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ORIGINAL ARTICLE

Chemiluminescence from thermal oxidation of amino acids and proteins

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Abstract Chemiluminescence (CL) with maximum emission in the range 550–650 nm is observed when proteins and certain amino acids are heated in air, and CL intensity is significantly reduced in nitrogen. Of the 20 common amino acids, lysine (Lys) has the highest thermal CL intensity by a factor of ~30 over arginine, threonine and asparagine. This finding differs from previous studies on amino acids and proteins oxidised using free radical initiators or singlet oxygen, where tryptophan was the dominant factor for CL emission. CL from heating solid Lys in air is accompanied by browning and the generation of fluorescent products which are characteristic of advanced glycosylation end products (AGEs) in thermally treated milk proteins. During thermal oxidation, Lys may react with its own carbonyl oxidation products to form fluorescent compounds similar to AGEs via the formation of Schiff bases. The mechanism of thermal oxidation of proteins may be similar to polyamide polymers, where reaction of free primary amino groups with carbonyls to form Schiff bases plays a key role.

Keywords Chemiluminescence · Protein · Amino acid · Schiff base · Maillard reaction · Polyamide

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Introduction

Living systems of both animal and plant origin can produce weak chemiluminescence (CL) emission when subjected to injury, invasive attack by microorganisms or to oxidative stress (Turrens et al. 1986; Cadenas et al. 1980; Yasui and Sakurai 2000; Lissi et al. 1988; Boveris et al. 1980; Totsune et al. 1993; Salin and Bridges 1981; Salin et al. 1985). Protein oxidation has been shown to be a significant mechanism involved in the generation of CL from biological materials. Addition of *t*-butyl hydroperoxide to bovine serum albumin (BSA) and to the amino acids tryptophan (Trp), tyrosine or histidine in the presence of haem resulted in CL emission (Barnard et al. 1993). Exposure of lipid-free BSA to peroxynitrite generated CL with a significant fraction of emitted light at wavelengths above 500 nm, suggesting that triplet states of tyrosine (Tyr) or Trp were involved (Watts et al. 1995). Exposure of the 20 common amino acids to peroxynitrite showed that Trp was the dominant CL emitter by an order of magnitude (Pollet et al. 1998).

More recent studies have shown that oxidation of amino acids and proteins by peroxy radicals (Aspee et al. 2001; Aspee and Lissi 2000) and by hypochlorite (Aspee and Lissi 2002) also result in the emission of visible CL. Trp was confirmed as the major source of CL in these studies, with Tyr also generating some CL in the presence of peroxy radicals. Further studies by the same group have shown that Trp is the major contributor to the CL observed following singlet oxygen-mediated oxidation of proteins, peptides and amino acids (Alarcon et al. 2007).

Our group has an interest in the thermal degradation of proteins and peptides, and recently reported on the thermal chemiluminescence (TCL) from fibrous proteins in the solid state for the first time (Millington et al. 2007). TCL

emission was observed in both oxygen and nitrogen atmospheres from wool and feather keratins, and from collagen and silk fibroin in non-isothermal studies conducted by monitoring total CL emission during temperature ramp experiments from 40 to 220°C at a rate of increase of 2.5°C/min. The TCL temperature profiles of these fibrous proteins in nitrogen are much weaker than those in oxygen and show a peak near 130°C, which in synthetic polymers is characteristic of polymer hydroperoxides (Strlic et al. 2000). This peak was not present in wool keratin following chemical reduction with sodium dithionite, which reduces any peroxides and hydroperoxides present. TCL studies on the amino acids that are particularly sensitive to oxidation in wool keratin [Trp, Tyr, phenylalanine, histidine and cystine (Millington 2006)] showed that only Trp gave a significant hydroperoxide peak near 130°C and that this feature was not present for freshly recrystallised material.

Assuming that the peak in the TCL temperature profile of fibrous proteins at 130°C is due to protein hydroperoxides, species other than TrpOOH must also be present in fibrous proteins to explain the peak observed for collagen, which contains no Trp. A series of papers by Gebicki and Gebicki (1993), Simpson et al. (1992), Gebicki (1997), Du and Gebicki (2004) and Fu et al. (1995) describes the importance of protein hydroperoxides in living systems and suggests that proteins may act as free radical traps for hydroxyl radicals. Measurement of the peroxide yields for individual amino acids oxidised by hydroxyl radicals generated directly by γ-radiation showed that valine, proline, leucine, isoleucine and lysine (Lys) produced the highest levels (Gebicki and Gebicki 1993). The peroxidation efficiency of Trp under these conditions was less than half that of the four amino acids producing the highest levels of hydroperoxides (Gebicki and Gebicki 1993).

In polymers the origin of TCL during oxidation is believed to be due to the formation of traces of peroxides and hydroperoxides, as shown in Scheme 1.

The emission of light may occur from the excited carbonyl groups generated via reactions (3) and (4), usually in the region near 415 nm, and for some polymers may also include a contribution from singlet oxygen dimol emission at 634 and 703 nm (Matisova-Rychla 2007). However, singlet oxygen dimol emission is not observed from simple hydrocarbons such as polypropylene (PP) (Tiemblo et al. 1999).

In this paper, further TCL studies on a range of globular proteins, amino acids and polyamino acids are presented. This work includes the measurement of the dispersed TCL spectrum of proteins and amino acids using a recently developed Fourier transfer CL spectrometer (FT-CL) (Tsukino et al. 2008) in an effort to identify the species responsible for CL emission.

