On the thermodynamics of peptide oxidation: anhydrides of glycine and alanine[1]

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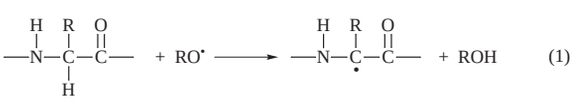
The α-amino C–H bond dissociation enthalpies of glycine anhydride and alanine anhydride and the C–O bond dissociation enthalpies of the corresponding anhydride peroxyl radicals have been measured by photoacoustic calorimetry in aqueous solution. Furthermore, the one-electron oxidation potentials of the C-centered radicals formed upon hydrogen abstraction from glycine anhydride and alanine anhydride have been measured by photomodulation voltammetry in aqueous solution. In addition, the C–H bond dissociation enthalpies of glycine anhydride and alanine anhydride and one-electron reduction potentials of the corresponding radicals have been calculated by *ab initio* methods [at the B3LYP/6-31G(D) level]. The resulting experimental gas-phase C–H bond dissociation enthalpies are 340 and 325 ± 15 kJ mol^{-1}, the C–O bond dissociation enthalpies are 56 and 64 ± 15 kJ mol^{-1} and the one-electron oxidation potentials are 0.175 and 0.086 V *vs*. NHE for the glycine anhydride and alanine anhydride related species, respectively. The calculated C–H bond dissociation enthalpies are 351.1, 334.7 and 332.7 ± 10 kJ mol^{-1} for glycine anhydride, L-alanine anhydride and D,L-alanine anhydride, respectively. The one-electron reduction potentials of the corresponding radicals are 1.19, 1.00 and 0.99 V *vs*. NHE. The thermochemical properties of amino acid anhydrides and the corresponding C-centered radicals and peroxyl radicals determined in this work are consistent with each other and with previously published observations on the radical chemistry of amino acid anhydrides and related species.

Introduction

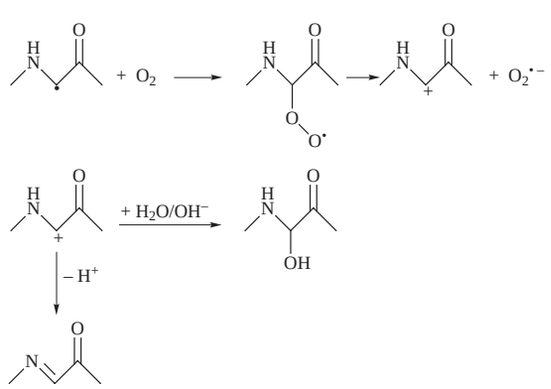
Oxidation of proteins has been suggested to be the cause of pathological disorders, such as protein turnover, cataractgenesis, atherosclerosis and tissue injury during ischemia-reperfusion.[2] The primary step in protein oxidation is the formation of a protein or peptide radical by hydrogen abstraction or one-electron oxidation of the protein or peptide. In principle, hydrogen abstraction can take place either on easily oxidized side-groups, *e.g.* from the phenol group in tyrosine, the thiol group in cysteine or from the indole group in tryptophane, or from the C–H bonds of the protein/peptide backbone [reaction (1)].



(1)

The latter possibility has attracted increasing attention during the last decade and a number of experimental[3,4] and theoretical studies[5–9] have been aimed at elucidating the α-amino C–H bond dissociation enthalpy (BDE) and the redox properties of various amino acids and peptides and the corresponding radicals. Most experimental studies have involved indirect determinations of the C–H bond dissociation enthalpies, however, there is an excellent agreement between the experimental studies and the independent theoretical studies, *e.g.* the C–H bond dissociation enthalpy of glycine has been estimated to ≤329 kJ mol^{-1} on experimental grounds[4] and to 331 kJ mol^{-1} by high level *ab initio* calculations.[6] In previous work we have established the methodology for the accurate calculation of α-C–H BDEs, by *ab initio* methods,[8] and have applied it to glycine, alanine, serine and threonine residues.[9] These C–H bonds are significantly weaker than other secondary or tertiary C–H bonds which can be explained by the so called captodative effect of the $-NH_2$ and $-CO_2H$ substituents.[7] According to theoretical studies, the C–H bonds in proteins and polypeptides are stronger and thus less susceptible to hydrogen abstraction and oxidation than neutral free amino acids.[8] The α-C–H bond dissociation enthalpy for a glycine peptide, $HCO-NHCH_2CONH_2$, has been calculated to 348 kJ mol^{-1}.[8] Recently, a value of 386 kJ mol^{-1} was reported for the C–H bond dissociation enthalpy of alanine anhydride.[10]

