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COMMUNICATION

Evidence for epoxidation by the chromium(VI)-glutathione system

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Abstract—Oxygen-dependent epoxidation and oxidative cleavage of *trans*-β-methylstyrene is shown to occur during reduction of chromium(VI) by glutathione. Oxygen-activated chromium(IV) or (V) intermediates are hence a possible pathway to the oxidative damage of DNA *in vitro*. © 1997 Elsevier Science Ltd

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Chromium(VI) is an established carcinogen [1]. *In vitro* and *in vivo* results indicate that lesions characteristic of oxidative damage can be caused by chromates [2]. *In vitro* it is known that the reduction of chromium is essential for the formation of lesions. Low-molecular weight cellular components such as glutathione (γ-glutamyl-cysteinyl-glycine, GSH) and ascorbic acid are believed to be among the important low-molecular weight compounds responsible for the reduction of chromate in biological systems [3]. A considerable number of papers have suggested that oxidative damage is mediated by Fenton or pseudo-Fenton chemistry [4] *in vivo* and systems such as chromate/GSH/hydrogen peroxide have been used to generate lesions such as strand breaks *in vitro*. However, it is now clear that chromate can generate strand breaks *in vitro* in the presence of molecular oxygen, but without added peroxide [5]. Chromium in oxidation states (IV) or (V) is known to be able to affect epoxidations [6]. In this communication we show, for the first time, that species generated during the reduction of chromium(VI) by GSH can epoxidate *trans*-β-methylstyrene. This simple model substrate has been used to probe the ability of the chromium(VI)/GSH system to generate peroxidic reactivity. The approach used is similar to that of Suga *et al.* [7] who studied the reduced iron(II)-bleomycin system which activated dioxygen and oxidized low-molecular weight alkenes.

Solid samples of the chromium(II), (III) or (V) compounds or a solution of the chromium(III) or (VI) compounds (1 mM) and a solution of GSH (1–50 mM) in dematallated phosphate buffer (0.1 M, pH 7.0) were added to a solution of *trans*-β-methylstyrene (97.5 mM) in methanol. This solution was stirred at room temperature for 2.5 h in the presence of air, or in an atmosphere of oxygen-free nitrogen. The products of the reaction were obtained by extractive work up [7] and were analysed by gas chromatography and were further identified using gas chromatography/mass spectroscopy. Corrections for differing response factors were made. For anaerobic experiments solutions and solvents were sparged with nitrogen for 45 min and were handled using standard syringe techniques before being stirred for 2.5 h under nitrogen. Sources of chromium in various oxidation states were: sodium dichromate(VI), sodium bis(2-ethyl-2-hydroxybutyrato) oxochromate (V) monohydrate [chromium (V)EHBA], chromium(III) chloride hexahydrate, chromium(III)-GSSG reaction products and chromium(II) acetate. Control experiments showed that little oxidation occurred in the absence of either chromium(VI) or GSH. Only the chromium(VI) experiments with added hydrogen peroxide (5 mM) showed significant substrate oxidation, 184% benzal-

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dehyde was formed (yields based on chromium) as hydrogen peroxide reduced chromium(VI). This system is known to cleave DNA [8], however, the level of hydrogen peroxide added was much higher than that likely to be found in cells and such a reaction is unlikely to be of any physiological significance.

In aerobic experiments with chromium(VI)/GSH *ca* 1.3–2.8% of the organic substrate was oxidized. The major products in all aerobic reactions were benzaldehyde and the *trans*-epoxide of β-methylstyrene (*trans*-β-methylstyrene oxides, Fig. 1). Some benzaldehyde was also formed in the absence of GSH, but yields in the presence of GSH were much greater (Fig. 2). The absence of any *cis*-epoxide product is probably due to the *trans* configuration being thermodynamically more stable than the *cis* and is not due to the reaction being regiospecific*. The presence of the epoxide suggests a pseudo-Fenton mechanism involving hydroxyl radicals is unlikely to be operating in this system. The amount of benzaldehyde formed seems to be related to the initial concentration of chromium(VI) (except in the case of the 1 : 1 reaction). However, the amount of *trans*-β-methylstyrene oxide formed seems to be dependent, in a non-linear fashion, on the initial GSH concentration. Several minor oxidation products were also formed in all experiments, the most significant of which was 7–14% of the alcohol $PhCHOHCH_2Me$.