Experimental

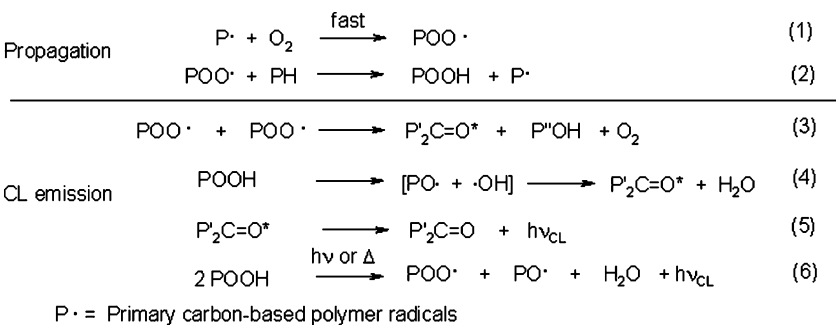
Materials

Amino acids were laboratory grade and used as supplied. Proteins and polyamino acids were supplied by Sigma-Aldrich.

Total thermal chemiluminescence

Thermal chemiluminescence experiments were carried out using a Lumipol 3 commercial photon counting instrument supplied by the Polymer Institute of the Slovak Academy of Sciences, Bratislava, Slovakia. Powdered samples were placed in small aluminium pan (9 mm diameter), and just enough material to cover the bottom of the pan was used (20 ± 5 mg). TCL peak temperatures and intensities are not very sensitive to sample mass but depend more upon the surface area of the sample. Using crystalline or powdered materials, previous studies have shown that variation of the sample mass by up to 50% results in a variation in intensity of within $\pm 10\%$ and no change in peak temperature.

Gas (O_2 or N_2) flow rates were maintained at 25 cm^3/min throughout each experiment. Temperature ramp studies were carried out from 40 to 220°C using a constant heating rate of 2.5°C/min. Samples were heated at 40°C for 10 min in N_2 to remove traces of adsorbed water and O_2 and to establish a steady baseline before starting the ramp study.



Scheme 1 Mechanism of CL emission from polymers in the presence of oxygen

Dispersed thermal chemiluminescence spectroscopy

Continuous CL spectra from solid proteins and amino acids at elevated temperatures were obtained using a MS-8310 FT-CL spectrometer (Japan Applied Technology, Tokyo). The optical system using a Savart plate and polarizer, and the data-acquisition system using a charge coupled device make it possible to collect weak emission from a wide-spread two-dimensional area over an extended measuring time. Details of this instrument have been reported elsewhere (Tsukino et al. 2008). Samples were heated in an aluminium dish (25 mm diameter) in air to 180°C to obtain a sufficiently high CL intensity to record a spectrum over an integration period as short as 30 s for some materials and up to 2 h for others.

Phosphorescence and fluorescence spectroscopy

Phosphorescence and fluorescence spectra were obtained using a Hitachi F4500 spectrophotometer. Phosphorescence spectra were obtained on solid materials placed in a quartz tube and cooled to 77 K in liquid nitrogen to avoid oxygen quenching.

Reaction of wool keratin with acetaldehyde

Wool fabric (30 × 30 mm) was swelled in distilled water (3 ml), and acetaldehyde solution (90%, 1 ml) was added. In the case of alkaline acetaldehyde treatment, 0.1 M NaOH solution (0.5 ml) was also added. The samples were left soaking overnight at room temperature, and then washed thoroughly with distilled water. Chemicals were purchased from Wako Pure Chemicals, Japan, and used as received.

Results and discussion

Total thermal chemiluminescence of proteins and polyamino acids in N_2

In our previous work on fibrous proteins we consistently observed a peak near 130°C in the TCL temperature profiles of wool and feather keratin, bovine skin collagen type I and silk fibroin in nitrogen, which for synthetic polymers is characteristic of polymer hydroperoxides (Strlic et al. 2000). For polymers heated in the absence of oxygen, TCL emission at low temperatures (<150°C) cannot be due to the bimolecular reaction of peroxy radicals generated via autoxidation, but must be due to the thermal degradation of traces of oxidised species already present in the polymer. We carried out a similar study on a further 11 proteins in nitrogen, and the TCL profiles are shown in Fig. 1. Seven of these show a peak present in the range 105–136°C, whereas four proteins (lysozyme, ribonuclease A, zein and gluten, shown in the inset) do not. The presence of the peak in N_2 does not correlate with the Trp content of the protein, since collagen, the gelatins, and protamine are Trp-free. This may indicate that some proteins are more resistant to hydroperoxide formation than others. However, whilst the thermal stability of fibrous proteins such as wool keratin is similar to synthetic polymers as its construction is based on strongly bonded networks, globular proteins of lower molecular weight thermally degrade at much lower temperatures and this may affect their TCL profiles. It would be necessary to carry out a hydroperoxide assay on these proteins to determine whether the presence of the TCL peak correlates with OOH content, but an accurate OOH assay applicable to a range of proteins, some of which have very limited aqueous solubility, was beyond the scope of this study.

A similar experiment was carried out using some available polyamino acids to determine whether specific amino acid residues could be associated with the hydroperoxide peak. The TCL temperature profiles are shown in Fig. 2. This figure is similar to that observed for proteins (Fig. 1), in that some polyamino acids [poly(Ala), poly(Gly), poly(Pro)] show no temperature peak.

For polymers, the TCL emission above 160°C in N_2 normally indicates thermal chain scission into free radicals followed by radical recombination, and is not dependent on adsorbed O_2. This will be similar for biopolymers such as peptides and proteins.

