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UV–visible spectral identification of the solution-phase and solid-phase permanganate oxidation reactions of thymine acetic acid

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Abstract—Solution-phase and solid-phase permanganate oxidation reactions of thymine acetic acid were investigated by spectroscopy. The spectral data showed the formation of a stable organomanganese intermediate, which was responsible for the rise in the absorbance at 420 nm. This result enables unambiguous interpretation of the absorbance change at 420 nm, as the intermediate permanganate ions could be isolated on the solid supports.
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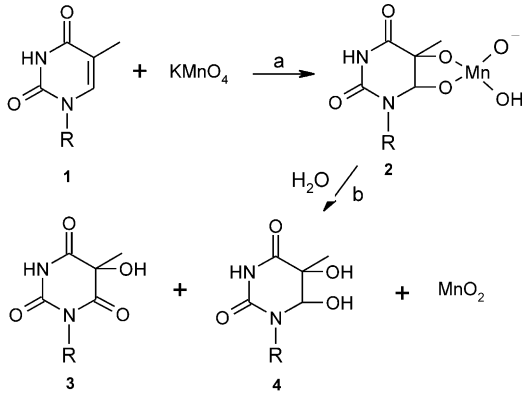
Potassium permanganate ($KMnO_4$) has been widely used as a chemical modification agent of DNA in chemical and biochemical research for many years.[1,2] $KMnO_4$ oxidizes selectively the C5–C6 double bond of an un-stacked thymine base within a DNA molecule and this property has established the fundamental concept for numerous biological applications (such as sequencing techniques, foot-printing assays, DNA interference assays, thymine dimer assays, detection of Z-DNA, B-Z & Z-Z junctions, detection of 8-oxoguanine sites, mutation detection as well as other spectroscopic methods associated with an absorbance at 420 nm).[3]

Although the mechanism of this reaction with thymine and other olefins has been well established for many years,[4,5] some specific questions associated with the nature of the absorbance at 420 nm developed during the reactions still remain unanswered.[3,6] It has been generally accepted that the permanganate oxidation reaction of thymine in a solution phase (Scheme 1) forms a cyclic organo-manganese intermediate **2** (soluble complex MnO_2 species) which then decomposes under acidic or basic conditions to give the corresponding oxidized products **3** and **4** plus colloidal soluble MnO_2 species (absorption at 420 nm). Both the intermediate **2** (R=H) and the colloidal product were suggested to contribute to a strong absorption band at 420 nm and/or around 320 nm.[6–8] These spectroscopic interpretations have been controversial, as the permanganate ions could not be separated in the reaction mixture.[6] Here, we report the oxidation reaction of thymine acetic acid **1** ($R=CH_2COOH$) in both solid-phase and solution-phase oxidation reaction conditions. Comparative study by spectroscopy indicated that the formation of the intermediate was responsible to the rise of the absorbance at 420 nm and the relationship between the organo-manganese species and the absorbance in this visible region has been confirmed.



Scheme 1. Solution-phase oxidation of thymine (R=H) and thymine acetic acid ($R=CH_2COOH$). (a) 0.1 M TEAC in DCM; (b) H_2O.

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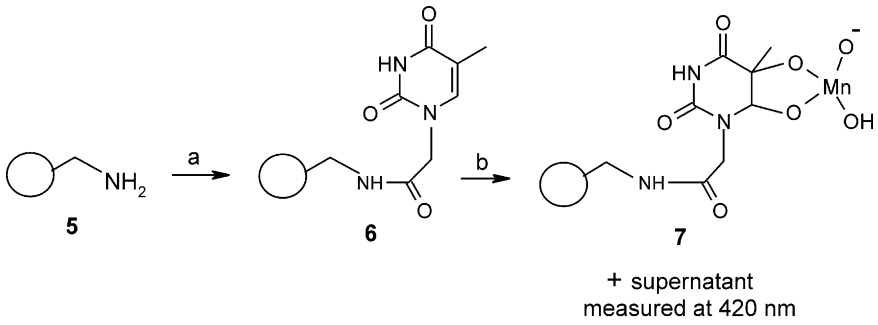

Scheme 2. Solid-phase oxidation of thymine acetic acid. (a) Thymine acetic acid/DIC/HOBt/50% DCM/DMF; (b) $KMnO_4$, 0.1 M TEAC in DCM.

Previously, we reported that the oxidation levels represented by the absorbance at 420 nm was highly correlated with the substrate concentrations ($KMnO_4$ and nucleotide bases).[9] The absobance wavelength was also used as a key signature for quantitative determination of the reaction rates and the oxidation levels when the comparative study was carried out with various different nucleotide bases and DNA.[10] For this purpose, our main objective was to establish the spectroscopic nature of the absorbance at 420 nm via the solid-phase oxidation reaction of thymine acetic acid. This nucleotide base was chosen for our convenient attachment to the solid supports (Scheme 2). Briefly, the commercially available aminomethyl-polystyrene resin **5** (100 mg, loading capacity of 120 μmol, 100–200 mesh, NOVABIOCHEM) was derivatised with thymine-acetic acid (22.1 mg, 120 μmol) using the standard coupling conditions [1-hydroxybenzotriazole, HOBt (20 mg, 148 μmol)/diisopropylcarbodiimide, DIC (18.6 mg, 148 μmol) in 10 mL of 50%, dimethyl formamide, DMF in dichloromethane, DCM]. After being washed, the resulting thymine-coupled resin **6** was then treated with $KMnO_4$ (20 μmol) in 10 mL of 0.1 M tetraethylammonium chloride (TEAC)/DCM solution. The reaction mixture was gently mixed and the supernatant was separated for repetitive scanning from 200 nm to 800 nm (for 60 min). The spectral data (Fig. 1) was obtained from the Cary-300 UV–visible spectrophotometer (Varian Inc.) indicated that the consumption of $KMnO_4$ was followed by a reduction of its absorbance in the UV–visible regions (at around 320 nm and 525 nm). However, $KMnO_4$ was almost transparent at the visible region of around 420 nm (the absorbance was unchanged around 420 nm).