The α-carbon-centered radicals formed upon hydrogen abstraction from peptides can react with molecular oxygen to form peroxyl radicals, which can expel superoxide anion radicals to give carbocations (inner-sphere electron transfer) which then hydrolyze to the corresponding alcohol or deprotonate to give an imine (Scheme 1).[11]



Scheme 1

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Another possible reaction mechanism would be direct outer-sphere electron-transfer between the C-centered radical and molecular oxygen. The α-amino acid radicals can also be oxidized by other relatively mild oxidizing agents, *e.g.* $IrCl_6^{2-}$ and $Fe(CN)_6^{3-}$.[11,12] The life-time of the alkylperoxyl radical strongly depends on the oxidation potential of the corresponding alkyl radical.

Amino acid anhydrides are the simplest model compounds for peptides and the kinetics of hydrogen abstraction and other radical reactions for these species have been thoroughly studied by Mieden *et al.*[11] For linear peptides, the decrease in bond dissociation enthalpy upon CH_3-substitution on the α-carbon has been found to be only 3.7 kJ mol^{-1} on theoretical grounds (optimized structures).[9] However, the difference between β-sheet and α-helix structure was found to be considerable. The corresponding difference for free amino acids has been calculated to 13.6 kJ mol^{-1}.[9]

In this work we have measured the α-C–H bond dissociation enthalpies for glycine and alanine anhydride and the C–O bond dissociation enthalpies for the corresponding peroxyl radicals by photoacoustic calorimetry. The one-electron oxidation potentials of the corresponding C-centered radicals have been measured by photomodulation voltammetry.

Furthermore, we have calculated the α-C–H bond dissociation enthalpies for glycine and alanine anhydride by *ab initio* methods. From these numbers we have also derived the one-electron reduction potentials of the corresponding C-centered radicals.

Experimental

All chemicals were of the purest grade available (Sigma and Aldrich) and were used as supplied unless otherwise stated.

Photoacoustic calorimetry

The photoacoustic calorimeter used in this work has been described in detail elsewhere.[13,14] Briefly, pulses from a nitrogen laser (Laser Photonics Model VSL 337ND, 337.1 nm, pulse width 10 ns, output 250 μJ/pulse, repetition rate 5 Hz) were used to photolyze solutions contained in a standard quartz flow cell (Hellma 171 QS). The laser light was attenuated to an incident energy of *ca.* 10 μJ/pulse by using a 1 mm iris so that only a fine beam passed through the sample cell. Variations in laser energy were monitored by splitting a small fraction of the incident light to a reference detector. Changes in the absorbance of the samples were determined by measuring the energy of the laser pulses transmitted through the solution with a second energy meter.

Each pulse initiates a predetermined free-radical reaction in the irradiated volume within the sample cell. The heat evolved by this reaction produces a thermal shock wave in the solution that is detected by using a piezoelectric transducer that was clamped to the wall of the quartz cell. A small quantity of silicone grease is applied to the detecting surface of the transducer to improve coupling with the cell. Signals from the transducer were amplified, averaged then stored using a digital oscilloscope (Tektronix 7D20).

In a typical experiment, an aqueous solution containing a substrate (10 mM) and hydrogen peroxide (0.3–1.75 M) was prepared and placed in a Pyrex reservoir where it was either deoxygenated by bubbling with nitrogen (when measuring the C–H bond dissociation enthalpy) or oxygenated by bubbling with oxygen (when measuring the C–O bond dissociation enthalpy). The apparatus was calibrated by using solutions of 2-hydroxybenzophenone, which converts all of the absorbed light energy into heat within a few nanoseconds.

The signal amplitude was corrected for any variations in the laser power by dividing the averaged photoacoustic response by the averaged laser power. This normalized response was plotted against the fraction of laser light absorbed by the hydrogen peroxide (by changing the hydrogen peroxide concentration). The data for the 2-hydroxybenzophenone calibration standard was treated in the same way. The slopes of the plots for the reaction of interest and the calibration standard are referred to as a_r and a_s, respectively.