Dispersed thermal chemiluminescence spectroscopy of proteins and amino acids in air

Whilst proteins and polyamino acids clearly generate TCL emission when heated in nitrogen, the origin or absence of the emission peak cannot be firmly assigned to protein hydroperoxides without confirmation by analysis. However, it is clear that the presence of Trp is not essential for TCL emission from proteins. It was therefore of interest to determine the spectra of the TCL emissions, as this information would be valuable in assigning the origin of the CL and a possible mechanism. However, to undertake such a study, higher intensities of CL were required than observed in nitrogen, so the proteins were heated isothermally to 180°C in air.

Figure 3 shows the TCL spectra of various proteins obtained by heating in air. All of the proteins show CL emission in the 450–750 nm range, with a peak occurring in most cases between 550 and 650 nm. Fine structure is evident for some proteins, but not for others. There is close similarity in the appearance of the spectra of proteins containing Trp with spectra of Trp-free proteins, for example

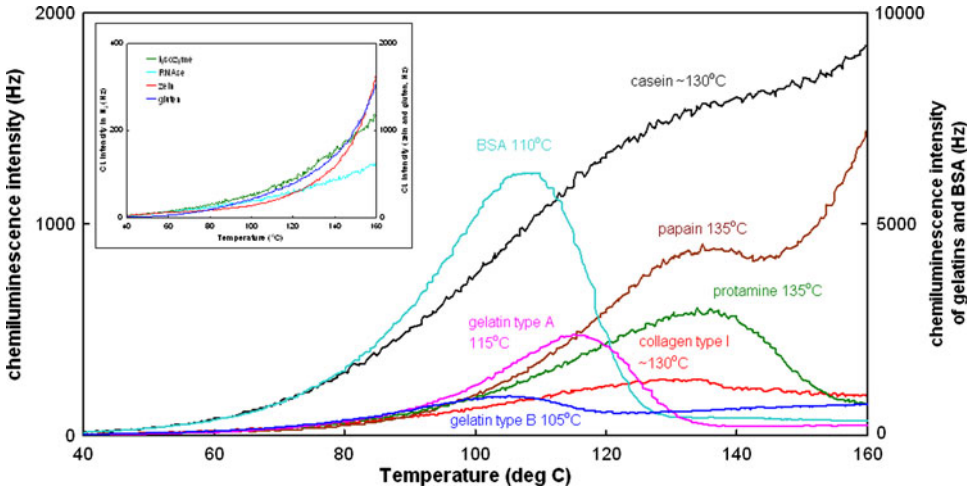


Fig. 1 Non-isothermal CL profiles of seven proteins in N_2 in the temperature range 40–160°C at a ramp rate of 2.5°C/min. Peak temperatures are marked on the *plot*. CL intensities for gelatin types A and B and BSA are significantly higher and plotted on a second *y*-axis. *Inset* shows the profiles of four proteins that do not produce a peak in the hydroperoxide region

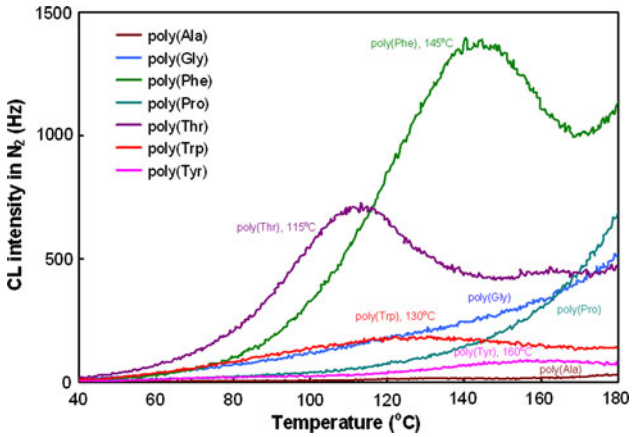


Fig. 2 Non-isothermal CL profiles of polyamino acids in N_2 in the temperature range 40–180°C at a ramp rate of 2.5°C/min. Peak temperatures, where present, are shown on the *plot*

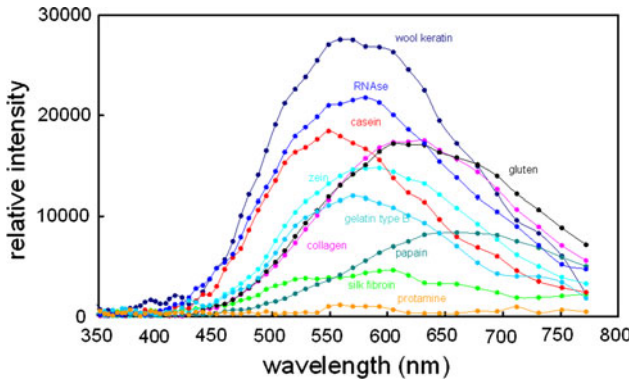


Fig. 3 Spectra of TCL emission from proteins heated at 180°C in air

wool keratin and ribonuclease A, which strongly suggests that the CL has a common origin other than Trp.

Similar TCL spectral analysis studies have been carried out previously on some synthetic polymers. For PP it was reported that the TCL emission peak occurs near 415 nm, which is characteristic of excited carbonyl species in an unoxidised aliphatic environment (Tiemblo et al. 1999). As the oxidation of PP proceeds, the peak is shifted significantly towards the red with a maximum above 510 nm being observed for highly oxidised material. This work confirms an earlier study on PP showing a similar red shift in the CL emission maximum following prolonged oxidation by Lacey and Dudler (1996). Also it was observed for PP that at the early stages of oxidation the emission at 415 nm is similar to the phosphorescence spectrum, but this is not the case for the longer wavelength emissions observed following extensive thermal degradation (Tiemblo et al. 1999).

