are shown in Fig. 3. The yield of the solvated electron in Ala and *n*-propylamine solutions were twice as high as for Ala_2 and Ala_4 solutions. We note that the formation of the solvated electrons correlates rather well with the excited state lifetime for amino acid and peptide derivatives, respectively (Table 1). The radical cation of the isoindole chromophores was observed at shorter absorption wavelengths than that of the solvated electron (Fig. 4).

In conclusion, the α-peptide derivatives have different excited state behavior compared to the Ala and *n*-propylamine derivatives. They give fewer solvated electrons, they have higher photodestruction quantum yields, they have lower fluorescence quantum yields and they have shorter fluorescence lifetimes. It is thus clear that photoionization and photodestruction are two different pathways. However, another deactivation channel for the excited states might be various degrees of intramolecular electron transfer (see below). Any triplet absorption could not be detected in the present experiments. This is consistent with our previous results and with findings on the indole and tryptophan systems in polar solvents [8,14].

Both with regard to substituent and solvent effects on fluorescence quantum yields, there are

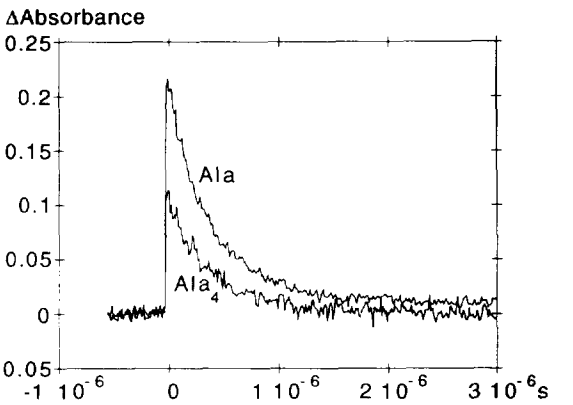

Fig. 3. Decay curves at 700 nm for solvated electrons ejected from Ala and Ala_4 derivatives (120 μ*M* in 50 m*M* borate buffer, pH 8.8).

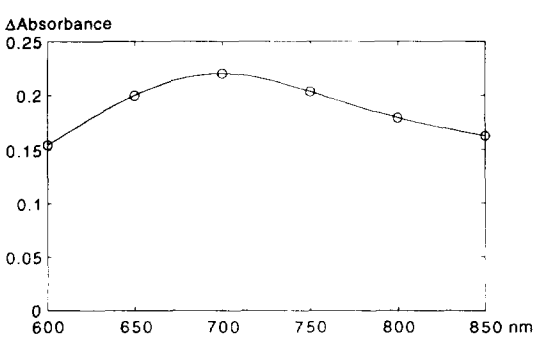

Fig. 2. Difference absorption spectrum of solvated electrons obtained by laser irradiation of an Ala solution (initially 120 μ*M* in 50 m*M* borate buffer, pH 8.8). The traces were sliced 100 ns after the laser pulse.

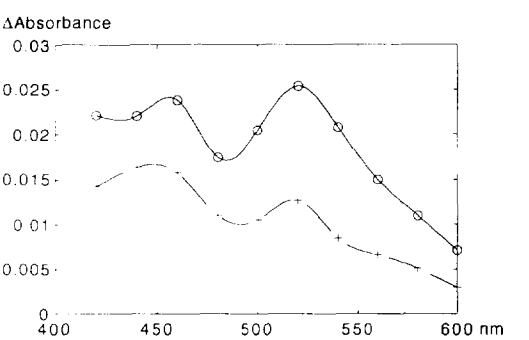

Fig. 4. Difference absorption spectra obtained by slicing traces 4 μs after the laser pulse at different detection wavelengths. OPA/βME-Ala solution (○) and OPA/βME-Ala_4 solution (+). The derivatives were initially 120 μ*M* in 50 m*M* borate buffer, pH 8.8.

some analogies between the isoindole derivatives in this study and various tryptophan derivatives [3,14–16]. In tryptophan and some analogues it is postulated that an important deexcitation process, in addition to fluorescence, results from an intramolecular excited state electron transfer reaction where the indole excited singlet states are electron donors and nearby functional groups are electron acceptors [17,18]. The amide functional group is a better electron acceptor than the carboxylate, which in turn is a better electron acceptor than the methylene group, thus it more effectively quenches the isoindole fluorescence (compare Φ_f for the *n*-propylamine, Ala and peptide derivatives in Table 1). In prior work, we have shown that β-Asp dipeptide derivatives, apparently, fluoresce as intensively as amino acid derivatives [19]. Thus, moving the peptide bond one C–C bond away from the isoindole nucleus reduces the quenching efficiency considerably. The factor of 3–4 increase in excited state lifetime brought about by separating the isoindole from the amide by one C–C bond is consistent with current estimates of the distance dependence (number of bonds) of electron transfer [20,21].

