Methylglyoxal detoxification by an aldo-keto reductase in the cyanobacterium *Synechococcus* sp. PCC 7002

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Aldo-keto reductases (AKRs) are a superfamily of enzymes that reduce aldehydes and ketones, and have a broad range of substrates. An AKR gene, *sakR1*, was identified in the cyanobacterium *Synechococcus* sp. PCC 7002. A mutant strain with *sakR1* inactivated was sensitive to glycerol, a carbon source that can support heterotrophic growth of *Synechococcus* sp. PCC 7002. It was found that the *sakR1* null mutant accumulated more toxic methylglyoxal than the wild-type when glycerol was added to growth medium, suggesting that SakR1 is involved in the detoxification of methylglyoxal, a highly toxic metabolite that can damage cellular macromolecules. Enzymic analysis of recombinant SakR1 protein showed that it can efficiently reduce methylglyoxal with NADPH. Based on immunoblotting, SakR1 was not upregulated at an increased cellular methylglyoxal concentration. A pH-dependent enzyme-activity profile suggested that SakR1 activity could be regulated by cellular pH in *Synechococcus* sp. PCC 7002. The broad substrate specificity of SakR1 implies that SakR1 could play other roles in cellular metabolism.

INTRODUCTION

Cyanobacteria are a diverse group of prokaryotes that carry out oxygenic photosynthesis and grow photoautotrophically. Some cyanobacteria can also grow heterotrophically in the presence of organic substrates (Rippka et al., 1979). The substrates that support heterotrophic growth of cyanobacteria include the sugars fructose and glucose. However, in the cyanobacterium *Synechococcus* sp. PCC 7002, glycerol is the only substrate that supports heterotrophic growth. A high concentration of exogenous glycerol is inhibitory to cyanobacterial growth, and it has been observed that glycerol-dependent growth of *Synechococcus* sp. PCC 7002 is only established after a period of adaptation to glycerol.

Although the pathway of glycerol metabolism has not been studied in cyanobacteria in detail, it is expected that it would be the same as that of other bacteria such as *Escherichia coli*, since, based on cyanobacterial genome sequences, cyanobacterial cells have all the genes to encode the enzymes required for glycerol metabolism (www.kazusa.or.jp/cyanobase; J. Zhao and D. A. Bryant, unpublished data). The inhibitory effect of glycerol to cyanobacterial growth could largely be due to toxic products of glycerol metabolism. One highly toxic product of cellular glycerol metabolism is methylglyoxal (Freedberg et al., 1971), which is an electrophile and reacts with cellular macromolecules such as proteins and DNA (Ferguson et al., 1998). One of the pathways leading to methylglyoxal production in most cells is the formation of methylglyoxal from dihydroxyacetone phosphate (DHAP) enzymically or non-enzymically (Ferguson et al., 1998; Kalapos, 1999). In most cells, detoxification of methylglyoxal is through glyoxalase I-II systems (Inoue & Kimmura, 1995; Kalapos, 1999). Another important mechanism is reduction of methylglyoxal by aldo-keto reductases (AKRs), which catalyse the formation of acetol from methylglyoxal (Kalapos, 1999).

AKRs are a large superfamily of related proteins that carry out NADPH-dependent reduction of various aldehydes and ketones (Jez et al., 1997, 2001; Ellis, 2002). A common feature of the superfamily is that they share a (α/β)8-barrel motif found in triose phosphate isomerase. The AKR superfamily consists of 14 families, based on their structures and sequences (Jez et al., 2001). Although a detailed mechanism has been revealed for some of the AKRs, the physiological function of most putative members of this superfamily is still unclear, partly due to their broad substrate specificity and partly because of the difficulty of genetic analysis, as many organisms have multiple genes that encode AKRs. In bacteria, a small number of AKRs have been characterized (Ellis, 2002). For example, YghZ of *E. coli* has been shown to reduce methylglyoxal and enhance methylglyoxal resistance when the gene is overproduced (Grant et al., 2003). Ko et al. (2005) recently showed that AKRs play an important role in vivo in methylglyoxal detoxification in *E. coli*.
In the study of glycerol metabolism in the cyanobacterium Synechococcus sp. PCC 7002, we noticed that a transposon-generated mutant with an insertion in an ORF encoding a putative AKR was sensitive to glycerol. Here we show that this gene encodes an AKR and is required for methylglyoxal detoxification in Synechococcus sp. PCC 7002.