To determine whether there is similarity between the TCL and the phosphorescence emission of a protein, the phosphorescence spectrum of wool keratin was measured at 77 K. Figure 4 shows a comparison of the TCL and normalised phosphorescence spectra of wool. Wool phosphorescence shows two features, the first excited at $\lambda_{ex} = 295$ nm and emitting at $\lambda_{em} = 440$ nm is due to the Trp residues (Ghiggino et al. 1975). A second, less intense feature ($\lambda_{ex} = 350$ nm, $\lambda_{em} = 495$ nm) has previously been assigned to the Trp oxidation product *N*-formylkynurenine (NFK) (Smith and Melhuish 1985). However, it is interesting to note that simple polyamides also show phosphorescence emission in the 400–500 nm region. By studying the mild oxidation of model amide compounds, Allen and McKellar (1978) showed that the phosphorescence in polyamides was due to the presence of α,β unsaturated carbonyl groups formed via oxidation.

The TCL emission from wool is significantly red-shifted by 85 nm relative to the phosphorescence emission at 495 nm. We repeated the phosphorescence and TCL studies on polyamide 6 (PA6) to determine whether a similar shift in emission was evident which may suggest that the phosphorescence and TCL emissions are related.

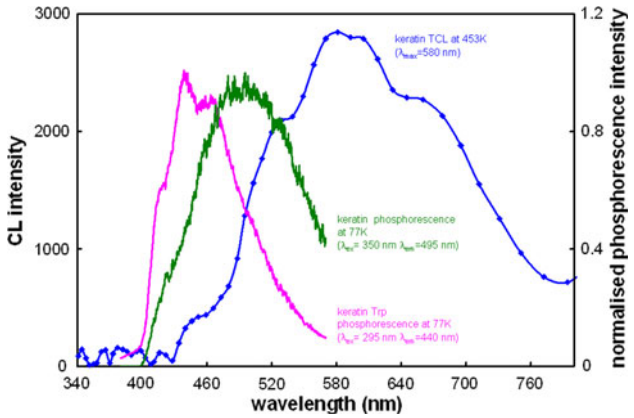


Fig. 4 Comparison of TCL emission of wool keratin at 453 K and the phosphorescence spectrum at 77 K

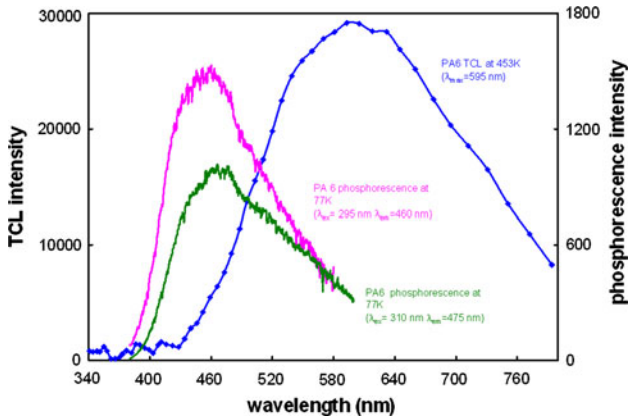


Fig. 5 Comparison of TCL emission of PA6 at 453 K and the phosphorescence spectrum at 77 K

TCL emission from PA6 has been reported previously (Matisova-Rychla et al. 1994; Lanska et al. 1998).

Figure 5 shows a similar red shift of 120 nm for the TCL emission from PA6 relative to phosphorescence. Together with the previous work by Tiemblo et al. (1999), who observed up to ~100 nm red shift between the phosphorescence and TCL emission in highly oxidised PP, it is possible that the TCL of both PA6 and wool keratin is similarly related to their phosphorescence emission.

We next examined the TCL emission spectra of the 20 common amino acids by heating the solids in air at 180°C, in the same manner as the proteins shown in Fig. 3. The aim of this was to determine whether this approach would be useful to identify the principal contributors to the TCL observed from proteins. The intensity of total TCL emission in O_2 was not significantly affected by recrystallisation of the amino acids. Figure 6 shows the spectra of the nine amino acids with the highest TCL emission intensities. Integration times of 30 min were used to capture the light from all of the amino acids except for Lys, which had a

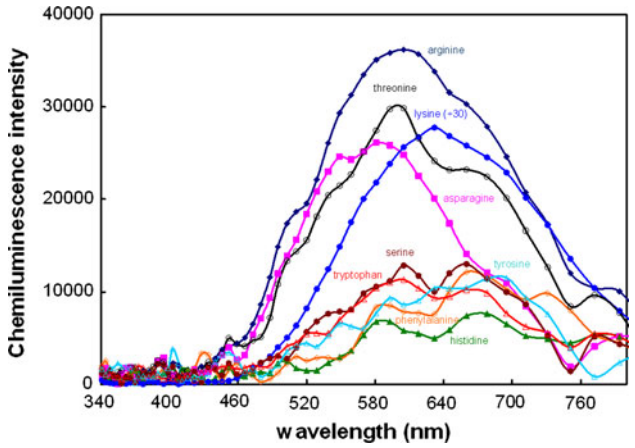


Fig. 6 TCL spectra of the nine amino acids with highest intensity emissions after heating at 180°C in air

very intense TCL emission and required only 1 min. Glutamine and glutamic acid decomposed at 180°C and TCL emission from the other nine amino acids not shown was weaker.

Surprisingly the four amino acids having the highest TCL emission intensity are Lys, arginine (Arg), threonine (Thr) and asparagine (Asn), but the intensity for Lys is around a factor of 30 higher than that of the other three. This contrasts with the previous published work of the groups led by Barnard et al. (1993), Watts et al. (1995), Pollet et al. (1998), Aspee et al. (2001), Aspee and Lissi (2000, 2002) and Alarcon et al. (2007), who showed that Trp was the major contributor to CL emission during oxidation of proteins and amino acids. Lys has a broad emission maximum at 630 nm and has a similar TCL spectrum to gluten and collagen (Fig. 3). A comparison of the maximum TCL intensities observed from amino acids during heating in air at 180°C is shown in Fig. 7.

