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Bleomycin-Dependent Damage to the Bases in DNA Is a Minor Side Reaction†

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ABSTRACT: The antitumor antibiotic bleomycin degrades DNA in the presence of ferric ions and H_2O_2 or in the presence of ferric ions, oxygen, and ascorbic acid. When DNA degradation is measured as formation of base propenals by the thiobarbituric acid assay, it is not inhibited by superoxide dismutase and scavengers of the hydroxyl radical or by catalase (except that catalase inhibits in the bleomycin/ferric ion/H_2O_2 system by removing H_2O_2). Using the technique of gas chromatography/mass spectrometry with selected-ion monitoring, we show that DNA degradation is accompanied by formation of small amounts of modified DNA bases. The products formed are identical with those generated when hydroxyl radicals react with DNA bases. Base modification is significantly inhibited by catalase and partially inhibited by scavengers of the hydroxyl radical and by superoxide dismutase. We suggest that the bleomycin–oxo–iron ion complex that cleaves the DNA to form base propenals can decompose in a minor side reaction to generate hydroxyl radical, which accounts for the base modification in DNA. However, hydroxyl radical makes no detectable contribution to the base propenal formation.

The bleomycins are a group of glycopeptide antitumor antibiotics that are used effectively in the treatment of several human cancers (Umezawa, 1978). Two features of the bleomycin molecule are related to its biological action: an ability to bind to DNA (largely determined by the bithiazole and terminal amine residues of the molecule) and an ability to bind metal ions (determined by the β-aminoalanine–pyrimidine–β-hydroxyhistidine part) (Umezawa, 1978; Dabrowiak, 1980; Burger et al., 1981). When ferrous ions are complexed to bleomycin in the presence of oxygen, the complex can cleave

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DNA strands, largely at G-C (5′ → 3′) and G-T (5′ → 3′) sequences, and this iron ion dependent DNA cleavage is thought to be the mechanism by which bleomycin exerts its antitumor effect (Sausville et al., 1976, 1978; Umezawa, 1978; Dabrowiak, 1980; Burger et al., 1981; Hecht, 1986; Ciriolo et al., 1989). DNA degradation by a bleomycin–Fe^{3+} complex can be brought about either by addition of a reducing agent, such as ascorbic acid, in the presence of oxygen or by addition of H_2O_2 (Burger et al., 1981; Gutteridge et al., 1985). DNA cleavage by bleomycin releases some free bases but also produces base propenals in amounts that are stoichiometric with strand cleavage (Burger et al., 1981, 1982; Giloni et al., 1981). When heated with thiobarbituric acid (TBA)[1] at low pH, base propenals rapidly decompose to give malondialdehyde (MDA), which combines with the TBA to form a pink $(TBA)_2MDA$ adduct (Gutteridge, 1979; Giloni et al., 1981; Gutteridge et al., 1985). Thus the TBA test is an accurate and sensitive measure of DNA strand cleavage by bleomycin and has been widely employed (Gutteridge, 1979; Giloni et al., 1981; Gutteridge et al., 1985). For example, a "bleomycin method" has been developed to measure non-transferrin-bound iron ions in human body fluids (Gutteridge & Hou, 1986; Gutteridge & Halliwell, 1987; Aruoma et al., 1988).

The mechanism of DNA degradation by iron ion–bleomycin complexes has not been fully elucidated [for a review, see Petering et al. (1990)]. Aerobic incubation of bleomycin with Fe^{2+}, or Fe^{3+} in the presence of a reducing system, has been suggested to produce highly reactive hydroxyl radical (•OH), as detected by a variety of assay procedures including spin trapping (Oberley & Buettner, 1979; Gutteridge et al., 1985; Mahmutoglu et al., 1987). However, DNA degradation by bleomycin/Fe^{3+} in the presence of oxygen and ascorbic acid is not inhibited by catalase, scavengers of •OH, or superoxide dismutase (SOD) (Sausville et al., 1976, 1978; Burger et al., 1981; Gutteridge & Shute, 1981; Rodriguez & Hecht, 1982; Gutteridge et al., 1985; Gutteridge & Halliwell, 1987; Petering et al., 1990). It is now widely believed that the DNA-cleaving species is an oxo–iron complex of bleomycin, such as bleomycin–Fe^{3+}–O_2H^- (Sugiura et al., 1981; Pratviel et al., 1989; Petering et al., 1990).