METHODS

Bacterial strains and growth conditions. Synechococcus sp. PCC 7002 was grown in A medium (Stevens et al., 1973), either in liquid form or on 1.5% agar plates. When grown in liquid medium, Synechococcus sp. PCC 7002 was bubbled with air plus 1% CO2 and illuminated with cool white fluorescent light at a light intensity of 100 μM photons m⁻² s⁻¹. When needed, kanamycin (100 μg ml⁻¹) or streptomycin (100 μg ml⁻¹) was added for growth of mutant strains of Synechococcus sp. PCC 7002. Growth measurement for Synechococcus sp. PCC 7002 in liquid medium was by monitoring OD730. Growth measurement for Synechococcus sp. PCC 7002 on solid medium was based on colony size after 3 weeks incubation on agar plates. The plates with colonies were first recorded by a digital camera, and colony sizes were measured with CS-Tian software (Tian-neng). For photographing growth, various amounts of glycerol from a 30% (w/v) stock were added to the growth medium, while the growth conditions were the same as those of photoautotrophic growth. E. coli was grown in LB medium at 37°C. The strain DH5α was used for all routine cloning purposes, and strain BL21(DE3) was used for overproduction of recombinant protein.

Gene inactivation and overexpression. Total genomic DNA was isolated from Synechococcus sp. PCC 7002 in exponential growth with a kit from Omega DNA. Inactivation of the Synechococcus sp. PCC 7002 sakR1 gene was performed as follows. A fragment containing sakR1 was amplified from genomic DNA by PCR using primers 5'-AGCCGAGAACTTGTGCCATGACAC-3' and 5'-AAGGATCCGTCGGAGAAGTGGCGG-3'. The amplified fragment was cloned into pGEM-T (Promega). The resulting plasmid pSAKR1 was used as template for inverse PCR using primers 5'-AACATATGACCCGCCACA-3' and 5'-CAGAATTCTCCCGTCCCCTG-3'. The amplified fragment was digested with HincII and ligated with a DNA fragment containing the npt gene encoding resistance to kanamycin. The orientation of the npt gene was confirmed by PCR with the primers 5'-AATTTGGGCCTGATCACCAG-3' and 5'-CAGAATTCTCCCGTCCCCTG-3'. For overproduction of the SakR1 protein, sakR1 was amplified from genomic DNA using the primers 5'-AACATATGACCCGCCACA-3' and 5'-TTTCTGAGCCTACATGAGGAGG-3'. The amplified fragment was digested with NdeI and XhoI and cloned into pET15b (Novagen). The resulting plasmid pET-sakR1 was transformed into E. coli strain BL21(DE3). Expression of sakR1 as a fusion gene was induced by IPTG at 0-5 mM for 12 h at 22°C. The cells were collected by centrifugation and broken with a French press at 168 MPA. The cell extracts were centrifuged at 10,000 g for 5 min and the pellet was discarded. The supernatant was loaded onto a nickel-chelating Sepharose column (Amersham Biosciences), and the column was washed several times with phosphate/NaCl buffer (20 mM NaKPO4, pH 7-4, 0-5 M NaCl), followed by elution in the same buffer containing various concentrations of imidazole from 50 to 500 mM. The His-tagged SakR1 was eluted at an imidazole concentration of 150 mM. The fractions containing the fusion protein were pooled and dialysed against phosphate buffer (20 mM NaKPO4, pH 7-4). The His tag was then removed by digestion with thrombin. The correct N-terminal sequence of the recombinant SakR1 was confirmed by protein sequencing.

Enzymic analyses of SakR1. The aldehyde- and ketone-reducing activity of SakR1 was measured by monitoring the substrate-dependent absorption change of NADPH at 340 nm (ε=6270 M⁻¹ cm⁻¹). Unless otherwise specified, the reactions were carried out at 25°C in phosphate buffer (100 mM NaKPO4, pH 6-0; initial NADPH concentration 0-2 mM). Values of Km and Vmax were determined by the initial rates of enzymic reactions at different concentrations of substrate using the curve-fit software Dynafit (Biokin). Substrates for measurement of SakR1 activities were purchased from Sigma-Aldrich, and were used at the concentrations indicated in Table 1. For measurement of SakR1 activities in reducing methylglyoxal at different pHs, the following buffer systems were used. In the range of pH 4-6, the following buffer systems were used. In the range of pH 4-6, the following buffer systems were used.