Thermal oxidation and TCL of Lys, Arg, Thr and Asn

Lysine is unique amongst amino acids in having a free primary amine group available. We were interested in the origin of the TCL from Lys, which was observed to undergo a significant browning following thermal oxidation. The fluorescence spectra of aqueous solutions of samples of Lys prepared before and after heating for 30 min at 180°C are shown in Fig. 8.

Thermal oxidation of solid L-Lys clearly produces fluorescent products that emit in the 420–430 nm region. It is interesting to note that oxidation of Lys is very important in the processing of protein foodstuffs, in particular in milk, since Lys is the most sensitive amino acid residue to damage during thermal processing (Leclere et al. 2001). Degradation of Lys residues can be detected by monitoring the appearance of advanced glycosylation end products

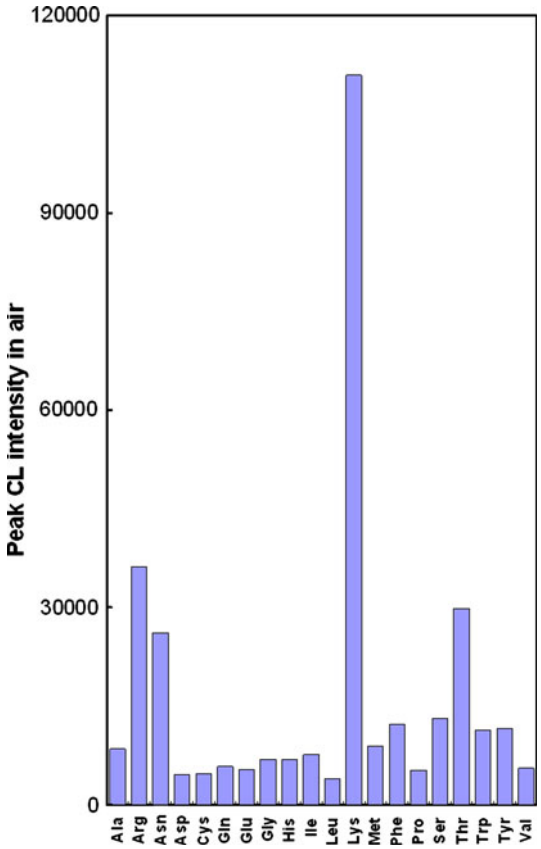


Fig. 7 Relative maximum chemiluminescence intensities emitted during heating of amino acids at 180°C in air

(AGEs, sometimes referred to as advanced Maillard products) which are formed by reaction of the free amino group of Lys with the aldehyde or ketone groups of reducing carbohydrates via a series of reactions involving Schiff bases, amino-ketones and α,β dicarbonyls (Leclere et al. 2001). These AGEs are highly conjugated and fluorescent with emission maxima around 430 nm, very close to the emissions observed by heating Lys in O_2 in Fig. 8.

Previous work showed that chemiluminescent products are formed at an early stage in the Maillard reaction (Namiki et al. 2007). The exact nature of the CL products is unknown, but CL emission from the simple Maillard reaction between methylglyoxal and methylamine in the presence of air produced a peak in the 580 nm region, very similar to that from the oxidised proteins and amino acids shown in Figs. 3 and 6 (Namiki et al. 2007). A CL peak was also observed at 580 nm for the Maillard reactions between glucose/methylamine, and for the glucose/β-alanine and xylose/β-alanine reactions was red-shifted to 680 nm. The authors claimed that this red shift to 680 nm demonstrates the presence of singlet oxygen (Namiki et al. 2007).

It therefore appears probable that when heated in air at 180°C, the free amino group of Lys may react with its own carbonyl oxidation products to form compounds similar to AGEs, which produce intense TCL. In a further experiment, a reducing sugar (D-glucose) was mixed with Lys and the total CL emission intensity at 180°C compared with pure Lys. A small reduction in CL intensity was observed in the presence of glucose, which suggests that sufficient carbonyl compounds may be generated during thermal degradation of Lys alone to produce the luminescent products.

The free primary amino terminal groups in proteins and polyamides can react similarly to free amine groups in Lys, and this may explain the similarity in the TCL spectra observed for proteins in Fig. 3. The lower abundance and more limited mobility of free amino groups in proteins and nylon may explain the large differences in intensity. Since the TCL intensity from Lys exceeds that from other amino acids by a factor of $\sim$30, TCL studies on proteinaceous foodstuffs may be a valuable means of determining the rate

