Dyes and Pigments 92 (2012) 1351–1354

Contents lists available at SciVerse ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Detection of singlet oxygen generated by commercial fine art organic pigments by means of a novel, robust chemical probe

Morena Silvestrini, Alessandra Ciappa, Fabrizio Fabris, Giuseppe Borsato, Ottorino De Lucchi*

Dipartimento di Scienze Molecolari e Nanosistemi, Università Ca' Foscari Venezia, Dorsoduro 2137, I-30123 Venezia, Italy

ARTICLE INFO

Article history:
Received 8 July 2011
Received in revised form
22 September 2011
Accepted 23 September 2011
Available online 8 October 2011

Keywords:
Singlet oxygen
Sensitizer
Paintings degradation
Organic pigments
Oxo-ene reaction
Singlet oxygen detection

ABSTRACT

A novel, robust chemical method for the detection of singlet oxygen is reported. It uses the oxo-ene reaction of the largely available and inexpensive dicyclopentadiene which is transformed by singlet oxygen into a hydroperoxide, quantitatively converted *in situ* by acetic anhydride and pyridine into a stable ketone, easily detectable by standard GC. The method has been tested in the detection of singlet oxygen as generated by standard organic pigments used in commercially available tubes for fine art paintings.

The quest for conservation commands chemical investigation of the processes involved in the deterioration of fine arts works. Weather and light fastness are becoming common terms in the choice and commercialization of fine art materials and especially of pigments for paintings. The coloured nature of the latter makes them virtual generators of singlet oxygen which is the activated excited state of molecular oxygen and as such, potentially more destructive than the fundamental triplet state oxygen. Besides being the cause of fading of the colour of the pigment itself, singlet oxygen may be the cause of damage of the binding materials for the pigments and of the varnishes composing the artwork, greatly contributing to its final deterioration. Actually, because of abundance, distribution and ease of formation, singlet oxygen can be predicted as one of the most disruptive agents of degradation [1].

In particular, the relevant amount of oxygen (nearly 20%) present in dry air as the lower energy diradical form with two unpaired electrons (triplet oxygen) can be paired by mean of light to occupy a single π orbital, to afford the excited state (*i.e.* singlet state), which, being higher in energy is also more reactive. The excitation is promoted by a specific sensitizer, which absorbs the visible light, reaching the excited state and transfers its energy to oxygen [2,3]. The postulated mechanisms involved in the excitation transfer were suggested by Schönberg, [4] and Kautsky [5]. Coloured dyes (*e.g.* tetraphenylporphyrin, rose bengal, methylene blue) behave as sensitizers for oxygen, finding practical applications in organic synthesis [6].

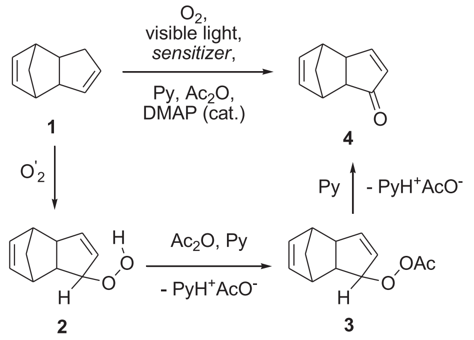
From the biochemical viewpoint, the detection of singlet oxygen is a long standing area of research where the action and the involvement of such activated form of oxygen is well documented. Customarily, molecular probes are used which are able to produce fluorescence when in presence of singlet oxygen. To the best of our knowledge, so far, the most active and sensible detection probe is by Posner et al., which makes use of *trans*-1-(2-methoxyvinyl) pyrene [7]. The latter reacts with $^1O_2$ to form (4 + 2) and (2 + 2) cycloadducts, which decompose emitting florescence that can be monitored and related with the amount of singlet oxygen produced. Though the system is extremely sensitive and reliable (able to detect up to picomoles of $^1O_2$), it presents drawbacks in the stability of the reagent which auto-oxidises in the presence of oxygen and easily hydrolyses.

In the search of a robust and selective method for the detection of $^1O_2$ that could be easily performed in organic chemistry laboratories and quantified by GC even after hours from the experiments we thought to use the oxo-ene reaction of dicyclopentadiene **1**. The latter with singlet oxygen is known to produce the unstable hydroperoxide **2**, which can be rapidly decomposed *in situ* by a mixture of acetic anhydride and pyridine (Scheme 1) [8,9]. The resulting dicyclopentadien-3-one **4** is stable and easily detectable, allowing simple and affordable analyses of the final mixtures.

---

* Corresponding author. Tel.: +39 41 234 8546; fax: +39 41 234 8517.
E-mail addresses: delucchi@unive.it, fabrisfa@unive.it (O. De Lucchi).

