Superoxide Dependence of the Toxicity of Short Chain Sugars*

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Erythrose inhibited the growth of a sodA sodB strain of Escherichia coli under aerobiciosis; but did not inhibit anaerobic growth of the sodA sodB strain, or the aerobic growth of the superoxide dismutase (SOD)-competent parental strain. A SOD mimic protected the sodA sodB strain against the toxicity of erythrose as did the carbonyl-blocking reagents hydrazine and aminoguanidine. Three carbon sugars, such as glyceraldehyde and dihydroxy acetone, and the two carbon sugar glycolaldehyde, were similarly toxic in an O2-sensitive manner. An unidentified dialyzable component in E. coli extract augmented the oxidation of short chain sugars, and this was partially inhibitable by SOD. The toxicity of the short chain sugars appears to be because of an O2-dependent oxidation to α,β-dicarbonyl compounds. In keeping with this view was the O2-independent toxicity of methylglyoxal.

We have previously noted that α-hydroxy carbonyl compounds can autoxidize with the production of O2 (1). Cyanide, and to a lesser extent other nucleophiles, strongly accelerated this autoxidation, and the augmenting effect of preincubation at elevated pH indicated the involvement of an endiolenic intermediate. This cyanide-catalyzed oxidation had been seen earlier with methylglyoxal (2, 3). Oxidation of α-hydroxy carbonyl compounds was subsequently seen to proceed as a chain reaction in which O2 could serve as both an initiator and a propagator (4). The autoxidation of such compounds have been studied by others, who also noted O2 production (5, 6) and the role of enolization (5). α-Amino carbonyl compounds appear to behave in a similar way (7–9). The mutagenicity of α-hydroxy carbonyl compounds in Salmonella typhimurium was attributed to their ease of autoxidation with attendant oxy radical production (10).

During an attempt to use erythrose as a carbon source for the growth of a sodA sodB strain of Escherichia coli, we noted an oxygen-dependent toxicity and set out to explore its mechanism. The data reported herein indicate a role for O2 in the toxicity of erythrose and shorter chain sugars. These results are relevant to the much slower process of nonenzymic glyca- tion seen with long chain sugars, such as glucose, which exist primarily as internal hemiacetals and are therefore less reactive (11–15).

RESULTS

Erythrose Toxicity Is Dependent on O2—Erythrose dose-dependently inhibited the growth of a sodA sodB strain of E. coli, as shown in Fig. 1 by lines 3, 2, and 1. The SOD-competent parental strain was much less affected (lines 6, 5, and 4). This suggested that an oxidation of erythrose initiated by, and/or producing, O2 was a factor in this toxicity. In that case the toxicity of erythrose should be markedly diminished under anaerobic conditions. The results in Fig. 2 demonstrate that this was the case. The slight growth inhibition by erythrose shown in Fig. 2 was certainly because of residual oxygen, because the cultures were exposed to air periodically when the Gas Pack jars were opened and A600 nm was measured. As a final demonstration of the importance of O2 in the toxicity of erythrose, the effect of a cell permeable mimic of SOD activity (MnTM-2-PyP) (19) was examined. The results in Fig. 3 show that this compound at 25 μM completely eliminated the growth inhibitory effect of 20 mM erythrose.

Three- and Two-carbon Sugars—Erythrose can ring close to a furanose form, but sugars shorter than four carbons cannot. Such sugars have previously been seen to prone to enolization and autoxidization (1–5). One would expect, therefore, that sugars containing two- or three-carbon atoms should equal or exceed the toxicity of erythrose and that their toxicities should be O2 and O2 augmented. Glyceraldehyde, dihydroxy acetone, and glycolaldehyde were all examined and all were more toxic to the sodA sodB than to the parental strain under aerobic conditions. Further, the toxicity of these compounds to the sodA sodB strain was O2-dependent and was eliminated by the SOD mimic MnTM-2-PyP (19). Thus Fig. 4 shows the growth inhibitory effect of 2.0 mM glyceraldehyde and the elimination of that growth inhibition by 25 μM MnTM-2-PyP. A control compound, ZnTM-2-PyP, which does not catalyze the dismuta- tion of O2, was without effect (not shown). It must be noted that ZnTM-2-PyP was tested in the dark. This was necessary because the zinc compound, unlike the manganese compound, exerted a photodynamic effect, which caused lethality in the light.

MATERIALS AND METHODS

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The abbreviations used are: SOD, superoxide dismutase; MnTM-2-PyP, manganese (III) mesotetraakis(N-methylpyridinium-2-yl)porphyrin.