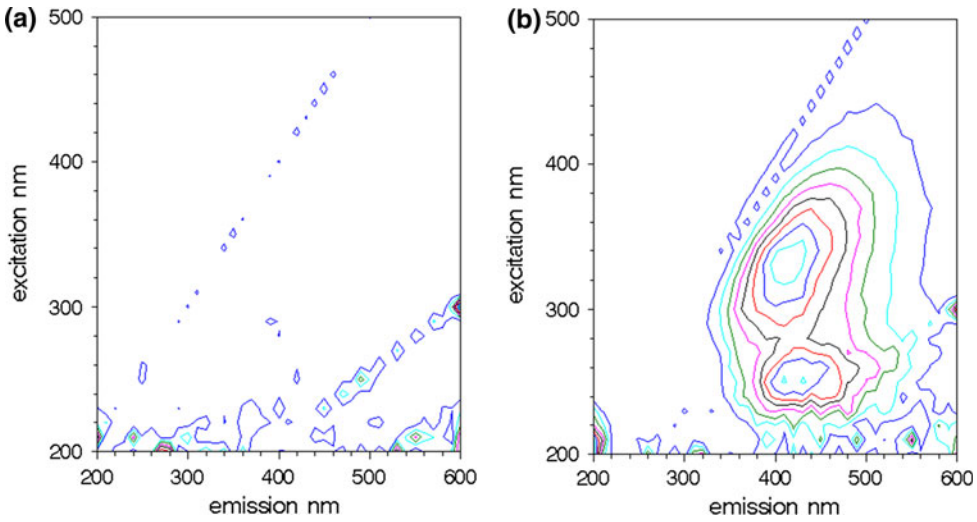


Fig. 8 Fluorescence spectra of aqueous solutions of samples of solid L-lysine **a** before and **b** after heating in oxygen for 30 min at 180°C

of oxidation of Lys residues during or cooking or thermal treatments.

The origin of the TCL from Arg, Thr and Asn is less clear. No browning or fluorescent products were observed for these amino acids following oxidation at 180°C in oxygen. Time course experiments were conducted on Lys, Arg, Thr and Asn for over 4 h to determine the rate of change of total TCL and their spectral emissions with time (Fig. 9). Thermogravimetric studies at 180°C over 4 h in N_2 showed that weight losses from Arg, Thr and Asp were minimal and <4% for Lys. The time course TCL studies showed a linear increase in total TCL emission with time for Arg and Asp, and their spectra showed a single dominant emission peak in the range 580–590 nm. However, TCL emission from Lys and Thr showed far more complex behaviour with many different time-dependent emission peaks evident, particularly in the case of Thr. It is clear from Fig. 9 that more complex thermal reactions must occur for Lys and Thr than occur for Arg and Asp. After an extended period of heating, the CL spectrum of Thr shows a large red shift and stronger CL emission at 660 and 712 nm. It is possible that the Thr oxidation products could liberate singlet oxygen, which shows dimol emission at 633 and 703 nm (Khan and Kasha 1963).

It was of interest to determine the effect on CL emission of modifying the free amino groups on a protein to form Schiff bases, since, as discussed later, Schiff bases may be a common precursor of CL emission from both polyamides and proteins. This was conveniently done by reacting wool fabric with acetaldehyde. Acetaldehyde reacts with the free terminal and Lys amino groups present in proteins to initially form Schiff bases which are intermediates in the formation of stable fluorescent products (Braun et al. 1995; Hoffmann et al. 1993). About 30% of the Lys residues in wool form Schiff bases (Friedman et al. 1974).The reaction was carried out at both neutral and alkaline pH, since deprotonation of the amino groups accelerates formation of Schiff bases. The wool fabric underwent significant visual browning following acetaldehyde treatment.

Figure 10 shows the time dependence of the TCL spectra of wool treated with acetaldehyde at 180°C in air, compared with untreated wool, and also the time course for total TCL emission from these samples. It is clear that following treatment with acetaldehyde the intensity of TCL from wool is increased by a factor of 9 under neutral and a factor of 13 under alkaline conditions. Figure 10d shows that whilst the total TCL of untreated wool (and wool treated with alkali only) increases with time due to increased oxidation, TCL of wool containing Schiff bases decreases. This may be due to the lower population of free amino groups remaining available for oxidation following acetaldehyde treatment. The peak emission of acetaldehyde-treated wool after 5 min heating is significantly red-shifted compared to the untreated sample (560–620 nm), and appears similar to the TCL emission from free Lys at 630 nm (Figs. 6, 9).

This study demonstrates the importance of the presence of free primary amino groups, and in particular Lys residues, in the thermal oxidation of proteins and the emission of TCL. Previous work on the thermal oxidation of polyamides has reached similar conclusions regarding the influence of free amino groups. Karstens and Rossbach (1989) have shown that Schiff bases (azomethines) derived from primary terminal amino groups play a key role in the thermal oxidation of polyamides (PA6 and PA6,6) and are responsible for the formation of yellow chromophores in polyamides maintained at high temperatures in the presence of oxygen. The mechanism involves reaction of carbonyl end groups on the PA chains, formed via oxidation during polymer processing, with the terminal amino groups to form Schiff bases. These groups polymerise to form conjugated (and hence highly coloured) oligoenimines and regenerate free amine groups (aggregative conversion), or oxidise to regenerate further free carbonyl groups (disaggregative conversion) as shown in Scheme 2 (Karstens and Rossbach 1989).

The conjugated oligoenimines in oxidised PA6 and PA6,6 have been characterised by fluorescence spectroscopy and chemical analysis (Karstens and Rossbach 1990). A similar mechanism could also occur for the thermal oxidation of proteins, particularly proteins that are rich in Lys. This may be an important finding for research on the mechanism of protein yellowing.

There have been few relevant studies on the products of thermal oxidation of proteins and amino acids in air or oxygen. Most studies on thermal decomposition or pyrolysis of amino acids, including Lys, have been carried out in vacuo or in inert atmospheres and therefore have limited relevance to this work (Breitbart et al. 1979). However, it is interesting to note that Lys is one of the primary targets for metal-catalysed oxidation in proteins, where aminoadipic semialdehyde (from Lys) and glutamic semialdehyde (from Arg and Pro) have been identified as the major carbonyl products (Requena et al. 2001).