3.3. Implications for LC–LIF detection

To be able to optimize LIF detection, knowledge about the photodegradation rate of the fluorescent species is important. The ratio Φ_f/Φ_D describes the mean maximal number of fluorescent photons that can be expected from a fluorophore when going to the limit of infinite laser irradiances [7,22]. These values are compiled in Table 2 together with the relative fluorescence intensity (peak height) for each derivative in CE experiments where a conventional low intensity light source is used (Fig. 5). If only fluorescence and photodestruction are taken into consideration, these data suggest that each chromophore on the average absorbs roughly two photons with this detector configuration. Further, at high light intensities, a 80–90-fold detection selectivity for amino acid over peptide derivatives is yielded (Table 2). OPA/βME- and fluoresceinisothiocyanate (FITC)-labelled amino acids are detected in a low-light-intensity regime with approximately the same sensitivity [4]. However, in LIF detection schemes where several hundreds or thousands of photons may be available to excite the chromophore, the detection limits for FITC-labelled amino acids can be 10^2–10^5 superior to OPA/mercaptopropionate-labelled amino acids [23,24]. The difference is most likely primarily due to a higher photostability of the FITC-amino acid chromophores.

Table 2
Calculated ratio between fluorescence quantum yields and photodestruction quantum yields and relative fluorescence intensity

Amine	Φ_f/Φ_D	RFI[a]
n-Propyl-NH_2	22.6	–
Ala	12.9	10 ± 0.5
Ala_2	0.17	1.0 ± 0.06
Ala_3	0.14	0.7 ± 0.06
Ala_4	0.14	0.7 ± 0.06
Ala_5	0.14	0.5 ± 0.09
Ala_6	0.15	0.4 ± 0.06
Gly_2	–	0.7 ± 0.05
Gly_3	–	0.3 ± 0.04
Gly_4	–	0.2 ± 0.04
Gly_5	–	0.3 ± 0.02
Gly_6	–	0.3 ± 0.02

[a] RFI = Relative fluorescence intensity, obtained from peak height measurements in CE experiments.

The choice of using OPA/βME for labelling of α-peptides must not be to achieve ultra-sensitive

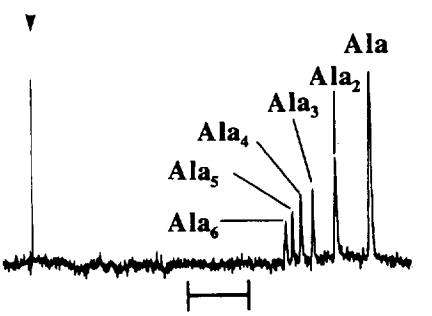

Fig. 5. Separation of a mixture of Ala and Ala peptide standards. A 510-μl volume of standard (100 μ*M* of each peptide and 20 μ*M* Ala) was derivatized with 60 μl of reagent (OPA, 500 m*M* and βME, 250 m*M*). Following a 2-min reaction time the solution was injected. CE conditions; potential 25 kV, current ca. 40 μA. Time bar is 1 min.

LIF detection as the derivatives are extremely light sensitive. However, these derivatives have some attractive features that would enable their selective determination. As an example, phase-modulated detection would enable highly selective determination of OPA/βME-labelled α-peptides over amino acid derivatives owing to their significantly shorter excited state lifetimes. Further, the high thermal stability of labelled α-peptides may be used to enhance the detection selectivity against amino acids (see below).