In recent papers, it has been shown that treatment of DNA with bleomycin/iron ions leads not only to cleavage of the DNA backbone but also to the formation of 8-hydroxyguanine (8-OH-Gua) residues in the DNA (Kohda et al., 1989; Gutteridge et al., 1990). 8-Hydroxyguanine is frequently used as a "marker" of •OH-induced damage to DNA (Kasai et al., 1986, 1989), since a sensitive HPLC-based assay has been developed to measure it (Floyd et al., 1986). However, •OH also produces a wide range of other modifications of the DNA bases, and it is the overall pattern of base modification rather than formation of any one product that appears diagnostic for •OH (Dizdaroglu, 1985; Aruoma et al., 1989a,b, 1991; Dizdaroglu & Gajewski, 1990; Halliwell & Aruoma, 1991). Indeed, measurement of these base modifications has already been used to show that DNA damage by a Cu^{2+}–phenanthroline complex in the presence of reducing agents involves •OH (Dizdaroglu et al., 1990). Bases modified in DNA by •OH attack can be accurately identified and quantitated, after hydrolysis of DNA followed by derivatization, by gas chromatography/mass spectrometry with selected-ion monitoring (GC/MS-SIM) (Dizdaroglu, 1985; Fuciarelli et al., 1989; Aruoma et al., 1989a; Dizdaroglu & Gajewski, 1990). In the present work, we have used this technique to investigate in detail the base modification taking place when DNA is cleaved by mixtures of bleomycin, Fe^{3+}, and ascorbic acid or mixtures of bleomycin, Fe^{3+}, and H_2O_2.

Table I: Degradation of DNA by a Bleomycin (BLM)/Fe^{3+} Complex in the Presence of Ascorbic Acid (asc) and Oxygen or of H_2O_2

reaction mixture	MDA equiv (nmol/mg of DNA)
DNA only	0
DNA, $FeCl_3$	0
DNA, asc	0
DNA, BLM/Fe^{3+}/asc	33
DNA, BLM/Fe^{3+}/asc/mannitol (100 mM)	31
DNA, BLM/Fe^{3+}/asc/DMSO (100 mM)	33
DNA, BLM/Fe^{3+}/asc/SOD (600 units/mL)[a]	31
DNA, BLM/Fe^{3+}/asc/catalase (4125 units/mL)[b]	36
DNA, BLM/Fe^{3+}/asc/albumin (1 mg/mL)	32
DNA, H_2O_2	0
DNA, BLM/Fe^{3+}/H_2O_2	41
DNA, BLM/Fe^{3+}/H_2O_2/mannitol (100 mM)	45
DNA, BLM/Fe^{3+}/H_2O_2/DMSO (100 mM)	45
DNA, BLM/Fe^{3+}/H_2O_2/SOD (600 units/mL)	42
DNA, BLM/Fe^{3+}/H_2O_2/catalase (4125 units/mL)	2
DNA, BLM/Fe^{3+}/H_2O_2/albumin (1 mg/mL)	45

[a] Units of SOD are defined as in McCord and Fridovich (1969). [b] Units of catalase are μmol of H_2O_2 decomposed/min under the reaction conditions defined by Sigma Chemical Co.