Table 1. Specific activities of recombinant SakR1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration (mM)</th>
<th>Specific activity (nmol min⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylglyoxal</td>
<td>1</td>
<td>1278 ± 87</td>
</tr>
<tr>
<td>2-Nitrobenzaldehyde</td>
<td>1</td>
<td>1206 ± 101</td>
</tr>
<tr>
<td>4-Nitrobenzaldehyde</td>
<td>1</td>
<td>1091 ± 67</td>
</tr>
<tr>
<td>3-Nitrobenzaldehyde</td>
<td>1</td>
<td>1072 ± 93</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>1</td>
<td>651 ± 66</td>
</tr>
<tr>
<td>Isopentanaldehyde</td>
<td>1</td>
<td>641 ± 35</td>
</tr>
<tr>
<td>DL-Glyceraldehyde</td>
<td>1</td>
<td>507 ± 26</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>1</td>
<td>373 ± 12</td>
</tr>
<tr>
<td>Succinic semialdehyde</td>
<td>1</td>
<td>225 ± 26</td>
</tr>
<tr>
<td>Ninhydrin</td>
<td>1</td>
<td>211 ± 15</td>
</tr>
<tr>
<td>Dihydroxycetone</td>
<td>1</td>
<td>48 ± 15</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td>1</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>Isatin</td>
<td>1</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>1</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>50</td>
<td>660 ± 22</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>50</td>
<td>364 ± 22</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>50</td>
<td>240 ± 31</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>50</td>
<td>109 ± 6</td>
</tr>
</tbody>
</table>
pH 4–0–5–5, a sodium acetate/acetic acid system (100 mM) was used; in the range pH 6–0–7–0, a phosphate buffer system (100 mM) was used; in the range pH 7–5–9–0, a Tris/HCl system (100 mM) was used.

**Analysis of cellular methylglyoxal.** Cellular methylglyoxal concentration was determined as follows. A *Synechococcus* sp. PCC 7002 culture was grown until the OD$_{730}$ reached 1–5. The cells were harvested by centrifugation and resuspended in fresh A$^+$ medium at an OD$_{730}$ of 1–5. The culture was then grown for 1 h at 37 °C at a light intensity of 100 μM photons m$^{-2}$ s$^{-1}$ before glycerol was added to a final concentration of 6 mM. Portions of the culture were collected and used for the measurement of methylglyoxal at the times indicated. The concentration of cellular methylglyoxal was determined according to Cordeiro & Freire (1996). To 20 ml of the culture mentioned above, 2 ml perchloric acid stock solution (5 M) was added and the solution was put in an ice/water bath for 10 min. The samples were centrifuged at 4 °C for 10 min at 12 000 g and immediately analysed by the methylinquinoline method (Cordeiro & Freire, 1996). Final analyses were performed by HPLC using a C18 column from Vydac (4.6 mm × 250 nm, 5 mm). The mobile phase was 40% (v/v) ammonium formate (25 mM, pH 3–4) and 60% (v/v) methanol at a flow rate of 0.8 ml min$^{-1}$. Derivatives of methylglyoxal were monitored spectrophotometrically at 320 nm.

**Other methods.** Chlorophyll concentration was determined according to MacKinney (1941). Protein concentration was determined according to Peterson (1974). SDS-PAGE was performed according to Laemmli (1970). Sequence comparison and alignment were performed using CLUSTAL W (Thompson et al., 1994).