3.4. Thermal stability of OPA/βME-peptide derivatives in solution

It is well known that several OPA/βME-amino acid derivatives decay rapidly [25]. Particularly unstable are Gly and Orn, whereas Ala belongs to an intermediate group while under the same conditions, the most stable are Val and Ile [26]. Here, both the Ala peptide and the Gly peptide derivatives were found to be severalfold more stable than their parent amino acid derivative. The peptide derivatives in the Ala series were considerably more stable than those in the Gly series, and the two Val dipeptides were by far the most stable (Table 3). Thus, the structural features of the N-terminal amino acid residue has a

Table 3
Apparent first-order decay constants and half lives for OPA/βME-derivatized amino acids and peptides (1 m*M*) with different concentrations of OPA in borate buffer (pH 10)

Amine	k_{app}/min^{-1}	$t_{1/2}$/min	[OPA]/m*M*
Gly	0.43	1.6	150
Gly_2	0.15	4.7	150
Gly_6	0.12	5.7	150
Ala	0.026	26.6	150
Ala_2	0.016	43.3	150
Ala_6	0.015	46.2	150
Val–Ala	0.0029	239	150
Val–Val	0.0032	217	150
Ala	0.058	11.9	495
Ala_2	0.019	36.5	495
Ala_6	0.017	40.8	495

The derivatives were generated by adding 60 μl of a reagent solution (150 or 495 m*M* OPA) to 65 μl of a mixed standard containing Ala 77 μ*M*, Ala_2 308 μ*M* and Ala_6 615 μ*M*. Separate experiments were performed for the Gly series, Val–Ala and Val–Val. Following a 2-min reaction time the derivatives were injected into the capillary and thereafter at 5–7-min intervals until 50 or 70 min ($n = 5–7$ in each experiment, except for Gly: $n = 3$).

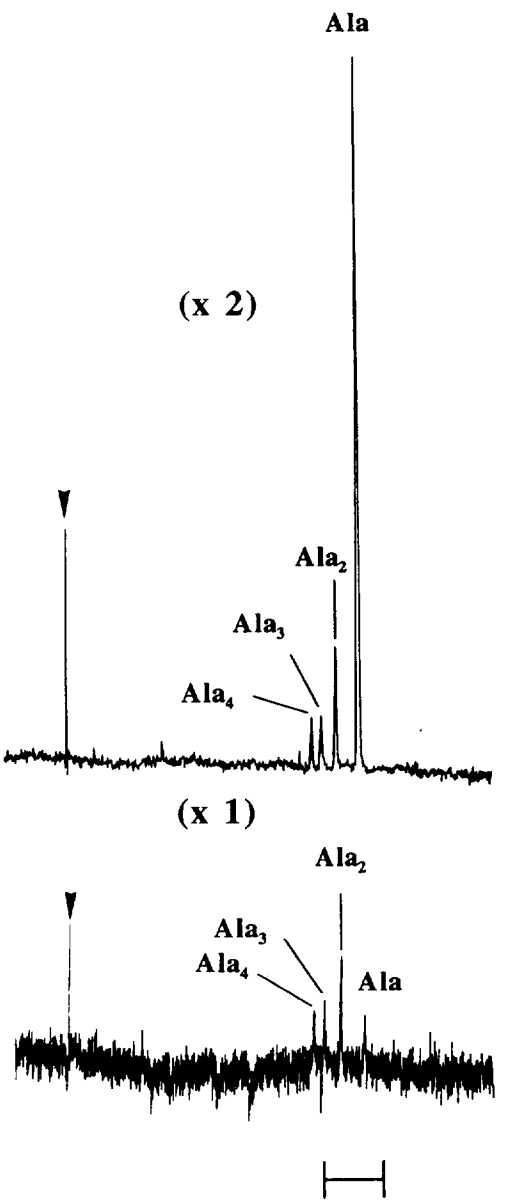

Fig. 6. Electropherograms of a derivatized standard. A 180-μl volume of Ala_2, Ala_3 and Ala_4 (278 μ*M* of each), Ala (167 μ*M*) and 60 μl reagent (OPA, 150 m*M* and βME, 75 m*M*), injected after a 2-min reaction time (upper trace) and following acidification for 6.5 min with 50 μl HCl (0.5 *M*) yielding pH 1.8. Time bar is 1 min.

large impact on the stability of the OPA/βME-peptide derivative which also is obvious when comparing the stability of the Val–Val and Val–Ala derivatives (Table 3). Thus, it appears safe to say that for peptides with aliphatic sidegroups, the derivative stability can be estimated from the identity of the N-terminal residue alone and ranked Val peptide > Ala peptide > Gly peptide. Extremely stable derivatives have been obtained with OPA/βME-labelled Leu–Ala and Phe–Ala (less than 3% absorbance drop after 24 h, borate buffer pH 9.7) [27]. This corroborates the above reasoning as the OPA/βME derivatives of Leu and Phe are among the most stable obtained with amino acids [26,27]. It has earlier been shown that the stability of 1-alkylthio-2-alkylisoindoles is largely dependent on the thiol and amine structure [28,29]. Extensive branching and increased steric bulk at C-10 (α-C in the native amino acid or peptide), and increased chain length renders derivatives with a higher stability [28,29]. The derivative stability ranking obtained in the present study are consistent with these observations. However, increasing the length of the peptide backbone does not produce any profound effects as realized for simple linear–CH_2–extensions, where an approximate 2-fold higher stability is observed per each methylene group incorporated [28].