Experimental Procedures

All reagents, including bleomycin, were from Sigma Chemical Co.[2] Superoxide dismutase (SOD) was the bovine copper- and zinc-containing enzyme. Catalase was type C40 (thymol-free). Reaction mixtures contained, in a final volume of 2 mL, the following reagents at the final concentrations stated: 10 mM sodium phosphate buffer (pH 7.4), 66 μM bleomycin, 54 μM $FeCl_3$, 4 mM $MgCl_2$, 7.5 mM KCl, 0.42 mg/mL DNA, and (where indicated) 200 μM ascorbic acid or 2.8 mM H_2O_2. Bleomycin concentration was determined by measurement of absorbance at 291 nm ($\epsilon_{291} = 1.7 \times 10^4$ M^{-1} cm^{-1}) (Ciriolo et al., 1989). In all cases, $FeCl_3$ and an excess of bleomycin were premixed to allow complex formation before addition to the reaction mixture. Reactions were started by adding ascorbic acid or H_2O_2. Mixtures were incubated for 1 h at 37 °C. For measurement of DNA base damage, reaction mixtures were placed in an ice bath immediately after incubation and then dialyzed against water at 4 °C. Aliquots of dialyzed samples were analyzed by GC/MS-SIM for base products in DNA, as described in previous papers (Dizdaroglu, 1985; Fuciarelli et al., 1989; Aruoma et al., 1989a; Dizdaroglu & Gajewski, 1990). For assays of base propenal formation, 100 μL of 0.1 M EDTA was added after incubation to reaction mixtures to stop the reaction. Base propenal measurement

[1] Abbreviations: TBA, thiobarbituric acid; MDA, malondialdehyde; BLM, bleomycin; O_2^-, superoxide radical; •OH, hydroxyl radical; GC/MS-SIM, gas chromatography/mass spectrometry with selected-ion monitoring; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; 5-OH-Hyd, 5-hydroxyhydantoin; 5-OHMeUra, 5-(hydroxymethyl)uracil; 5,6-diOH-Cyt, 5,6-dihydroxycytosine; FapyAde, 4,6-diamino-5-formamidopyrimidine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 8-OH-Ade, 8-hydroxyadenine; 8-OH-Gua, 8-hydroxyguanine; SOD, copper–zinc superoxide dismutase; asc, ascorbic acid; DMSO, dimethyl sulfoxide.

[2] Certain commercial equipment or materials are identified in this paper in order to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table II: Yields of Base Products (nmol/mg of DNA[a]) Formed in DNA by Treatment with the BLM/asc/Fe^{3+} System

	treatment					
product	1[b]	2	3	4	5	6
5-OH-5-MeHyd	0.082 ± 0.005	0.112 ± 0.006	0.099 ± 0.019	0.114 ± 0.008	0.114 ± 0.014	0.118 ± 0.012
5-OH-Hyd	0.028 ± 0.006	0.042 ± 0.001	0.032 ± 0.003	0.034 ± 0.008	0.106 ± 0.013	0.043 ± 0.006
Cyt glycol	0.204 ± 0.025	0.430 ± 0.055	0.428 ± 0.148	0.410 ± 0.070	0.388 ± 0.021	0.325 ± 0.027
5-OHMeUra	0.018 ± 0.007	0.030 ± 0.005	0.026 ± 0.002	0.040 ± 0.006	0.035 ± 0.005	0.033 ± 0.004
Thy glycol	0.188 ± 0.019	0.335 ± 0.051	0.243 ± 0.031	0.265 ± 0.079	0.252 ± 0.034	0.305 ± 0.067
5,6-diOH-Cyt	0.007 ± 0.001	0.024 ± 0.001	0.016 ± 0.002	0.020 ± 0.004	0.029 ± 0.004	0.016 ± 0.002
FapyAde	0.113 ± 0.004	0.212 ± 0.056	0.226 ± 0.037	0.226 ± 0.014	0.356 ± 0.021	0.191 ± 0.014
8-OH-Ade	0.517 ± 0.122	0.443 ± 0.107	0.510 ± 0.136	0.463 ± 0.065	0.424 ± 0.050	0.397 ± 0.035
FapyGua	0.171 ± 0.02	0.459 ± 0.016	0.311 ± 0.062	0.301 ± 0.067	0.379 ± 0.087	0.243 ± 0.078
8-OH-Gua	1.087 ± 0.247	1.885 ± 0.047	1.449 ± 0.066	1.307 ± 0.354	1.340 ± 0.257	0.732 ± 0.122
total	2.415 ± 0.459	3.972 ± 0.345	3.240 ± 0.051	3.180 ± 0.675	3.423 ± 0.506	2.403 ± 0.367