Conclusions

Both fibrous and globular proteins emit CL in the range 450–750 nm when heated isothermally in air at 180°C. Weaker CL is also observed when proteins are heated in nitrogen, and in non-isothermal temperature ramp experiments an emission peak between 100 and 140°C is observed for some proteins, but absent for others. For synthetic polymers, a peak in this region is characteristic of the presence of hydroperoxides, which are key

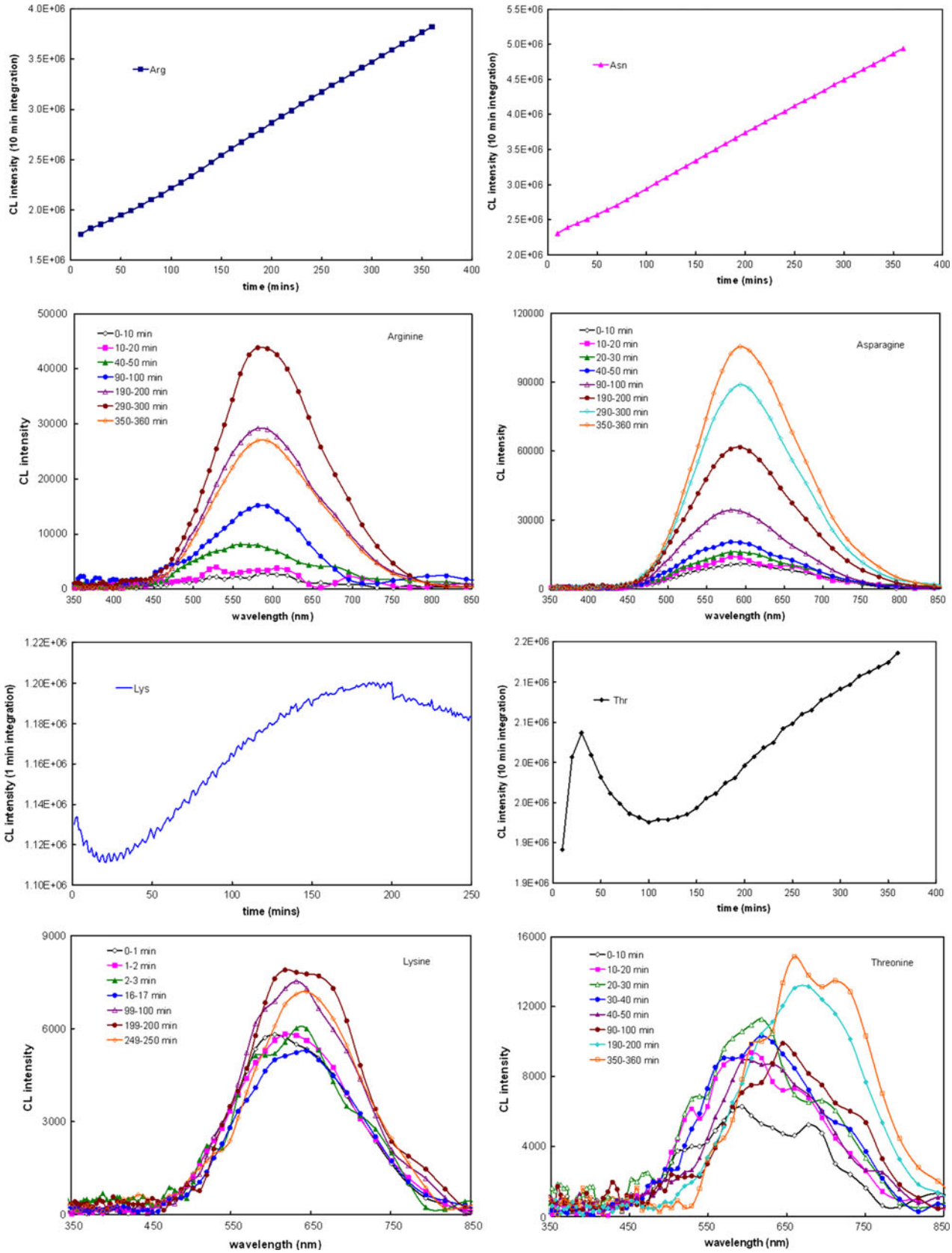


Fig. 9 Changes in total chemiluminescence emission and CL spectra with time for Arg, Asp, Lys and Thr heated at 180°C