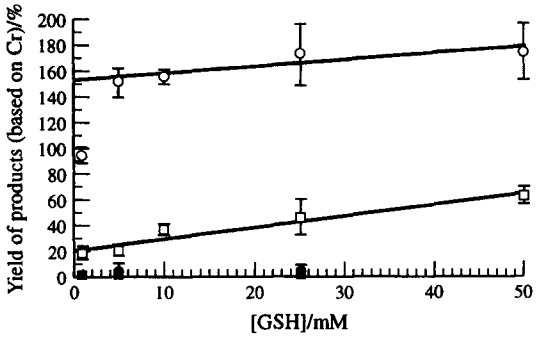


Fig. 1. The major oxidation products as measured by GC: (benzaldehyde; circles, *trans*-epoxide; squares) chromium(VI) (1 mM), GSH (1–50 mM) and *trans*-β-methylstyrene (97.5 mM) in methanol in the presence (open markers) and absence (filled markers) of dioxygen. Values are mean ± SD of 3–5 experiments.

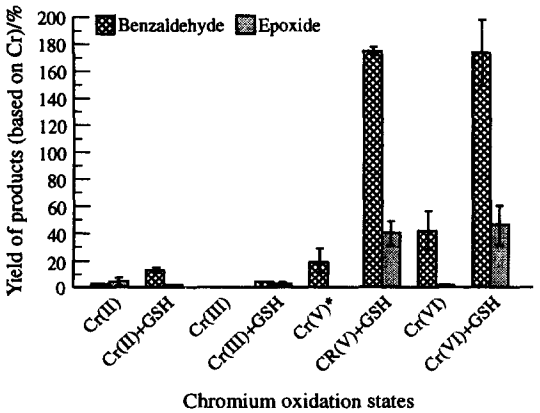


Fig. 2. The major oxidation products as measured by GC: with different chromium oxidation states (1 mM), with and without GSH (25 mM) and *trans*-β-methylstyrene (97.5 mM) in methanol in the presence of dioxygen (*150% yield of *trans*-stilbene based on chromium). Values are mean ± SD of 2–3 experiments.

In experiments using ascorbate (25 mM) and cysteine (25 mM) as the reducing agent the alcohol was the only product observed. The rapid nature of the chromium(VI)/ascorbate or cysteine reaction means any reactive chromium intermediates have only a short contact time with the substrate and little opportunity to react. The formation of oxidation products was shown to be dependent on dioxygen (Fig. 1). In the anaerobic systems substrate oxidation was almost completely inhibited, despite the complete reduction of the chromium(VI). *In vitro* the reaction of chromium(VI) and GSH in the presence of DNA leads to the formation of lesions such as strand breaks or apurinic/apyrimidinic sites [5] only in the presence of molecular oxygen. Studies using a variety of different chromium oxidation states confirm that high-oxidation chromium compounds reduced by GSH cause substrate oxidation (Fig. 2). The chromium(II) oxidation state has been suggested as being capable of causing DNA damage [9], however, in this system it did not epoxidize or cleave *trans*-β-methylstyrene.