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Protective Effect of Hydrazines—Tautomerization to the enediolate has been shown to precede oxidation of short chain sugars by O₂ or by O₂⁻ (1). In that case, blocking the carbonyl group, through formation of hydrazides, should prevent enolization and consequently oxidation. We examined the effect of hydrazine and of aminoguanidine on the oxidation of erythrose, monitored in terms of the CN⁻ catalyzed reduction of cytochrome c (which was inhibitable by SOD). Fig. 5 illustrates the inhibitory effect of hydrazine (line 2) and of aminoguanidine (line 3). In panel A the hydrazines were added before the cyanide and thus had time to convert much of the open chain form of erythrose to its hydrazide. When the aminoguanidine was added after the cyanide, its inhibitory effect was much diminished (panel B) because of the conversion of the carbonyl form of the sugar to the cyanohydrin, before the addition of the aminoguanidine.

The interactions of aminoguanidine with erythrose and with methylglyoxal were followed in terms of increased absorbancies at 230 and 318 nm, respectively, at pH 7.8 in 50 mM potassium phosphate at 25 °C. Both reactions could be followed on a time scale of minutes, but the reaction with methylglyoxal was more rapid than that with erythrose probably because of the existence of the bulk of the sugar in the furanose form. Somewhat surprisingly the reaction of methylglyoxal with aminoguanidine exhibited a fast initial rate followed by a somewhat slower

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**Fig. 1. Toxicity of erythrose to aerobic E. coli.** Overnight cultures of J1132 and AB1157 in LB medium were diluted 200-fold into M9CA medium ± erythrose. Line 1, J1132 + 20 mM erythrose; line 2, J1132 + 8 mM erythrose; line 3, J1132; line 4, AB1157 + 20 mM erythrose; line 5, AB1157 + 8 mM erythrose; line 6, AB1157.

**Fig. 2. Toxicity of erythrose to anaerobic E. coli.** Overnight cultures of J1132 and AB1157 were diluted 200-fold into M9CA ± 8 mM erythrose, and the cultures were placed in Gas Pack jars that were opened every 2 h to allow turbidimetry. Line 1, J1132 + erythrose; line 2, J1132; line 3, AB1157 + erythrose; line 4, AB1157.

**Fig. 3. MnTM-2-PyP protects J1132 against erythrose.** Conditions are the same as in Fig. 1. Line 1, 20 mM erythrose; line 2, no erythrose; line 3, 20 mM erythrose + 25 μM MnTM-2-PyP.

**Fig. 4. Glycolaldehyde toxicity and protection by MnTM-2-PyP.** Conditions are the same as in Fig. 1 except that erythrose was replaced by glycolaldehyde. Line 1, J1132 + 2 mM glycolaldehyde; line 2, J1132; line 3, J1132 + 2 mM glycolaldehyde + 25 μM MnTM-2-PyP.

**Fig. 5. CN⁻-augmented oxidation of erythrose and inhibition by hydrazines.** Reaction mixtures contained 0.27 mM erythrose and 10 μM cytochrome c ± hydrazine or aminoguanidine in 50 mM potassium phosphate at pH 7.8 and 25 °C. Cyanide was added to 0.10 mM where indicated. Panel A, line 1, no other additions; line 2, 30 mM hydrazine; line 3, 30 mM aminoguanidine. Panel B, the same as in panel A except that aminoguanidine was added after the CN⁻.
and linear rate. Because the shape of the absorption spectrum did not change with time, this probably reflects the partition of methylglyoxal between anhydrous (carbonyl) and hydrated (gem diol) status with the former reacting rapidly and then being replenished by a rate-limiting dehydration of the gem diol. If a mechanism of enolization and oxidation was involved in the toxicity of erythrose, then hydrazines might protect. Aminoguanidine was selected for this test and the results in Fig. 6 show that it protected the sodA sodB strain against the growth inhibitory effect of erythrose.

Nucleophile in E. coli—It has previously been seen that strong nucleophiles, such as CN, catalyze the oxidation of short chain sugars (1–3). The possibility that nucleophiles within E. coli might similarly augment the oxidation of short chain sugars was examined. An extract of the sodA sodB strain stimulated the reduction of cytochrome c by erythrose, and this was partially inhibited by SOD. An extract of the parental strain was also effective, but added SOD did not then inhibit; presumably because the reaction was already maximally inhibited by the endogenous SOD. The active component in the E. coli extracts was seen to be dialyzable but was not further characterized.