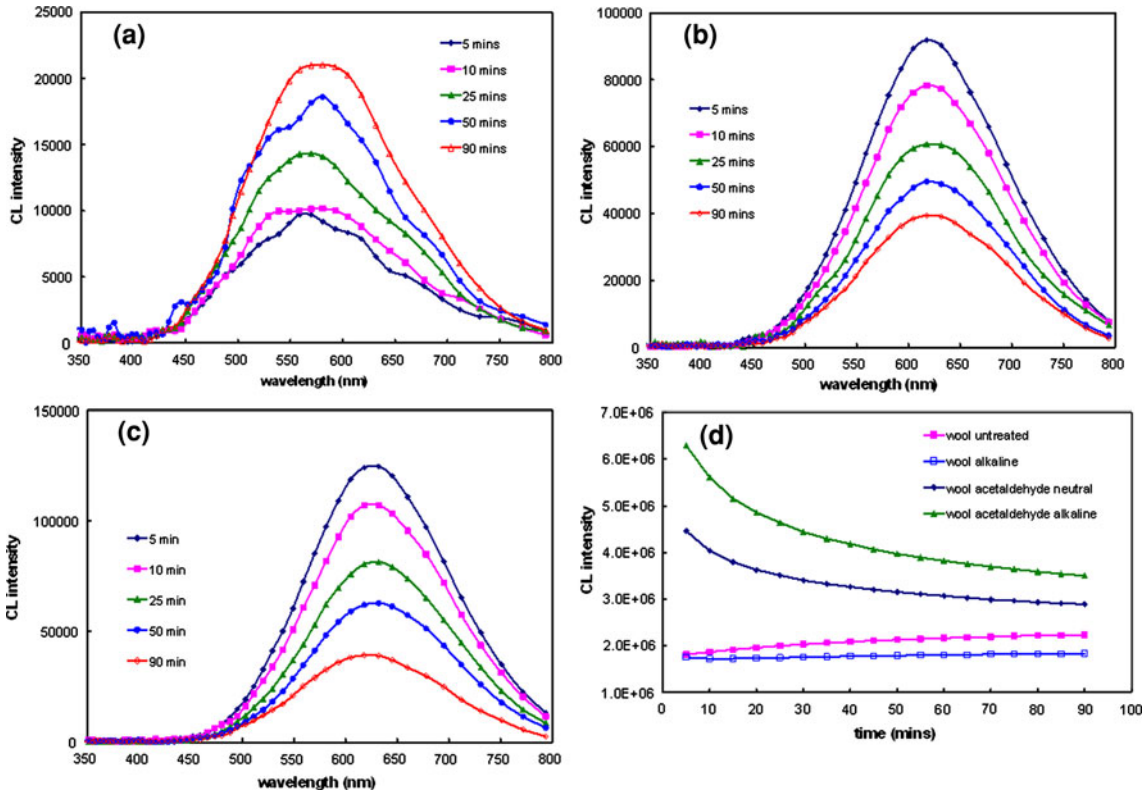
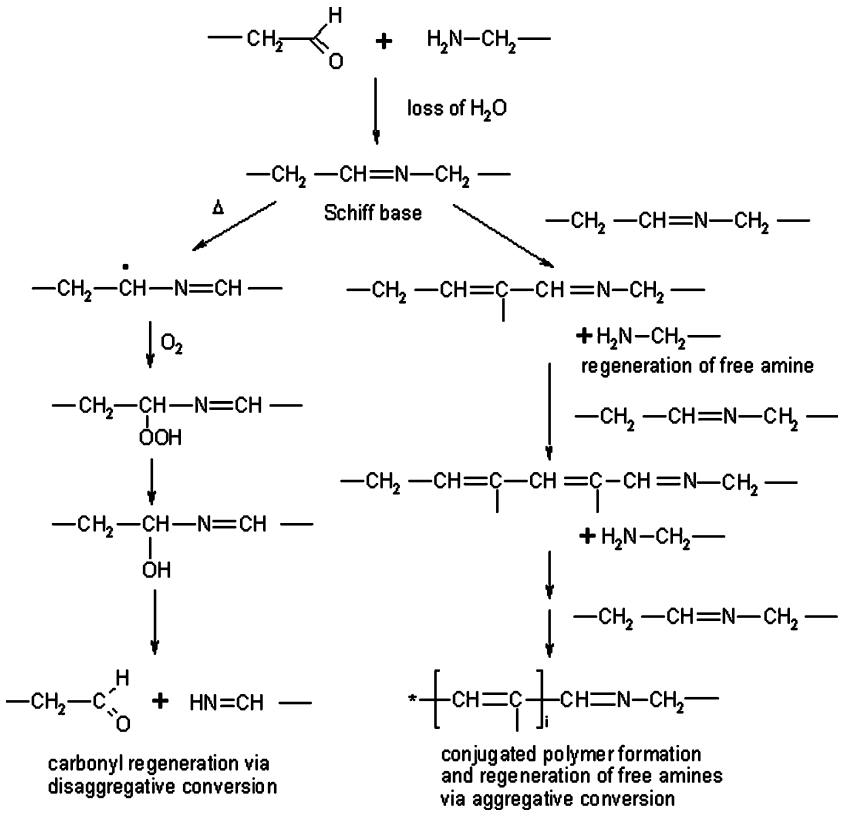


Fig. 10 Changes in TCL emission spectra with time at 180°C in air for **a** untreated wool, **b** acetaldehyde-treated wool at neutral pH, and **c** acetaldehyde-treated wool at alkaline pH. Plot **d** shows the change in total TCL emission of these samples with time under similar conditions

Scheme 2 Karstens and Rossbach mechanism for thermo-oxidative degradation of polyamides via Schiff bases (Karstens and Rossbach 1989)



intermediates in the free radical oxidation of proteins. TCL emission over similar wavelength range to that observed from proteins in air or oxygen is observed for simple polyamides such as PA6, suggesting a common factor for emission of CL may be the presence of free amino groups. It is likely that the thermal oxidation of proteins occurs via the formation of Schiff bases, as shown previously for polyamides. Thermal CL spectra of the 20 common amino acids showed that Lys, Arg, Thr and Asp had the highest emission intensities, with Lys having the highest intensity by a factor of thirty. Significant browning of Lys occurs on thermal oxidation at 180°C and fluorescent products are formed with an emission maximum near 430 nm. This is similar to the fluorescence emission from AGEs formed from Lys residues via Schiff bases in foods. During thermal oxidation in the presence of oxygen, Lys may react with its own carbonyl oxidation products to form compounds similar to AGEs. Reaction of a model protein (wool keratin) with acetaldehyde results in significant browning and the formation of Schiff bases. TCL emission from acetaldehyde-treated wool was of significantly higher intensity, by a factor of 9 under neutral and a factor of 13 under alkaline conditions. Since the CL intensity from Lys is much larger than from other amino acids, thermal CL studies on proteinaceous foodstuffs may be valuable in determining the rate of oxidation of Lys residues during cooking or thermal treatments.

Acknowledgments The authors are grateful to Mike Jones for technical assistance with this research.

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