[a] All values represent the mean ± standard deviation from triplicate measurements. [b] 1, DNA; 2, DNA/BLM/asc/Fe^{3+}; 3, DNA/BLM/asc/Fe^{3+}/mannitol (50 mM); 4, DNA/BLM/asc/Fe^{3+}/DMSO (50 mM); 5, DNA/BLM/asc/Fe^{3+}/SOD (600 units/mL); 6, DNA/BLM/asc/Fe^{3+}/catalase (1100 units/mL).

Table III: Yields of Base Products (nmol/mg of DNA[a]) Formed in DNA by Treatment with the BLM/H_2O_2/Fe^{3+} System

	treatment					
product	1[b]	2	3	4	5	6
5-OH-5-MeHyd	0.082 ± 0.005	0.059 ± 0.005	0.079 ± 0.020	0.094 ± 0.013	0.105 ± 0.002	0.504 ± 0.015
5-OH-Hyd	0.028 ± 0.006	0.026 ± 0.010	0.017 ± 0.002	0.031 ± 0.014	0.034 ± 0.001	0.222 ± 0.010
Cyt glycol	0.204 ± 0.025	0.268 ± 0.042	0.308 ± 0.067	0.279 ± 0.113	0.413 ± 0.226	1.439 ± 0.204
5-OHMeUra	0.018 ± 0.007	0.021 ± 0.010	0.020 ± 0.004	0.034 ± 0.003	0.041 ± 0.023	0.070 ± 0.010
Thy glycol	0.188 ± 0.019	0.144 ± 0.022	0.115 ± 0.020	0.126 ± 0.017	0.183 ± 0.004	1.023 ± 0.060
5,6-diOH-Cyt	0.007 ± 0.001	0.010 ± 0.003	0.011 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.251 ± 0.037
FapyAde	0.113 ± 0.004	0.110 ± 0.030	0.103 ± 0.018	0.089 ± 0.004	0.099 ± 0.001	1.018 ± 0.056
8-OH-Ade	0.517 ± 0.122	0.583 ± 0.067	0.523 ± 0.121	0.472 ± 0.081	0.626 ± 0.176	2.024 ± 0.096
FapyGua	0.171 ± 0.023	0.174 ± 0.021	0.101 ± 0.020	0.109 ± 0.020	0.073 ± 0.010	0.483 ± 0.019
8-OH-Gua	1.087 ± 0.247	1.211 ± 0.068	1.007 ± 0.034	1.303 ± 0.588	1.024 ± 0.113	7.992 ± 0.385
total	2.415 ± 0.459	2.606 ± 0.278	2.284 ± 0.308	2.552 ± 0.865	2.607 ± 0.558	15.026 ± 0.892

[a] All values represent the mean ± standard deviation from triplicate measurements. [b] 1, DNA; 2, DNA/BLM; 3, DNA/H_2O_2; 4, DNA/Fe^{3+}; 5, DNA/BLM/Fe^{3+}; 6, DNA/BLM/H_2O_2/Fe^{3+}.

was performed by the TBA test (Gutteridge, 1979) by adding 1 mL of 1% (w/v) TBA in 0.05 M NaOH and 1 mL of 25% (v/v) HCl. The mixtures were then heated at 100 °C for 15 min. The chromogen was extracted into butan-1-ol, and the absorbance of the upper (organic) phase was measured at 532 nm. Results were converted into malondialdehyde equivalents by using a calibration curve with tetraethoxypropane as a standard.