0143-7208/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2011.09.018



Scheme 1. Model reaction considered for the study of the generation of singlet oxygen.

To test the feasibility of the method, Rose Bengal (RB) [10] supported on cross-linked polystyrene [11] was irradiated in the presence of **1** and the acetylating mixture during oxygen bubbling (Table 1, entry 17). This solid dye required 6 h to convert 40% of the starting material **4** into the final product **6**, as detected by GC. In order to exclude the effect of the intense visible light radiation (500 Watt) in the formation of the product via a radical process, a blank experiment in absence of any sensitizer was performed and considered as zero level of conversion (Table 1, entry 0).

Under the same conditions and after the same time, no reaction was monitored without light or without oxygen, demonstrating that the reagents are stable in the absence of singlet oxygen. A certain amount of oxidized product is observed when both light and oxygen are used without sensitizers and this offset value was subtracted from the observed data. Even though the absolute quantity of generated singlet oxygen cannot be assessed, the method is of practical application when used to establish the amount of $^1O_2$ produced by pigments in comparison with the standard RB system because both systems are constituted by insoluble sensitizers.

Keeping the irradiation time constant for all the experiments, standard pigments obtained from commercial water colours (Maimeri SpA) were considered. The coloured matter as squeezed by the tube was repeatedly washed with water, ethanol, hexanes and separating by centrifugation the solid material subsequently to every washing. These solid materials were tested as sensitizers for the generation of singlet oxygen with irradiation of **1**, acetylating mixture and a stream of oxygen [12]. Results are summarized in Table 1.

Data in Table 1 are obtained after subtracting the offset as obtained with no sensitizer. In all cases a modest amount of product **4** was obtained, showing that there is no great production of $^1O_2$. All pigments however proved to produce $^1O_2$, except Permanent Orange 62 (*N*-(2,3-dihydro-2-oxo-1H-benzimidazol-5-yl)-2-[2-(4-nitrophenyl)diazenyl]-3-oxo-butanamide, entry 2) and Pigment Red 254 (1,4-Diketo-3,6-bis(4-chlorophenyl)pyrrolo[3,4-*c*]pyrrole, entry 7) which actually seems to act as quenchers. The highest values of conversion were observed with Pigment Blue 16 (29H,31H-phtalocyanine, entry 5), and Vat Red 41 (thioindigo, entry 10), confirming their active role in the formation of singlet oxygen, as previously observed by other researchers [13,14]. The higher activity of the phthalocyanine can be associated with the similarity in structure with tetraphenylporphyrine, a well known dye for singlet oxygen sensitisation. Fig. 1

In conclusion, with this simple and viable methodology we could prove that standard pigments present in commercial fine art materials can act as modest sensitizers for singlet oxygen. Though we are far from an accurate quantification of the amount produced, from our data it can be assumed to be between 1 and 10% of immobilised RB which is presently one of the best solid, insoluble sensitizers for $^1O_2$. It can be concluded that organic pigments

Table 1
Effect of solid dye in the production of singlet oxygen, as trapped by **1** to afford **4**.

Entry	Colour	Colour Index Name	CAS Registry #	Ketone **4** (mmol)–[%]	Ketone **4** neat of $^3O_2$ effect (mmol)–[%]	Efficiency (% relative to RB)
0	–	–	–	0.111–0.88	0.000–0.00	0.00
1	🟫	PR206	71819-76-6	0.190–1.51	0.080–0.64	1.57
2	🟧	PO62	52846-56-7	0.079–0.63	−0.031 to −0.25	−0.61
3	🟦	PB60	81-77-6	0.150–1.19	0.040–0.32	0.78
4	🟧	PY139	36888-99-0	0.126–1.00	0.016–0.13	0.31
5	🟫	PR88	522-75-8	0.823–6.55	0.713–5.68	13.95
6	🟥	PR168	4378-61-4	0.395–3.14	0.285–2.27	5.58
7	🟥	PR254	84632-65-5	0.103–0.82	−0.007 to −0.06	−0.14
8	🟩	PG36	14302-13-7	0.443–3.53	0.333–2.65	6.52
9	🟩	PG7	1328-53-6	0.459–3.65	0.349–2.78	6.83
10	⬛	PB16	574-93-6	1.218–9.70	1.108–8.82	21.69
11	🟪	PV23	6358-30-1	0.387–3.08	0.277–2.21	5.42
12	🟪	PR122	980-26-7	0.554–4.41	0.444–3.54	8.69
13	🟨	PY97	12225-18-2	0.174–1.39	0.064–0.51	1.25
14	🟥	PR209	3573-01-1	0.395–3.14	0.285–2.27	5.58
15	🟧	PO43	4424-06-0	0.206–1.64	0.096–0.76	1.88
16	🟫	PV49	1047-16-1	0.332–2.64	0.222–1.77	4.34
17	–	Rose Bengal B polystyrene	–	5.22–41.56	5.110–40.68	100.00