The reactions taking place in the deoxygenated solutions are given by reactions (2) and (3) with the overall reaction given by reaction (4).

$$H_2O_2 + h\nu \longrightarrow 2HO^{\bullet} \quad (2)$$

$$2HO^{\bullet} + 2RH \longrightarrow 2H_2O + 2R^{\bullet} \quad (3)$$

$$H_2O_2 + 2RH \longrightarrow 2H_2O + 2R^{\bullet} \quad (4)$$

The overall enthalpy change for the process is given by eqn. (5).

$$\Delta H_{aq} = 2\Delta H_f(R^{\bullet})_{aq} - 2\Delta H_f(RH)_{aq} + 2\Delta H_f(H_2O)_{aq} - \Delta H_f(H_2O_2)_{aq} \quad (5)$$

The relation between $\Delta H_{4(aq)}$ and the PAC signal is given by eqn. (6), where $h\nu$ is the laser photon quantum energy

$$\Delta H_{4(aq)} = \frac{h\nu}{\Phi_2}\left(1 - \frac{a_r}{a_s} + \frac{\Phi_2 \Delta V_4}{\chi_s h\nu}\right) \quad (6)$$

(354.8 kJ mol^{-1}), Φ_2 is the quantum yield for hydrogen peroxide photolysis [reaction (2)], ΔV_4 is the net volume change in the overall reaction [reaction (4)] and χ_s is the adiabatic expansion coefficient for the solvent (water) and is defined as $\beta M/\rho C_p$ where β is the thermal expansion coefficient, M is the molecular weight, ρ is the density and C_p is the heat capacity. For water, χ_s has a value of 0.062 cm^3 kJ^{-1}. Under the present experimental conditions, the quantum yield Φ_2 approaches unity. The value of ΔV_4 is not known for the homolysis of hydrogen peroxide in water. However, this difficulty can be handled by a more empirical approach (see below).

The C–H bond dissociation enthalpy, $D_{C-H(aq)}$, is given by: $[\Delta_f H°(H^{\bullet}) + \Delta_f H°(R^{\bullet}) - \Delta_f H°(RH)]$, and can be calculated from eqn. (7), which is obtained from eqn. (5) by rearranging

$$D_{C-H(aq)} = \frac{\Delta H_4}{2} - \frac{\Delta H_{corr}}{2} + 404 \text{ (kJ mol}^{-1}\text{)} \quad (7)$$

and inserting the value of $\Delta_f H°(H^{\bullet})_{aq}$.[13] In eqn. (7), ΔH_{corr} is an empirical correction factor for a given solvent that includes both the contribution from the volume change and from the change in solvation upon converting one mole of H_2O_2 into two moles of water. This approach has been described in detail elsewhere.[14] Briefly, the PAC experiment provides an apparent value of $\Delta H_{4(aq)}$ [eqn. (6)]. If one assumes that $D_{C-H(aq)} = D_{C-H(g)}$ for simple alkyl systems, then ΔH_{corr} is the difference between the apparent $\Delta H_{4(aq)}$ from the PAC experiment and the gas phase value that can be calculated from literature values. Such data has been reported by Griller and co-workers.[13] Values of $\Delta H_{4(aq)} - \Delta H_{4(g)}$ for the alkyl C–H bonds of methanol, ethanol, propan-2-ol and 2-methylpropan-2-ol were found to be −51.8, −40.5, −47.7 and −41.0 kJ mol^{-1}, respectively. This provides an average value for ΔH_{corr} of −45 kJ mol^{-1}.

Since the contribution to ΔH_{corr} from hydrogen bonding to the newly formed water molecules has been estimated to be −33 kJ mol^{-1} [13] the contribution from the volume change can be estimated to be *ca.* −12 kJ mol^{-1}. In oxygen purged solutions reaction (8) takes place in addition to those listed above and the overall reaction is now given by reaction (9). Further reactions

$$2R^{\bullet} + 2O_2 \longrightarrow 2RO_2^{\bullet} \quad (8)$$

$$H_2O_2 + 2O_2 + 2RH \longrightarrow 2H_2O + 2RO_2^{\bullet} \quad (9)$$

involving the peroxyl radicals are too slow to be seen in the time-window of the experiment. Since the volume change for reaction (9) is opposite in sign to that for reaction (4) (*i.e.* the contribution of the volume change to ΔH_{corr} is $\geqslant$12 kJ mol^{-1}) then a lower limit of $\Delta H_{corr} \geqslant -21$ kJ mol^{-1} can be assigned.