Results

Aerobic incubation of DNA with bleomycin/Fe^{3+}/ascorbic acid, under our experimental conditions, produced extensive DNA degradation to give base propenals, as measured by the TBA test (Table I). In agreement with previous results [for reviews, see Gutteridge and Halliwell (1987) and Petering et al. (1990)], there was no inhibition of the formation of TBA-reactive material by SOD, catalase, or the •OH scavengers mannitol or dimethyl sulfoxide (DMSO). It seems that free •OH, O_2^-, or H_2O_2 made no significant contribution to strand cleavage. Extensive DNA degradation also occurred in reaction mixtures containing bleomycin/Fe^{3+} and H_2O_2. Again, SOD and •OH scavengers had absolutely no inhibitory effect. Catalase inhibited formation of the TBA-reactive material by removing H_2O_2, but albumin, added as a protein control, did not (Table I).

DNA from reaction mixtures incubated under identical conditions was analyzed for chemical changes in the pyrimidine and purine bases. The commercial DNA used already contained some base products (Table II), as noted previously (Aruoma et al., 1989a,b). The structures of the products measured are given elsewhere (Dizdaroglu et al., 1990). Incubation of the DNA in reaction mixtures containing bleomycin alone, H_2O_2 alone, ascorbic acid alone, $FeCl_3$ alone, $FeCl_3$ and bleomycin, or $FeCl_3$ and H_2O_2 produced no significant increase in the amounts of modified bases detected. Tables II and III show results from some representative control experiments. However, the bleomycin/Fe^{3+}/ascorbic acid system gave reproducible increases in the amounts of modified bases present (Table II), although an increase of only approximately 65% over the background amounts was observed. For example, in the experiment given in Table II, approximately 1.5 nmol of modified bases/mg of DNA was detected in total, after allowing for the background level of such bases in the DNA, although the DNA degradation was much higher as assessed from the amount of the TBA-reactive material detected (33 nmol/mg of DNA) (Table I). When the concentration of bleomycin was increased (up to 264 μM), the amounts of base modification detected were not significantly different from those given in Table II. The formation of base products was inhibited by inclusion of catalase in the reaction mixtures (Table II). The •OH scavengers mannitol and DMSO also appeared to diminish the base damage slightly (Table II), and there was a suggestion that SOD had a small inhibitory effect. It should be emphasized that, apart from the effects of catalase, the results obtained with SOD and •OH scavengers are tentative because of the very small amount of base modification that occurred.

Incubation of DNA with bleomycin/Fe^{3+} and H_2O_2 also produced base modification, this time in significantly larger amounts (Table III), although again the amount of base modification (approximately 15 nmol/mg of DNA) was smaller than that of base propenal formation (45 nmol/mg of DNA) (Table I). Catalase (1100 units/mL, approximately 0.46 nM) almost completely inhibited the base modification,

Table IV: Yields of Base Products (nmol/mg of DNA[a]) Formed in DNA by Treatment with the BLM/H_2O_2/Fe^{3+} System