Growth Inhibition by α,β-Dicarbonyls—Among the products of oxidation of α-hydroxycarbonyl compounds are α,β-dicarbonyl compounds. It appeared possible that some of the toxicity of the short chain sugars might have been because of such dicarbonyl oxidation products. Lines 5 and 6 in Fig. 7 show that 5 or 10 mM methylglyoxal completely inhibited the growth of the sodA sodB strain, whereas lines 2 and 3 demonstrate that 20 mM aminoguanidine completely eliminated that toxicity. Lines 1 and 4 are controls with aminoguanidine alone, or no additions, respectively. The growth inhibiting effect of methylglyoxal shown in Fig. 7 was seen even under anaerobic conditions and with the SOD-competent, as well as with the SOD-null strain, and was thus not dependent on O$_2$ or on further oxidation (data not shown).

**DISCUSSION**

Short chain sugars and their phosphate esters, in which the carbonyl group is not largely blocked by cyclization to the furanose or to more stable pyranose rings, can tautomerize to enediols, which are prone to autoxidation. O$_2^-$ has been shown to serve as both an initiator and a propagator of these autoxidations (4). Short chain sugars are thus potentially capable of synergizing with O$_2^-$ in causing toxicity.

The results reported herein demonstrate that this does occur. Thus short chain sugars can cause a growth inhibition and a lethality to E. coli, which is O$_2$-dependent and blocked by SOD or by an exogenous SOD mimic. CN$^-$ augments the autoxidation of short chain sugars and their phosphates, and dialyzable unidentified nucleophiles, present in extracts of E. coli, exerted a similar effect.

Pentoses and hexoses are much less reactive in this regard than are the tetrose and trioses because of the stabilizing effect of ring closure to pyranose forms by hemiacetal formation. However, some carbonyl reactivity remains, even with glucose (14) and it leads to nonenzymic glycation, which generates ε-amino fructosyl lysine derivatives of proteins. These derivatives can then autoxidize leading to advanced glycation products such as N$^\alpha$-carboxymethyl lysine derivatives (12, 20). O$_2^-$ is involved in this glycoxidation process, as shown by the inhibitory effect of SOD (20). Aminoguanidine, shown here to protect E. coli against the toxicity of short chain sugars, has been shown to ameliorate the cardiovascular and renal pathologies seen in aging rats (11). The protection against erythrose toxicity by aminoguanidine shown in Fig. 6 could have been because of prevention of the enolization and subsequent oxidation of the erythrose, because of formation of the hydrazide derivative (20, 23), or to conversion of the a,β-dicarbonyl oxidation product of erythrose to the asymmetrical triazine (24–26) or to both actions. The profound toxicity of methylglyoxal and the complete protection against that toxicity provided by aminoguanidine indicate that much of the effect of the short chain sugars was because of a,β-dicarbonyl oxidation products and that much of the protective effect of aminoguanidine was because of the trapping of these dicarbonyls, probably by conversion to triazines. Hirsch et al. (24) have reported that the reaction of aminoguanidine with a,β-dicarbonyl oxidation products of D-glucose to yield triazines is rapid at neutral pH, being complete within 5 min at 37°C. The protective effect of aminoguanidine may thus be because of its dual action in preventing the O$_2^-$-producing oxidation of open chain forms of sugars and in trapping the a,β products of such oxidations.

Short chain sugars are produced during metabolism as the corresponding phosphates, and it is now clear that one of the functions of the SODs is to protect these metabolic intermediates against oxidations initiated and/or propagated by O$_2^-$ It is probably also important that the steady state concentrations of erythrose-4-phosphate, dihydroxyacetone phosphate, and...
3-phosphoglyceraldehyde be kept low. The toxicities of short chain sugars to E. coli can be exploited to shed light on the deleterious consequences of the much slower process of nonenzymic glycation followed by oxidation, by long chain sugars, which also seem to involve oxygen-derived free radicals. Thus the teratogenic effect of high glucose was diminished by superoxide dismutase, catalase, and glutathione peroxidase added to the culture medium (27). This was demonstrated more conclusively with transgenic mice overexpressing Cu,Zn-superoxide dismutase, which exhibited much less embryopathy when diabetic than did nontransgenic controls (28). That high glucose imposes on oxidative stress was also indicated by the observation that it induced Cu,Zn-superoxide dismutase in cultured endothelial cells (29).

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