The enthalpy for the overall process, $\Delta H_{9(aq)}$ is given by eqn. (10) and (10) minus eqn. (5) gives eqn. (11). Thus $D_{C-OO(aq)}$ is the

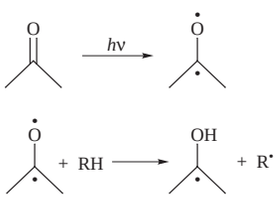
$$\Delta H_{9(aq)} = 2\Delta H_f(RO_2^{\bullet})_{aq} - 2\Delta H_f(RH)_{aq} + 2\Delta H_f(H_2O)_{aq} - 2\Delta H_f(O_2)_{aq} - \Delta H_f(H_2O_2)_{aq} \quad (10)$$

$$D_{C-O(aq)} = \frac{\Delta H_{9(aq)} - \Delta H_{4(aq)}}{2} = \Delta H_f(RO_2^{\bullet})_{aq} - \Delta H_f(R^{\bullet})_{aq} - \Delta H_f(O_2)_{aq} \quad (11)$$

difference in the enthalpy changes measured in the solutions bubbled with oxygen and nitrogen.

Photomodulation voltammetry

The instrument has been described in detail previously.[15] The carbon-centered α-amino acid radicals were generated in the electrochemical cell by photolysis, through an optically transparent gold electrode, of acetone (10% v/v) in water (unbuffered solutions) to form a triplet [reaction (12)] which abstracts the α-hydrogen from the amino acid or peptide (1 mM conc.).



(12)

The supporting electrolyte used was 1 M KCl in water. For the electrochemical experiments water was deionized and distilled.

The output from the lamp was modulated with a light chopper so the light intensity (and therefore the radical concentration) rose and fell as a sine wave. The electrochemical cell was fully iR compensated and was controlled with a PAR Model 174 polarographic analyzer. The AC component of the faradic current was detected with a Stanford Research Systems Model SR530 lock-in amplifier and plots of the AC current, as a function of potential, were recorded on a HP 7045 B X-Y recorder. All experiments were made at 100 Hz modulation frequency and the scan rate was 20 mV s^{-1}. Potential calibration in water was accomplished using ferricyanide (E° = 0.37 V *vs.* NHE).[16]

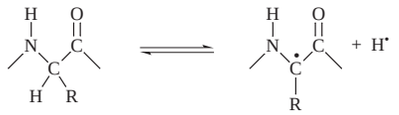
Computational details

All *ab initio* calculations presented here were performed with the Gaussian-92/94 molecular orbital packages.[17] The geometry optimizations and frequency calculations were carried out at the B3LYP/6-31G(D) level by the hybrid HF-DFT procedure implemented in the Gaussian molecular orbital packages with the keyword INT=FINEGRID. The vibrational frequencies were scaled by a factor of 0.98 in considering the zero-point energy.[18]

The values of $H^0_{298} - H^0_0$ required to obtain heats of reaction at 298 K and the entropies needed in the evaluation of reduction potentials were calculated by standard statistical thermodynamic methods, based on the rigid rotor-harmonic oscillator model[19] and using the frequencies obtained at B3LYP/6-31G(D) level. The frequencies were scaled by a factor of 0.98 in the calculation of these thermodynamic functions.[18]

The α-C–H bond dissociation enthalpies (BDEs), D_{C-H}, are defined as the heat of reaction (13), $\Delta H^0_{(13)}$.

As a means of reducing residual errors due to basis set and



(13)

correlation effects, the BDEs, D_{C-H}, were derived from the heats of isodesmic reactions.[20] These reactions can be represented by process (14).

$$\text{Anhydride radical} + \text{AH} \rightleftharpoons \text{Anhydride} + \text{A}^{\bullet} \quad (14)$$

AH is a reference molecule for which the BDE, D_{C-H}(AH), is known accurately. For each anhydride the heat of reaction (14), $\Delta H^0_{(14)}$, was evaluated from the energies obtained in the *ab initio* calculations at the B3LYP/6-31G(D) level, which was shown to give reliable results for the glycine model peptides.[8,9] In the context of bond dissociation enthalpies, D_{C-H}(Anhydride) is then given by eqn. (15).

$$D_{C-H}(\text{Anhydride}) = D_{C-H}(\text{AH}) - \Delta H^0_{(14)} \quad (15)$$