product	treatment					
	6[b]	7	8	9	10	11
5-OH-5-MeHyd	0.504 ± 0.015	0.344 ± 0.050	0.312 ± 0.020	0.343 ± 0.065	0.130 ± 0.065	0.347 ± 0.078
5-OH-Hyd	0.222 ± 0.010	0.109 ± 0.007	0.089 ± 0.011	0.184 ± 0.032	0.045 ± 0.003	0.233 ± 0.036
Cyt glycol	1.439 ± 0.204	0.961 ± 0.134	0.744 ± 0.140	0.881 ± 0.094	0.269 ± 0.026	0.934 ± 0.148
5-OHMeUra	0.070 ± 0.010	0.051 ± 0.006	0.048 ± 0.013	0.050 ± 0.013	0.024 ± 0.001	0.056 ± 0.008
Thy glycol	1.023 ± 0.060	0.381 ± 0.045	0.322 ± 0.057	0.472 ± 0.139	0.176 ± 0.023	0.605 ± 0.166
5,6-diOH-Cyt	0.251 ± 0.037	0.079 ± 0.008	0.054 ± 0.009	0.175 ± 0.032	0.020 ± 0.001	0.180 ● 0.056
FapyAde	1.018 ± 0.056	0.461 ± 0.058	0.291 ± 0.031	0.612 ± 0.042	0.153 ± 0.013	0.824 ± 0.105
8-OH-Ade	2.024 ± 0.096	1.207 ± 0.081	1.198 ± 0.065	1.258 ± 0.045	0.507 ± 0.034	1.453 ± 0.070
FapyGua	0.483 ± 0.019	0.301 ± 0.067	0.209 ± 0.052	0.270 ± 0.054	0.153 ● 0.025	0.273 ± 0.090
8-OH-Gua	7.992 ± 0.385	4.526 ● 0.231	3.908 ± 0.258	4.467 ± 0.395	1.377 ± 0.233	5.579 ± 1.033
total	15.026 ± 0.892	8.420 ± 0.687	7.175 ± 0.656	8.712 ± 0.911	2.854 ± 0.381	10.484 ± 1.790

[a] All values represent the mean ± standard deviation from triplicate measurements. [b] 6, DNA/BLM/H_2O_2/Fe^{3+}; 7, DNA/BLM/H_2O_2/Fe^{3+}/mannitol (50 mM); 8, DNA/BLM/H_2O_2/Fe^{3+}/DMSO (50 mM); 9, DNA/BLM/H_2O_2/Fe^{3+}/SOD (600 units/mL); 10, DNA/BLM/H_2O_2/Fe^{3+}/catalase (1100 units/mL); 11, DNA/BLM/H_2O_2/Fe^{3+}/albumin (0.5 mg/mL).

whereas albumin, added as a protein control at a greater molar concentration (16.7 nM), had a small inhibitory effect (Table IV). The reason for this is not known. Because of the greater amount of base modification in the bleomycin/Fe^{3+}/H_2O_2 system, it was possible to demonstrate convincingly the existence of a partial inhibitory effect of the •OH scavengers mannitol and DMSO (Table IV). Partial inhibition of product formation by SOD (600 units/mL, 6.25 nM) was also observed.

Discussion

The bleomycin/Fe^{3+}/ascorbic acid and bleomycin/Fe^{3+}/H_2O_2 systems cleaved DNA very extensively under our experimental conditions, as shown by base propenal formation. By comparison, modification of the bases in DNA was very limited, although slightly greater in the bleomycin/Fe^{3+}/H_2O_2 system. In agreement with Kohda et al. (1989) and Gutteridge et al. (1990), there was an increase in the amount of 8-OH-Gua, but increases in the amounts of many other products were also detected. Base products measured in the present work are typical products arising from reaction of •OH with the DNA bases [for reviews, see Teoule and Cadet (1978), von Sonntag (1987), and Dizdaroglu (1991)]. This pattern of products suggests that the small base modification in DNA by the bleomycin/Fe^{3+}/ascorbic acid and bleomycin/Fe^{3+}/H_2O_2 systems is caused by •OH derived from H_2O_2. Indeed, partial inhibition by •OH scavengers and almost complete inhibition by catalase are consistent with this view. The small inhibition by SOD suggests that O_2^- may play some role in the bleomycin/Fe^{3+}/H_2O_2 system. However, •OH scavengers, SOD, and catalase (except in the bleomycin/Fe^{3+}/H_2O_2 system) had no detectable inhibitory effect on base propenal formation; i.e., •OH does not make a significant contribution to DNA strand breakage. We therefore suggest that base propenal formation by bleomycin is not caused by attack of •OH but may be brought about by an oxo-iron complex, in agreement with previous proposals (Sugiura et al., 1981; Rodriguez & Hecht, 1982; Hecht, 1986; Pratviel et al., 1989). We suggest that this oxo-iron complex might decompose in a minor side reaction to give •OH. It might also produce O_2^- and H_2O_2, which could form •OH by Fenton-type reactions in the presence of iron ions bound to bleomycin. Formation of •OH in a minor side reaction would explain why base modification is so small and could account for previous reports claiming that •OH is produced in bleomycin/iron ion systems (Gutteridge et al., 1985; Oberley & Buettner, 1979; Mahmutoglu et al., 1987) and that 8-OH-Gua is produced in DNA exposed to such systems (Kohda et al., 1989; Gutteridge et al., 1990).