Both the chromium(VI) and (V) compounds need to be reduced by GSH in order for the oxidation of *trans*-β-methystyrene to occur. These results suggest that a chromium(IV) reaction intermediate is involved in oxidizing the substrate. This intermediate is probably activated by dioxygen to form a hypervalent oxo or superoxo complex which epoxidizes/cleaves the substrate (Scheme 1). Recent indirect evidence that chromium(IV) is formed during the reduction of chromium(VI) by GSH [10] supports the idea that chromium(IV) is important in oxidative DNA damage. The reactivity of oxygen activated hypervalent chromium species towards the unsaturated carbon–carbon bond of *trans*-β-methystyrene gives an insight into oxidative DNA damage in the absence of added hydrogen peroxide. Although epoxidation of the unsaturated carbon–carbon bond is not a direct model for DNA damage this experiment shows that the chromium(VI)/GSH system is capable of generating a stable peroxo species. In biological systems this species could be hydrolysed to give free superoxide ion as required for Haber–Weiss/pseudo-Fenton chemistry

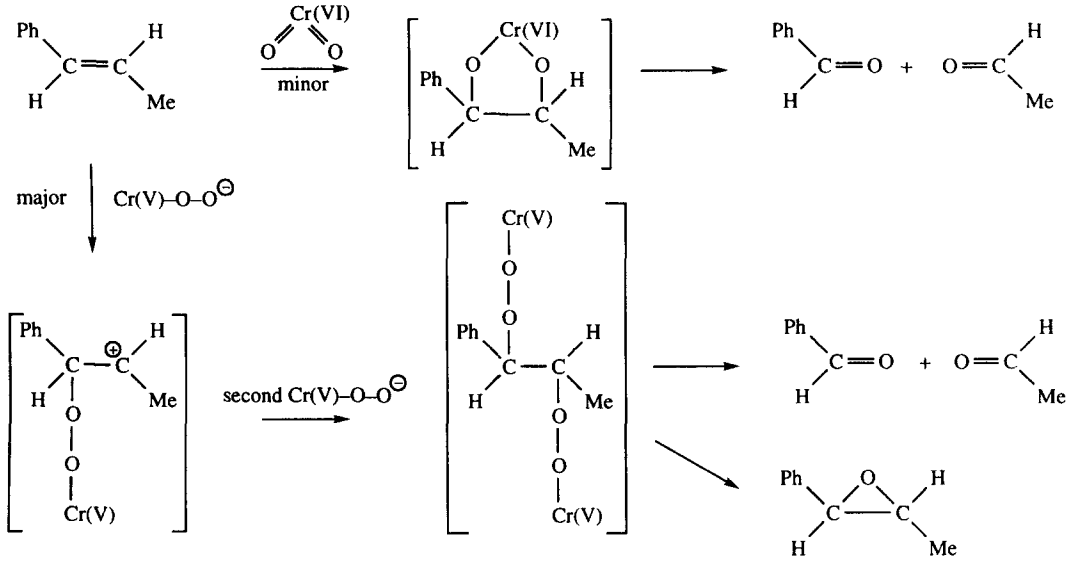
* Preliminary work on chromium(VI)/GSH with *cis*-B-methystyrene as the substrate shows that benzaldehyde and *trans*-β-methystyrene-oxide are the major products.

1. Formation of oxidising species:

$$Cr(VI) \xrightarrow{2GSH} Cr(IV)$$

$$Cr(IV) \xrightarrow{O_2} Cr(V) \simeq O/Cr(V)\text{–}O\text{–}O^{\ominus}$$

2. Mechanism of substrate oxidation:



Scheme. 1.

[11]. This leads to the generation of hydrogen peroxide:

$$2O_2^{-} + 2H^{+} \rightarrow H_2O + O_2$$

which may explain the observed damage to DNA. The mechanism of attack on DNA is unknown, but a likely damage site is on the sugar moiety, perhaps at the C4′ position as for the antitumor antibiotic neocarstatin [5]. Hydrogen abstraction at this site leads to both strand breaks and 4′-hydroxylated apurinic/apyrimidinic sites. Hydroxyl radicals generated from a chromium(V)-superoxo complex would be capable of causing this type of lesion, but there is no need to invoke the reaction of indigenous peroxides with chromium to explain this chemistry.

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