In order to obtain the most effective cancellation of residual errors, the structures of the reference molecule and radical used in reaction (14) should be related as closely as possible to those of the anhydride and its radical, respectively. Ideally AH should have both an adjacent amino group and a carbonyl group so that the special feature of the captodative effect can be taken into account. Previous studies have shown[9] that H_2NCH_2-COOH (AH(Gly)) is the most suitable reference molecule to give reliable values of D_{C-H}. The magnitude of D_{C-H} for glycine itself (331.0 kJ mol^{-1}),[6] was not directly available from experiment. However, it has been derived from a number of isodesmic reactions with heats of reaction based on G2(MP2) calculations.[6] Values of D_{C-H} for the amino acids alanine, serine and threonine were obtained previously using an isodesmic reaction similar to reaction (14) with glycine as reference.[9]

Results and discussion

Structures

The anhydrides of glycine and L-alanine have C_2 symmetry (Fig. 1) while the anhydride of racemic alanine has a centre of inversion. The structure of the ring is little perturbed by the presence of *cis* or *trans* methyl groups. Upon radical formation at the α-C centre, the ring adopts a somewhat more planar geometry and systematic changes occur in the lengths of the bonds near the radical site. Bonds connected to the radical site are shortened by 0.08 Å (0.04 Å in the case of the bond to the methyl group), and the amide C–N bonds are somewhat lengthened.

Entropies and $H^0 - H^0_0$

The entropies and $H^0 - H^0_0$ values in Table 1 show the anticipated increases with molecular size. For each radical the $H^0 - H^0_0$ value differs by only a small amount from that of the parent, which means that the corrections, which were needed in estimating heats of isodesmic reactions at 298 K, are small. Rather interesting is the fact that L-alanine anhydride, the form with the methyl radicals *cis* to each other, is 2 kJ mol^{-1} more stable than the D,L-anhydride, which has the methyls *trans* to each other.

BDEs

The 2 kJ mol^{-1} greater stability of the D,L-anhydride causes an identical difference in the values of D_{C-H} for the two forms, *viz.* 334.7 and 332.7 kJ mol^{-1}, respectively. However, both of these are significantly less than the calculated D_{C-H} for glycine anhydride, 351.1 kJ mol^{-1}.

The anhydrides of amino acids have been used as models of peptide linkages in long chain polypeptides.[11] In ref. 10, values

Table 1 Theoretical energies, thermodynamic properties and bond dissociation energies of the anhydrides of glycine and alanine

Species	Energy[a]/ hartree	ZPE/ kJ mol^{-1}	$H^0_{298} - H^0_0$/ kJ mol^{-1}	S^0_{298}/ kJ K^{-1} mol^{-1}	D_{C-H}[b]/ kJ mol^{-1}	$\Delta G°$/ kJ mol^{-1}	$E°$/V *vs.* NHE
Glycine[c]	−284.423 45	210.2	17.6	316.6	331		
Glycine radical[c]	−283.790 39	176.2	17.0	310.2			
Glycine anhydride	−416.006 84	289.4	21.9	358.2	351.1	−115.0	1.19
Glycine anhydride radical	−415.365 87	254.7	21.3	355.1			
L-Alanine[d]	−323.739 64	284.7	21.2	345.5	317		
L-Alanine radical[d]	−323.111 76	249.9	21.3	348.4			
L-Alanine anhydride	−494.642 79	437.5	29.4	414.7	334.7	−96.7	1.00
D,L-Alanine anhydride	−494.642 18	437.8	29.5	416.8	332.7	−95.3	0.99
Alanine anhydride radical	−494.008 30	402.7	29.5	418.1			

[a] B3LYP/6-31G(D). [b] Except for Gly, from isodesmic reaction: $X^{\bullet} + NH_2CH_2COOH \rightarrow HX + NH_2CHCOOH^{\bullet}$. [c] From ref. 6. [d] From ref. 9.