It is interesting to compare the DNA damage in the bleomycin system with that mediated by Cu^{2+}-phenanthroline complexes in the presence of reducing agents (Sigman, 1986). The latter system produces much more extensive damage to the bases in DNA, and the pattern of products formed is characteristic of attack by •OH (Dizdaroglu et al., 1990). Indeed, the DNA strand scission can occur at sites slightly removed from the binding site of the Cu^{2+}-phenanthroline complex, suggesting that it is mediated by a species that can diffuse over a very limited range (Williams et al., 1988), i.e., •OH. By contrast, cleavage by the bleomycin system occurs exactly at the binding site (Williams et al., 1988), consistent with our conclusion that it is not mediated by •OH and that •OH generation and subsequent formation of modified bases are minor side reactions.

Our data raise two other points. First, measurement of 8-OH-Gua or other base products would not be a good index of damage to DNA by bleomycin in vivo, since so little base modification actually takes place in relation to the amount of DNA strand breakage achieved. Second, there have been frequent proposals in the literature that the reactive species produced in Fenton-type systems is not •OH but some form of iron-oxo species [for a review, see Halliwell and Gutteridge (1990)]. Yet the bleomycin oxo-iron complex apparently failed to modify the DNA bases. The results obtained in the present work support our previous proposals that measuring the pattern of DNA base modification is an excellent method of identifying the involvement of •OH in biochemical systems (Aruoma et al., 1989a,b, 1991; Dizdaroglu et al., 1990; Halliwell & Aruoma, 1991).

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Interaction of the tRNA^{Phe} Acceptor End with the Synthetase Involves a Sequence Common to Yeast and *Escherichia coli* Phenylalanyl-tRNA Synthetases†

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ABSTRACT: Modified lysines resulting from the cross-linking of the 3′ end of tRNA^{Phe} to yeast phenylalanyl-tRNA synthetase (an enzyme with an $\alpha_2\beta_2$ structure) have been characterized by sequencing the labeled chymotryptic peptides that were isolated by means of gel filtration and reversed-phase chromatography. The analysis showed that Lys131 and Lys436 in the α subunit are the target sites of periodate-oxidized tRNA^{Phe}. Mutant protein with a Lys → Asn substitution established that each lysine contributes to the binding of the tRNA but is not essential for catalysis. The major labeled lysine (K131) belongs to the sequence IALQDKL (residues 126-132), which shares three identities with the peptide sequence ADKL found around the tRNAox-labeled Lys61 in the large subunit of *Escherichia coli* phenylalanyl-tRNA synthetase [Hountondji, C., Schmitter, J. M., Beauvallet, C., & Blanquet, S. (1987) *Biochemistry 26*, 5433-5439].

Periodate-oxidized tRNA (tRNAox)[1] has been used as a specific affinity label in a number of aminoacyl-tRNA synthetases to probe the lysine residues that interact with the CCA end of tRNA (Baltzinger et al., 1979; Fayat et al., 1979; Hountondji et al., 1979, 1985, 1986a, 1987; Hill & Schimmel, 1989). Covalent cross-linking of tRNA proceeds via the formation of a Schiff base between the 2′- or 3′-aldehyde group

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[1] Abbreviations: PheRS, phenylalanyl-tRNA synthetase; SDS, sodium dodecyl sulfate; tRNAox, periodate-oxidized tRNA; HPLC, high-performance liquid chromatography.

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