Isoindole degradation rates are strongly dependent on the concentration of *cis*- and *trans*-phthalandiols (hydrated OPA), [29]. These authors postulated that degradation occurs by two parallel routes:

$$k_{app} = k_0 + k_1[OPA]$$

where k_{app} is the apparent first-order decay constant, k_0 and k_1 are the rate constants for the OPA-independent and OPA-dependent processes, respectively. Under most analytical conditions this appears over all first order and the OPA-dependent process is by far the most important [28,29]. The observed differential rate of degradation comparing amino acid and peptide derivatives provides a possible avenue to achieve improved selectivity for peptides over amino acids. It was found that the degradation of the peptide derivatives is less dependent on the OPA concentration than the amino acid derivatives (Table 3). Further, OPA/βME derivative degradation is augmented following acidification [8,28,30,31]. We previously used acidification in order to degrade various amino acid and peptide

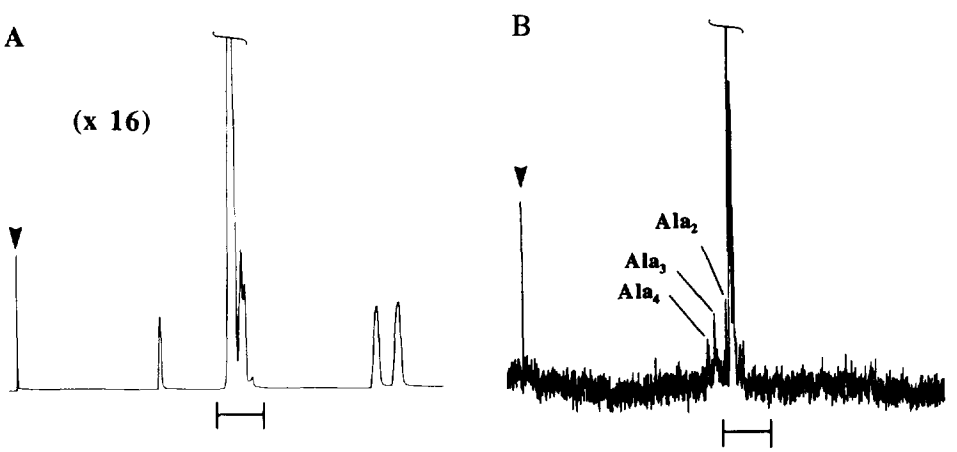

Fig. 7. Electropherogram of a derivatized standard. A 120-μl volume of Ala_2, Ala_3 and Ala_4 (250 μM of each) and 312 μM of each amino acid in a combined amino acid standard AA-S-18 was derivatized with 60 μl reagent (OPA, 150 mM and βME, 75 mM) injected after a 2-min reaction time (A) and the same standard following acidification for 6.5 min with 50 μl HCl (0.5 M) yielding pH 1.8 (B). Time bar is 1 min.

derivatives to enable selective determination of highly stable glutathione and γ-glutamylcysteine derivatives [8]. However, upon acidification (HCl, pH ≈ 1.8) after the completion of the derivatization reaction, the degradation rates increasingly diverged, and the decay of the Ala derivative was estimated to be approximately six times faster than for Ala_2 and Ala_6 derivatives, using merely a 45-fold excess OPA (Fig. 6). Selectivity-enhanced detection of peptides in certain types of samples should thus be feasible by this approach. This was tested in an amino acid standard spiked with three Ala peptides where most of the amino acids could be completely degraded after a 6.5-min acid treatment (Fig. 7). At the same time, the peptide derivatives were destroyed to a lesser extent and are clearly visible in the electropherogram in Fig. 7B. Certain thermally stable peptide derivatives, e.g. those with N-terminal Val residues may be favourably detected using that approach. As an example A-VI-5, a bradykinin potentiating peptide seems to be a suitable for such purposes.