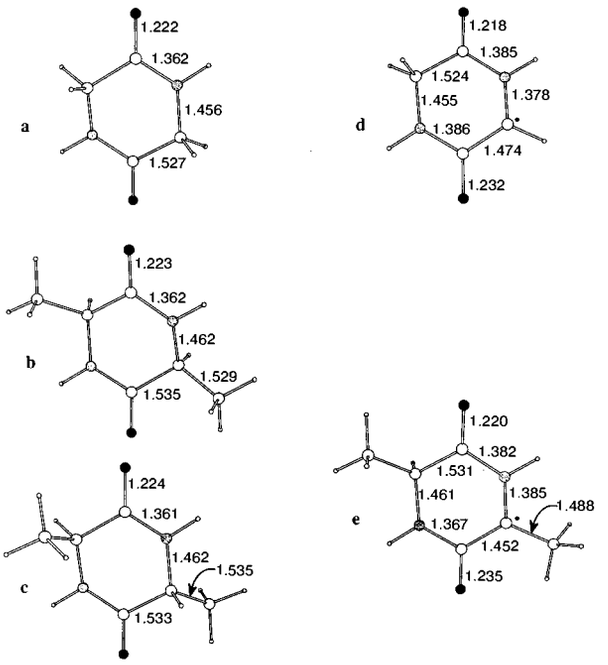


Fig. 1 B3LYP/6-31G* structures: (**a**) glycine anhydride; (**b**) L-alanine anhydride; (**c**) D,L-alanine anhydride; (**d**) α-C radical of glycine anhydride; (**e**) α-C radical of L-alanine anhydride

of D_{C-H} equal to 348 and 344 kJ mol^{-1} were estimated for α-C–H bonds of glycine and alanine residues, respectively, at mid chain positions in polypeptides with fully relaxed geometries. The calculated BDEs of the anhydrides computed here are the same within the ±10 kJ mol^{-1} uncertainty. The increase in BDE over the free amino acid in each case is due to the replacement of the H_2N and COOH groups by –CONH– amide groups, which are poorer electron donating and electron withdrawing groups and contribute less to the captodative stabilization of the radicals.[8,9]

In Table 2 the measured C–H bond dissociation enthalpies for the amino acid anhydrides and the C–O bond dissociation enthalpies for the anhydride peroxyl radicals are given.

For the more stable L-alanine anhydride the difference in the calculated D_{C-H} from glycine anhydride is 16.4 kJ mol^{-1}, quite similar to the difference of 15 kJ mol^{-1} observed experimentally. The agreement between the actual experimental and calculated D_{C-H} values for the two anhydrides is not as good. In both cases the experimental value, 340 kJ mol^{-1} for the glycine derivative and 325 kJ mol^{-1} for the alanine derivative, is ~10 kJ mol^{-1} less than the calculated one: 351 kJ mol^{-1} for glycine and 335 kJ mol^{-1} for alanine (Table 1). However, these differences are within the uncertainties, which are estimated to be: ±10 kJ mol^{-1} for the calculated values and ±15 kJ mol^{-1} for the experimental ones. The experimental and *ab initio* calculated C–H bond dissociation enthalpies are considerably higher than the numbers calculated by AM1 semiempirical quantum chemical calculations (315 and 298 kJ mol^{-1} for glycine anhydride and alanine anhydride, respectively).[21] Previous photoacoustic calorimetry measurements of C–H bond dissociation enthalpies in aqueous solution have also yielded gas-phase bond dissociation enthalpies that are somewhat lower, by *ca.* 5–10 kJ mol^{-1}, than values reported in the literature.[13] We would like to stress though, that the uncertainties in our measurements are relatively high (±15 kJ mol^{-1}) since the acoustic signals are very weak in aqueous systems.

Table 2 C–H and C–O bond dissociation enthalpies of glycine anhydride and alanine anhydride and the peroxyl radicals, respectively, measured by photoacoustic calorimetry

Anhydride	$\Delta H_{4(aq)}{}^{obs}$/ kJ mol^{-1}	D_{C-H}/ kJ mol^{-1}	$\Delta H_{9(aq)}{}^{obs}$/ kJ mol^{-1}	D_{C-O}/ kJ mol^{-1}
Glycine	−172 ± 30	340 ± 15	−260 ± 30	56 ± 15
Alanine	−202 ± 30	325 ± 15	−310 ± 30	64 ± 15

Clearly, the recently reported α-C–H bond dissociation enthalpy for the alanine anhydride, 386 kJ mol^{-1},[10] deviates markedly from both our experimental and calculated values. A reason for this discrepancy could be that the value of 386 kJ mol^{-1} is based on the kinetics of decomposition of the alanine anhydride dimer and several corrections are needed to obtain the α-C–H bond dissociation enthalpy.

