Bioorganic Chemistry **30,** 133–137 (2002)
doi:10.1006/bioo.2002.1238, available online at http://www.idealibrary.com on IDEAL®

Comparative Study of Permanganate Oxidation Reactions of Nucleotide Bases by Spectroscopy

Chinh T. Bui[1] and Richard G. H. Cotton

Genomic Disorders Research Centre (the University of Melbourne), 7th Floor, Daly Wing, 41 Victoria Parade, Fitzroy, Melbourne, Victoria 3065, Australia

Received August 20, 2001

The permanganate oxidation of free nucleotide bases was successfully studied in aqueous solution of tetraethylammonium chloride using spectroscopic techniques. The reaction was highly selective toward thymine and uracil, less with cytosine, very little reaction on guanine, and no reaction on adenine.

Key Words: $KMnO_4$; oxidation reaction; nucleotide bases; spectroscopy.

INTRODUCTION

The permanganate oxidation of free nucleotides and its mutagenic activity toward DNA has been well established for many years (*1,2*). Chemical Cleavage Mismatched method (CCM) was developed on the basis of reactivity of mismatched bases to detect mutation within DNA molecules (*3*). The technique partly relies on the chemical modification of nucleotide bases with $KMnO_4$ and requires cleavage with piperidine. The resulting DNA fragments are subsequently resolved by gel-electrophoresis to analyze the mismatched bases (*4*). Our recent study indicated that the mismatched bases including thymine, cytosine, guanine and adenine responded differently towards permanganate ions (*5*). However, the reactivities of individual mismatched bases were not substantially elucidated at a molecular level as the reactions were complicated by multiple reactivities of $KMnO_4$ with other bases nearby to the mutation site. This question prompted us to study the oxidation reaction of $KMnO_4$ with free nucleotide bases using the five commercially available compounds: thymine, uracil, cytosine, guanine and adenine. Here, we report a new and simple assay via spectroscopy to monitor activities of permanganate ion species during the oxidation of the carbon–carbon double bond within the base molecules.

[1] To whom correspondence and reprint requests should be addressed. Fax: 61-3-9288-2989. E-mail: chinhbui@mail.medstv.unimelb.edu.au.

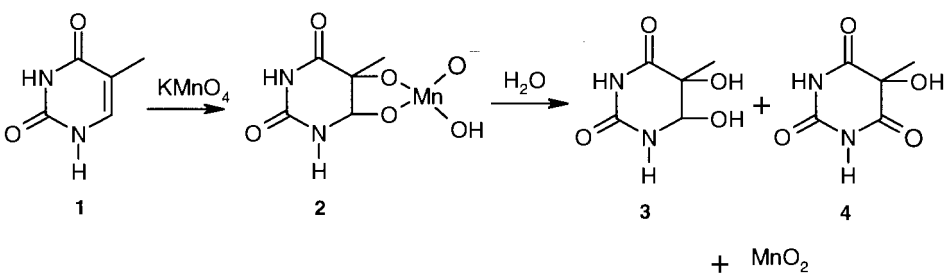
0045-2068/02 $35.00

MATERIALS AND METHODS

Chemicals and solvents were purchased from Aldrich Chemical Company (Castle Hill, Australia). Oxidation reactions of nucleotide bases with potassium permanganate were carried out in quartz 1.2-ml cuvette and the spectral data were obtained from Cintra-10 spectrophotometer (GBC Scientific Equipment Pty Ltd, Victoria, Australia) by recording the absorbance vs time curves at preselected wavelengths and/or by repetitive scanning of the ultraviolet-visible region (200 to 800 nm). ES–MS analysis: all oxidized nucleotide bases were analyzed by electrospray mass spectrometry using facility at the department of chemistry (University of Melbourne, Australia). Mass spectra were recorded on a Quattro II spectrometer and all samples were solubilized in methanol or 50% acetonitrile: H_2O.

Typical oxidation reaction with $KMnO_4$ for spectrophotometric analysis: 10 μl of $KMnO_4$ solution (0.158 mg, 1.0 μmol) and 20 μl of aq. thymine solution (0.21 mg thymine, 1.65 μmol) were sequentially added into 970 μl of 3 M tetraethylammonium chloride (TEAC) solution in a 1.2-ml cuvette. The reaction mixture was mixed and the absorbance was immediately measured at 420 and 525 nm at 25°C (every 5 min) over 50 min. Similar conditions were applied for uracil, cytosine and adenine: 20 μl of aq. uracil solution (0.185 mg, 1.65 μmol) or 20 μl of aq. cytosine solution (0.18 mg, 1.65 μmol) or 20 μl of aq. adenine solution (0.21 mg, 1.65 μmol). As guanine is poorly soluble in water, 160 μl of aq. guanine solution (0.25 mg, 1.65 μmol) was applied in this experiment. In the control experiments, permanganate oxidation reactions were carried out without reductants and no oxidation reactions were observed in all cases (Figs. 3 and 4).

The oxidation reactions were confirmed by disappearance of starting materials and formation of the oxidized products via mass spectrometric analysis. In one typical reaction, $KMnO_4$ (1.58 mg, 10 μmol) and thymine (1.26 mg thymine, 10 μmol) were incubated in 10 ml of 3 M tetraethylammonium chloride (TEAC) solution at 25°C. After 30 min, sodium bisulfite solution was added to the reaction mixture to terminate the oxidation. The reaction mixture was centrifuged and the supernatant was subjected to mass spectrometric analysis. Several protonated fragments including the ones associated with diol **3** and/or ketone **4** (Scheme 1) were observed: For thymine (*m*/*z*: 159.01, 161.13); cytosine (*m*/*z*: 144.20, 146.20) plus starting material (*m*/*z*: 112.10) and uracil (*m*/*z*: 145.07, 147.04). For the purpose of our research, all oxidized products were not isolated for further characterization.



SCHEME 1. Oxidation reaction of thymine with $KMnO_4$.