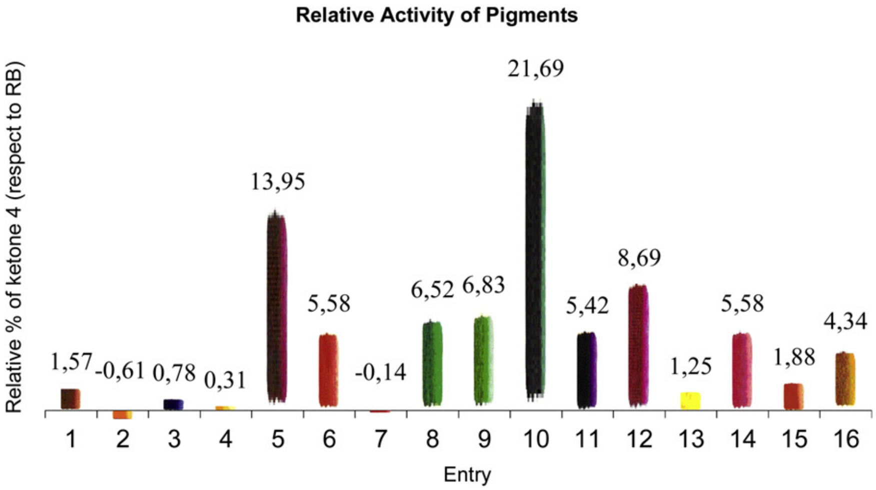


Fig. 1. Histogram reporting the amounts of **4** obtained from **1** relative to RB (subtracted of the value obtained without any pigment or sensitizer and in percent to the value obtained).

cannot be used in synthetic applications, but indeed in long times in the presence of light and oxygen can be cause of degradation of paintings for this reason.

1. Experimental

All reagents were used as purchased by suppliers without any further purification. Water colours were obtained from Maimeri S.p.A. Gas-chromatographic analysis were performed using a 15 m length, 025 mm i.d. column with 5% polydiphenysiloxane/95% polydimethylsiloxane 0.25 μm thick stationary phase, constant nitrogen flow 1.0 mL/min. Detector: FID. Linear correlation between product **4** and internal standard (*n*-tetradecane) was determined before analyses. Temperature ramp: 5 min at 50 °C followed by an increasing of 10 °C at each minute until the reachment of 300 °C, kept constant for 10 min. Typical retention times for substances: dicyclopentadiene **1** (8′45″), dicyclopentadien-3-one **4** (12′20″), *n*-tetradecane (14′30″).

1.1. Preparation of the pigment (removal of arabic gum and additives)

Water colour (approx. 1.0 g) was dispersed in water (10 mL) and the resulting slurry was centrifuged for 15 min. The supernatant was removed with the aid of a Pasteur pipette and the operation was repeated two times with the same solvent. The resulting wet solid was dispersed in ethanol (10 mL) and centrifuged for 20 min, removing the liquid with a Pasteur pipette. The operation was repeated with cyclohexane as solvent and the resulting pigment was dried in *vacuum* for 30 min.

1.2. Preparation of Rose Bengal B bound to polystyrene (swelling of the polystyrene matrix)

Rose Bengal B bound to polystyrene from Sigma–Aldrich (approx 100 mg) was added to dichloromethane (5 mL) and the mixture was stirred for 20 min at RT. The solvent was removed by filtration and the solid was dried in vacuum for 5 min, it was carefully weighted and used straight.

1.3. Pigment-induced singlet oxygen generation and trapping

In a water-cooled jacketed test tube (*h* 150 mm, *d* 15 mm) fitted with a reflux condenser topped with a rubber septum punctured with a needle and a 0.25 i.d. teflon® cannula, a magnetically stirred mixture of dicyclopentadiene (1.660 g, 12.56 mmol), acetic anhydride (1.3 mL, 1.404 g, 13.75 mmol), pyridine (2.0 mL, 1.956 g, 24.73 mmol), 4-(dimethylamino)pyridine (DMAP) (218 mg, 1.77 mmol), *n*-tetradecane (internal standard, 1.08 mL, 0.823 g, 4.17 mmol) and Rose Bengal B bound to polystyrene or pigment (100 mg) was irradiated for 6 h while oxygen was bubbled (6.66 mL/min) with a halogen lamp (500 W, 4.0 cm far from the reactor, wrapping lamp and apparatus with an aluminium foil). The mixture was poured onto water (10 mL) and extracted with cyclohexane (2 × 20 mL). Combined organic layers were dried over $MgSO_4$, filtered by suction and analyzed by GC.