**RESULTS**

**Cloning and inactivation of sakR1 encoding an AKR from *Synechococcus* sp. PCC 7002**

During the study of glycerol metabolism in *Synechococcus* sp. PCC 7002, we isolated a transposon-generated mutant that, compared to the wild-type, showed sensitivity to glycerol at millimolar concentrations. Characterization of the mutant showed that the transposon was inserted into an ORF that encoded a putative AKR, and the gene was named sakR1. Fig. 1 shows a sequence alignment of the deduced SakR1 protein with those of several other closely related AKRs. The sakR1 gene has two potential translation initiation sites. Based on the immunoblotting result (see Fig. 5B) and a prediction of codon usage preference in this organism (data not shown), we assigned the first ATG as the translation start site. The deduced SakR1 from *Synechococcus* sp. PCC 7002 has a high similarity to AKRs from other cyanobacteria and a moderately high similarity (57%) to YhdN from *Bacillus subtilis* (Kunst et al., 1997). It is shown in Fig. 1 that four key catalytic residues of SakR1, Asp60, Tyr65, Lys90 and His123, are conserved in *Synechococcus* sp. PCC 7002, as in AKRs from other species. Based on the sequences shown in Fig. 1, SakR1 from *Synechococcus* sp. PCC 7002 belongs to family 11 of the AKR superfamily and has been assigned the name AKR11B3 according to the AKR nomenclature system (Jez et al., 1997, 2001; www.med.upenn.edu/akr). The enzyme activity of the sakR1 gene product was confirmed using a recombinant protein (see below).

To further study sakR1, we reconstructed a mutant by partial deletion of the gene through a double recombination event. As shown in Fig. 2, the *spr* gene encoding kanamycin resistance replaced part of the coding sequence of sakR1, and the mutant was completely segregated, as confirmed by Southern hybridization. The mutant was named AKm1.

**Characterization of AKm1**

It has previously been observed that only a small portion of *Synechococcus* sp. PCC 7002 cells plated onto medium containing glycerol survives (J. Zhao and D. Xu, unpublished results). As mentioned above, the transposon-generated mutant of sakR1 was more sensitive to glycerol. The strain AKm1 had the same phenotype as the transposon-generated mutant when plated onto glycerol-containing plates (Fig. 3). When the glycerol concentration reached 6 mM, approximately one in 300 cells formed colonies in the wild-type, while only one in 100 000 AKm1 cells formed colonies, showing that glycerol was more inhibitory to the growth of AKm1 than to that of the wild-type. Complementation of AKm1 strains with a wild-type sakR1 gene on a shuttle vector restored the wild-type phenotype. The growth rates of the wild-type and AKm1 in A$^+$ media were measured, and we observed that there was a longer lag time before exponential growth when AKm1 cells were transferred to new liquid medium in the absence of glycerol (Fig. 4A). However, once the mutant cells reached the exponential growth phase, their growth rate was similar to that of the wild-type. In contrast to the growth in liquid medium, AKm1 cells showed a much slower growth rate on solid medium. The mean size of AKm1 colonies was 55% that of wild-type colonies after 3 weeks growth on solid medium (Fig. 4B).

To understand why AKm1 was sensitive to glycerol, we measured the cellular concentration of methylglyoxal, which in most organisms is a toxic intermediate of glycerol metabolism. As demonstrated in Fig. 5A, when 6 mM glycerol was present in the growth medium of the wild-type, cellular methylglyoxal increased rapidly from 0 to 0.6 μM within 1 h. The methylglyoxal level then gradually declined to the initial level within 24 h. In AKm1, a similar but faster initial increase of methylglyoxal was observed after the addition of 6 mM glycerol. Unlike the wild-type, however, the cellular methylglyoxal level in AKm1 increased until it reached its highest level (2.5 μM) 24 h after the addition of glycerol. These results show that AKm1 is unable to efficiently detoxify methylglyoxal. Although intracellular methylglyoxal levels reached a maximum 1 h after the addition of glycerol and declined during the following 12 h in the wild-type, as demonstrated in Fig. 5A, SakR1 protein levels did not increase during that period (Fig. 5B). Immunoblotting also showed that a protein cross-reacting with antibodies against recombinant SakR1 was absent in AKm1, confirming that the mutant was fully segregated. The molecular mass of the native protein detected by immunoblotting was very close to that of recombinant SakR1, supporting our assignment of the first ATG as the initiation codon shown in Fig. 1.
The toxic effect of exogenous methylglyoxal to the growth of Synechococcus sp. PCC 7002 was studied and the results are shown in Fig. 6. The wild-type and AKm1 responded to exogenous methylglyoxal differently. The methylglyoxal concentrations in the growth medium that reduced growth rates by 50% were 1.1 and 2.0 mM for AKm1 and the wild-type, respectively (Fig. 5B). At a concentration of 3 mM, exogenous methylglyoxal completely inhibited the growth of both strains.