4. Conclusions

It is shown that thermal and photoinduced degradation of OPA/βME-amino acid and -peptide derivatives are unrelated. In addition to fluorescence, photoionization and photodestruction are important pathways for deactivation of the first excited singlet state. α-Peptide derivatives are the most light sensitive and with high irradiances of the excitation light, a 80–90-fold detection selectivity for amino acid derivatives over peptide derivatives is yielded. Furthermore, our results show that a given OPA/βME-α-peptide derivative has higher thermal stability compared to the derivative obtained where the N-terminal amino acid in the peptide is reacted as a free amino acid.

Acknowledgements

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References

[1] M. Roth, *Anal. Chem.*, 43 (1971) 880.
[2] P. Lindroth and K. Mopper, *Anal. Chem.*, 51 (1979) 1667.
[3] R.F. Chen, C. Scott and E. Trepman, *Biochim. Biophys. Acta*, 574 (1979) 440.
[4] M. Albin, R. Weinberger, E. Sapp and S. Moring, *Anal. Chem.*, 63 (1991) 417.
[5] S.L. Pentoney, X. Huang, D.S. Burgi and R.N. Zare, *Anal. Chem.*, 60 (1988) 2625.
[6] M.H. Joseph and P. Davies, *J. Chromatogr.*, 277 (1983) 125.
[7] R.A. Mathies, K. Peck and L. Stryer, *Anal. Chem.*, 62 (1990) 1786.
[8] O. Orwar, M. Sundahl, M. Sandberg and S. Folestad, *Anal. Chem.*, 66 (1994) 4474.
[9] G. Gauglitz and S. Hubig, *J. Photochem.*, 30 (1985) 121.
[10] C.G. Hatchard and C.A. Parker, *Proc. R. Soc. London A*, 235 (1956) 518.
[11] S. Hamai and F. Hirayama, *J. Phys. Chem.*, 87 (1983) 83.
[12] M.S. Matheson and L.M. Dorfman, *Pulse Radiolysis*, M.I.T. Press, Cambridge, MA, 1969.
[13] R. Lesclaux and J. Joussot-Dubien, in J.B. Birks (Editor), *Organic Molecular Photophysics*, Wiley, New York, 1973.
[14] Y. Hirata, N. Murata, Y. Tanioka and N. Mataga, *J. Phys. Chem.*, 93 (1989) 4527.
[15] R.W. Cowgill, *Arch. Biochem. Biophys.*, 100 (1963) 36.
[16] R.W. Cowgill, *Biochim. Biophys. Acta*, 133 (1966) 6.
[17] J. Teraoka, P.A. Harmon and S.A. Asher, *J. Am. Chem. Soc.*, 112 (1990) 2892.
[18] S. Arnold, L. Tong and M. Sulkes, *J. Phys. Chem.*, 98 (1994) 2325.
[19] O. Orwar, S. Folestad, S. Einarsson, P. Andiné and M. Sandberg, *J. Chromatogr.*, 556 (1991) 39.
[20] J.J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.*, 71 (1974) 3640.
[21] S. Priyadarshy, S.M. Risser and D.N. Beratan, submitted for publication.

[22] T. Hirschfeld, *Appl. Optics*, 15 (1976) 3135.
[23] B. Nickerson and J.W. Jorgenson, *J. High. Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 878.
[24] S. Wu and N. Dovichi, *J. Chromatogr.*, 480 (1989) 141.
[25] M.C.G. Alvarez-Coque, M.J.M. Hernandez, R.M.V. Camanas and C.M. Fernandez, *Anal. Biochem.*, 178 (1989) 1.
[26] V.-J.K. Svedas, I.J. Galaev, I.L. Borisov and I.V. Berezin, *Anal. Biochem.*, 101 (1980) 188.
[27] R. Rowlett and J. Murphy, *Anal. Biochem.*, 112 (1981) 163.
[28] W.A. Jacobs, M.W. Leburg and E. Madaj, *Anal. Biochem.*, 156 (1986) 334.
[29] J.F. Stobaugh, A.J. Repta, L.A. Sternson and K.W. Garren, *Anal. Biochem.*, 135 (1983) 495.
[30] S.S. Simons, Jr. and D.F. Johnson, *Anal. Biochem.*, 82 (1977) 250.
[31] S.S. Simons, Jr. and D.F. Johnson, *Anal. Biochem.*, 90 (1978) 705.