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An improvement in thymine specific chemical DNA sequencing

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Potassium permanganate ($KMnO_4$) can be employed as a thymine specific reagent in the Maxam Gilbert technique for chemically sequencing DNA (1). However, this procedure is susceptible to ambiguities, with particular thymines in denatured DNA failing to react significantly with the chemical at 23°C. To overcome this problem, double strand (ds) DNA can be reacted with $KMnO_4$ at 94°C. A comparison of these two procedures (Figure 1, 23°C **b**, and 94°C), demonstrates that by ensuring stacking interactions between the bases are minimal with high temperature, a relatively equal and unambiguous reactivity with $KMnO_4$ occurs at each thymine. This modification increases the value of $KMnO_4$ as a convenient thymine specific chemical reagent for plasmid DNA sequencing (1), and facilitates the generation of thymine specific marker ladders for experiments such as DNA footprinting. This procedure should also be useful in the various methods for chemical sequencing genomic DNA (2,3). Interestingly, when ds DNA is treated with 25 uM $KMnO_4$ (as in 23oC **a**) between 70-90°C, a dramatic increase in the modification of thymines can occur over 2-3°C (J.McC. and M. Athanassiou, unpublished data), suggesting that use of this probe may aid studies on the conformational changes associated with temperature induced DNA denaturation. **Methods** 5' labeled 285 bp HindIII-HaeII fragment (^{32}P at HindIII) from the lac region of pUC 18, was reacted in 100 uL 50 mM sodium cacodylate pH 7, and 2 mM EDTA. Reactions: (23°C **a**) preincubated at 23°C for 15 minutes, then reacted with 3 ul of 2.5 mM $KMnO_4$ for 2 minutes; (23°C **b**) heated to 94°C for four minutes, quick chilled on ice for 3 minutes and then preincubated at 23°C for 15 minutes. Denatured DNA was then reacted with 1 uL 2.5 mM $KMnO_4$ for two minutes at 23°C; (94°C) DNA preincubated at 94°C for 2 minutes, then reacted with 1 ul 2.5 mM $KMnO_4$ for two minutes at this temperature. Reactions terminated by ice cold mix of 3 ul beta-mercaptoethanol, 3 ul tRNA (2 ug/ul) and 294 ul of ethanol. Then 10 ul 3 M sodium acetate was added, and DNA precipitated at -70°C for one hour. DNA was resuspended in 200 ul H_2O on ice, and reprecipitated with 20 ul 3M sodium acetate and 500 ul ethanol. The pellet was washed once with 70% ethanol, dried, then resuspended in 40 ul 1 M piperidine, and heated to 90°C for 30 minutes (4). The piperidine was removed by butanol precipitations (5). DNA was then resuspended in 200 ul H_2O, precipitated, washed twice with 70% ethanol, dried, and resuspended in denaturing buffer for electrophoresis on an 8% DNA sequencing gel.

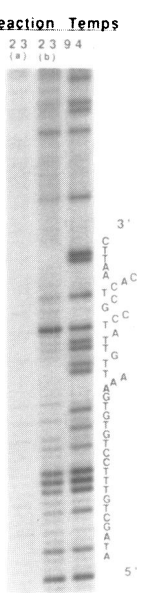

Figure 1.

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