Acknowledgements

This work was co-founded by MIUR (Rome) within the national PRIN framework.

References

[1] Mills JS, White R. The organic chemistry of museum objects. Butterworth-Heinemann; 1994.

[2] (a) Mulliken RS. Electronic structures of polyatomic molecules and valence. II. General considerations. Physical Review 1932;41(1):49–71;
(b) Mulliken RS. The interpretation of band spectra. Reviews of Modern Physics 1932;4(1):1–86.

[3] (a) Frimer AA. In: Patai S, editor. The chemistry of functional groups, peroxides. John Wiley and Sons, Ltd; 1983. p. 202–34;
(b) Wasserman HH, Ives JL, Turro NJ, editors. Modern molecular photochemistry. Menlo Park: Benjamin/Cummings; 1978. p. 579–611;
(c) Kearns DR. Physical and chemical properties of singlet molecular oxygen. Chemical Review 1971;71(4):395–427;
(d) Foote CS. Photosensitized oxygenations and the role of singlet oxygen. Accounts of Chemical Research 1968;1(4):104–10.

[4] Schonberg A. Notiz über die photochemische Bildung von Biradikalen. Annalen 1935;518(1):299–302.

[5] Kautsky H, de Bruijn H. Naturwissenschaften 1931;19:1043.

[6] (a) Wasserman HH, DeSimone RW. In: Paquette LA, editor. Encyclopedia of reagents for organic synthesis. John Wiley and Sons, Ltd; 2005. p. 4478–84;
(b) Singlet oxygen in organic synthesis. Tetrahedron 1981;37(10):1825–52.

[7] Thompson A, Lever JR, Canella KA, Miura K, Posner GH, Seliger HH. Chemiluminescence mechanism and quantum yield of synthetic vinylpyrene analogs of benzo[a]pyrene-7,8-dihydrodiol. Journal of the American Chemical Society 1986;108(15):4498–504.

[8] Mihelich ED, Eickhoff DJ. One-pot conversion of olefins to α,β-unsaturated carbonyl compounds. An easy synthesis of 2-cyclopentenone and related compounds. Journal of Organic Chemistry 1983;48(22):4135–7.

[9] (a) Pozzi G, Mercs L, Holczknecht O, Martimbianco F, Fabris F. Straightforward synthesis of a fluorous tetraarylporphyrin: an efficient and recyclable sensitizer for photooxygenation reactions. Advanced Synthesis and Catalysis 2006; 348(12–13):1611–20;
(b) Borsato G, De Lucchi O, Fabris F, Lucchini V, Frascella P, Zambon A. Synthesis and evaluation of new chiral diols based on the dicyclopentadiene skeleton. Tetrahedron Letters 2003;44(17):3517–20;

(c) Benaglia M, Danelli T, Fabris F, Sperandio D, Pozzi G. Poly(ethylene glycol)-supported tetrahydroxyphenyl porphyrin: a convenient, recyclable catalyst for photooxidation reactions. Organic Letters 2002;4(24): 4229–32.

[10] (a) Lamberts JJM, Neckers DC. Rose Bengal derivatives as singlet oxygen sensitizers. Tetrahedron 1985;41(11):2183–90;
(b) Lamberts JJ, Neckers DC. Rose bengal and nonpolar derivatives. Journal of the American Chemical Society 1983;105(25):7465–7.

[11] Martinez-Utrilla R, Botila JM, Sastre R. Quantitative evaluation of polymeric photosensitizers. Polymer Bulletin 1984;12(2):119–26.

[12] In order to exclude leeching of the solid dye, a mixture of 1 and acetylating mixture with each solid sensitizer was stirred for 2 hours, filtered and submitted to irradiation in a stream of oxygen, without observing any significant reactivity.

[13] Pigment Blue 16 Krestyn E, Kolarova H, Bajgar R, Tomankova K. Photodynamic properties of ZnTPPS4, ClAlPcS2 and ALA in human melanoma G361 cells. Toxicology in Vitro 2010;24(1):286–91.

[14] (a) Vat red 41 Gandra N, Frank AT, Le Gendre O, Sawwan N, Aebisher D, Liebman JF, et al. Possible singlet oxygen generation from the photolysis of indigo dyes in methanol, DMSO, water, and ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate. Tetrahedron 2006;62(46):10771–6;
(b) Haugen CM, Bergmark WR, Whitten DG. Singlet oxygen mediated fragmentation of amino alcohols, 1,2-diamines, and amino ketones. Journal of the American Chemical Society 1992;114(26):10293–7.