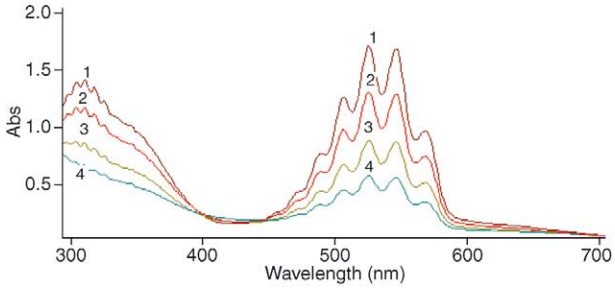

Figure 1. Solid-phase oxidation of resin coupled with thymine acetic acid **6**. Absorption spectrum of $KMnO_4$ in the supernatant: disappearance of permanganate ions at 2 min (scan 1), 10 min (scan 2), 20 min (scan 3) and 60 min (scan 4).

In the solution-phase experiments (Scheme 1), the permanganate ($KMnO_4$, 20 μmol) oxidation of free thymine acetic acid (25 μmol) in 10 mL of 0.1M TEAC/DCM was carried out (with and without the presence of the thymine-coupled resins) and 1 mL aliquot of each reaction mixture was transferred to a cuvette for UV–visible scanning from 200 to 800 nm. For both cases, the spectral data showed a dramatic increase of the absorbance at 420 nm. The former experiment in the presence of the thymine-coupled resins (i.e., both thymine acetic acid and thymine-coupled resins were present in the reaction mixture) indicated that $KMnO_4$ preferentially reacted with the free thymine acetic acid and the result is illustrated in Figure 2. The reaction mixtures of both solution-phase experiments developed strong yellow solutions and finally they became cloudy due to the precipitation of solid MnO_2 (after 2 h). Removal of the brown precipitates by centrifuge gave the colorless reaction mixtures, which showed completely transparent within the UV–visible region (curve 4 almost coincides with the base line, Fig. 2).

By comparing the two spectral data (Figs. 1 and 2), the rising of the absorbance at 420 nm in the solution-phase reaction was clearly assigned to the formation of the complex MnO_2 intermediate, which was not observed in the supernatant of the previous oxidation reaction on the solid-supports. In fact, the solid beads also turned strong yellow due to the formation of the complex on

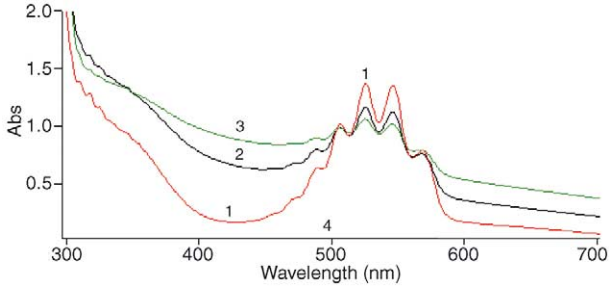

Figure 2. Solution-phase oxidation of soluble thymine acetic acid **1** ($R = CH_2COOH$) (both soluble thymine acetic acid and the thymine-coupled resins were used in the reaction mixture). The absorption spectrum of the intermediate cyclic complex in the supernatant: at 2 min (scan 1), 10 min (scan 2), 20 min (scan 3). Disappearance of permanganate ions due to the precipitate of insoluble MnO_2 at 120 min (scan 4). A similar spectral pattern was also observed in the experiment without the thymine-coupled resins.

the solid supports during the reaction. The result was consistent with previous observation of the 'relatively long-lived' manganese intermediate,[11] which was assumed to protect the oxidized products against further secondary oxidation by $KMnO_4$. In our study, this adduct survived in the reaction mixture for only a few hours and finally spontaneously decomposed to afford **3** and **4**.

Extraction of products derived from the oxidation reactions of thymine acetic acid (Scheme 1) gave a (ca. 9:1) mixture of the oxidized products **3** [Electronspray-mass spectroscopy, ES–MS: 254.9 $(M+K)^+$] and **4** {ES–MS: 256.9 $(M+K)^+$}. Further esterification of the carboxylic acids **3** & **4** (H^+/MeOH) confirmed the corresponding methyl esters by mass spectroscopy [ES–MS: 269.0 and 271.0 $(M+K)^+$].

This study has indicated that the absorbance change at 420 nm was derived from the formation of the manganese intermediate. It is also emphasized that only this wavelength is useful in quantification of the oxidation level, as the starting material $KMnO_4$ is almost transparent to the source light at this visible region (the absorbance regions outside this wavelength is interfered by both the starting material $KMnO_4$ and the intermediate ions). This study provides a direct spectroscopic evidence for the relationship between the organo-manganese species and the application wavelength at 420 nm.

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