The ~15 kJ mol^{-1} lower D_{C-H} for alanine (15, 16.4 and 17 kJ mol^{-1} from experiment, *ab initio* calculations and semiempirical calculations, respectively) can be attributed to the extra stabilization of the C-centered radical by the CH_3 group. A similar additional stabilization is seen in the difference in D_{C-H} between $H_2NCH(CH_3)COOH$ and H_2NCH_2COOH (Table 1). The lowering of the C–H bond dissociation enthalpy by *ca.* 15 kJ mol^{-1} upon α-CH_3 substitution is larger than the difference between the bond dissociation enthalpies of propane (secondary C–H bond) and isobutane (tertiary C–H bond), 9 kJ mol^{-1}.[22]

It is obvious, however, that the bond dissociation enthalpies of amino acid anhydrides are very low, particularly in aqueous solution, and that the α-C–H bonds are therefore susceptible to hydrogen abstraction even by less efficient hydrogen abstractors than the hydroxyl radical, *e.g.* peroxyl radicals formed in the reaction between peptide radicals and molecular oxygen. The O–H bond dissociation enthalpy for an alkyl hydroperoxide is *ca.* 365 kJ mol^{-1}[23] and the thermodynamic driving force for hydrogen abstraction from amino acid anhydrides by alkyl peroxyl radicals is consequently >14 kJ mol^{-1} according to the present study. Hydrogen abstraction from amino acid anhydrides in the presence of oxygen could thus result in a chain-reaction analogous to lipid peroxidation.[24] The fact that no chain-reaction has been observed may be ascribed to the instability of the amino acid anhydride peroxyl radicals in combination with a slow rate of hydrogen abstraction (*i.e.* short chain lengths).[25]

The C–O bond dissociation enthalpies of the amino acid

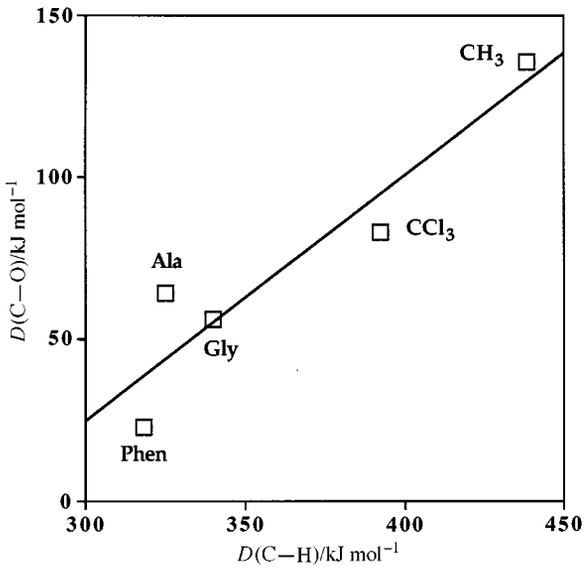


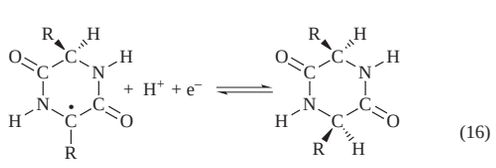
Fig. 2 The C–O bond dissociation enthalpies of peroxyl radicals plotted against the C–H bond dissociation enthalpies of the corresponding hydrocarbons for the phenylalanine derived hydroxycyclohexadiene (Phen), alanine anhydride (Ala), glycine anhydride (Gly), CCl_3H (CCl_3) and CH_4 (CH_3). Data taken from this work and refs. 26–29.

anhydride peroxyl radicals in Table 2 fall in the range 56–64 kJ mol^{-1} and are low compared to simple primary, secondary and tertiary alkyl peroxyl radicals, *e.g.* the C–O bond dissociation enthalpy for the methyl peroxyl radical has been reported to be 135.4 kJ mol^{-1}.[26] The difference of course reflects the stabilization of the α-C-centered anhydride radicals, which is normally defined as $D_{Me-H} - D_{C-H(RH)}$. The C–O bond dissociation enthalpy for $CCl_3OO^{\bullet}$ has been reported to be 83.3 kJ mol^{-1} [27] which is significantly lower than the value for $CH_3OO^{\bullet}$. Furthermore, the C–O bond dissociation enthalpy for the peroxyl radical formed upon reaction between the phenylalanine-derived hydroxycyclohexadienyl radical and molecular oxygen has been experimentally determined to 23 kJ mol^{-1}.[28] Judging from the plot in Fig. 2, the C–O bond dissociation enthalpy of these peroxyl radicals follows the C–H bond dissociation enthalpy[29] of the corresponding hydrocarbon quite closely. In view of this, our experimental data on the C–H and C–O bond dissociation enthalpies of the amino acid anhydrides and the corresponding peroxyl radicals seem reasonable.

The observation that the measured C–O bond dissociation enthalpy for the alanine anhydride peroxyl radical is higher than that of the glycine anhydride peroxyl radical is probably only an artifact due to the relatively large experimental uncertainties.

Reduction potentials

The standard reduction potentials for the half reactions (16) and (17) are listed in Table 3 along with the pK_as of the



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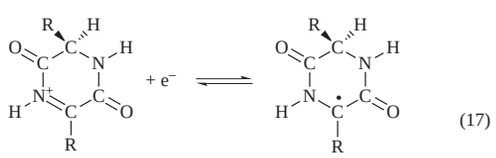

(17)

C-centered radicals[11] and the corresponding peroxyl radicals[12] (deprotonation from the nitrogen).

The E°s for reduction of the α-C-centered radicals to the anhydrides, $E^{\circ}_{(16)}$, were calculated from the D_{C-H} values and the entropies in Table 1 by the method described in ref. 9. They are similar to those of the glycine and alanine residues reported in ref. 9, and confirm the comments made above in relation to oxidation of the peptide α-CH groups. The values for the reduction of the protonated cyclic imine derivatives of the anhydrides to the α-C-centered radicals, $E^{\circ}_{(17)}$, are derived from the measured oxidation potentials of the anhydride radicals, which are assumed to be reversible. One may note that these are considerably lower than that of the *tert*-butyl radical (0.33 V *vs.* NHE in acetonitrile)[31] but higher than those of aminoalkyl radicals (<−0.68 V *vs.* NHE in acetonitrile).[32] It has been suggested that the thiyl radical of glutathione abstracts a hydrogen atom in an intramolecular fashion from the glutamyl group under physiological conditions.[3] The carbon-centered radical thus formed reduces methylviologen rapidly at pH 10.5 which indicates that its oxidation potential is lower than −0.45 V *vs.* NHE, the reduction potential of methylviologen.[33] The oxidation potentials for the amino acid anhydride radicals in Table 3 are thus considerably higher than the oxidation potential of the GSH amino acid radical. In the presence of oxygen, it is more likely that an amino acid radical reduces oxygen directly without any intermediate formation of a peroxyl radical or that the peroxyl radical is very short-lived. The potentials of both the glycine anhydride radical and the alanine anhydride radical are higher than the reduction potential of oxygen in aqueous solution.[34] Thus, direct electron transfer from these radicals to oxygen is not thermodynamically favorable. Nevertheless, superoxide anion radicals are formed in the reaction between amino acid anhydride radicals and molecular oxygen in a base-induced elimination from the peroxyl radicals. As expected, the oxidation potential of the alanine anhydride radical is *ca.* 90 mV lower than the oxidation potential of the glycine anhydride radical (a consequence of the electron-donating properties of the methyl group). The same trend has been observed for amino alkyl radicals.[32] The difference in oxidation potential between the glycine anhydride radical and the alanine anhydride radical is reflected by the differences in rate constants for the one-electron reduction of $Fe(CN)_6^{3-}$ (3.1×10^8 and 7.8×10^8 M^{-1} s^{-1}, respectively)[11] and for the OH^- induced expulsion of superoxide from the corresponding peroxyl radicals (1.6×10^5 and 3.7×10^6 s^{-1}, respectively).[12]

Table 3 Redox potentials and pK_as of amino acid anhydride α-C-centred radicals

Anhydride	E°_{red}/ V *vs.* NHE	E°_{ox}/ V *vs.* NHE	pK_a (R$^{\bullet}$)	pK_a ($RO_2^{\bullet}$)
Glycine	1.19	0.18	9.8[a]	10.7[b]
Alanine	0.99	0.09	10.6[a]	11.2[b]

[a] Ref. 11. [b] Ref. 12.

In conclusion, the thermochemical properties of amino acid anhydrides and the corresponding C-centered radicals and peroxyl radicals determined in this work are consistent with each other and with previously published observations on the radical chemistry of amino acid anhydrides and related species. Both theory and experiment indicate substantial stabilization of the anhydride radicals, with D_{C-H} values near 345 and 330 kJ mol^{-1} for glycine and alanine respectively. The result for alanine does not agree with a value of 386 kJ mol^{-1} derived from a recent study of the kinetics of decomposition of the dimer.[10] This also is not in keeping with the experimental studies in references 3 and 4.

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