Enzymic characterization of sakR1 product

To determine whether SakR1 had the ability to reduce methylglyoxal, we overproduced His-tagged SakR1 protein in E. coli (Fig. 7A). The His tag was later removed with thrombin. N-terminal sequencing of the recombinant SakR1 revealed that it had the sequence SGMTRQKNE-LMKTRQ, as predicted from the expression construction. The activities of the recombinant SakR1 were measured.
with various substrates, and the results in Table 1 show that SakR1 was able to reduce a variety of aldehydes and ketones. These activities are comparable to those of E. coli YghZ (Grant et al., 2003) and rat AKR7A1 (Ellis et al., 1993). At a methylglyoxal concentration of 1 mM, SakR1 had a high methylglyoxal-reducing activity, while it had low reducing activities for sugars such as xylose and glucose. We also determined apparent kinetic constants of SakR1 with selected substrates (see Table 2). The affinity of SakR1 for methylglyoxal was quite high ($K_m$ = 80 $\mu$M), suggesting that it could reduce methylglyoxal in vivo under physiological conditions. The highest ratio of $k_{cat}/K_m$ was obtained with methylglyoxal as substrate, and the lowest ratios were obtained with sugar substrates such as galactose and glucose. The specificity ($k_{cat}/K_m$) of SakR1 to methylglyoxal was $5.9 \times 10^5$, suggesting that an efficient reduction of methylglyoxal could be catalysed by SakR1. At concentrations of 50 mM, the $k_{cat}/K_m$ ratios obtained with the sugars tested in Table 2 were low, suggesting that these sugars were unlikely to be the substrates of SakR1 in vivo. The pH-dependent enzymic activity of SakR1 was also determined (Fig. 7B). The highest activity was obtained at pH 6; however, the enzymic activity declined rapidly when pH values were higher than 7.5 or lower than 5.

**DISCUSSION**

Among cyanobacteria that can grow heterotrophically with organic substrates, *Synechococcus* sp. PCC 7002 is the only species known to utilize glycerol efficiently (Rippka et al., 1979). Glycerol metabolism in many organisms leads to an increase of cellular methylglyoxal, which is a toxic by-product of carbon metabolism (Freedberg et al., 1971; Burke & Tempest, 1990; Russell & Cook, 1995). Methylglyoxal production has been found in nearly all cells. The major route of methylglyoxal production related to glycerol metabolism is the methylglyoxal synthase pathway (Hopper & Cooper, 1971). In *Synechococcus* sp. PCC 7002 and most other cyanobacteria, genes encoding methylglyoxal synthases are missing, based on BLAST searches of cyanobacterial genome sequences. It is speculated that the production of methylglyoxal from glycerol in *Synechococcus* sp. PCC 7002 (Fig. 5) is by a non-enzymic reaction from DHAP (Phillips & Thornalley, 1993). The highly toxic methylglyoxal is removed promptly from cells by glyoxalase detoxification pathways and through AKRs that convert methylglyoxal to acetol (Ferguson et al., 1998; Kalapos, 1999; Booth et al., 2003).
There are several lines of evidence that show that SakR1 is responsible for methylglyoxal detoxification. The strain AKm1 is more sensitive to glycerol (Fig. 3), suggesting that the gene product is involved in the protection of cells from toxic metabolites of glycerol. In E. coli, an uncontrolled carbon metabolism results in an increase in cellular methylglyoxal (Kim et al., 2004), and AKRs are required for its detoxification (Ko et al., 2005). The addition of glycerol to the growth medium induces a drastic increase in cellular methylglyoxal concentration in AKm1 (Fig. 5), while the glycerol-induced increase of the cellular methylglyoxal concentration in the wild-type is only transient and the methylglyoxal levels return to normal within 1 h. Both the wild-type and the AKm1 mutant were quite resistant to exogenous methylglyoxal. The growth of both strains was normal at methylglyoxal concentrations in the growth medium of less than 1 mM (Fig. 6), indicating that Synechococcus sp. PCC 7002 has an effective mechanism to protect its cells from exogenous methylglyoxal. However, at higher concentrations of methylglyoxal in the growth medium, the growth of AKm1 was more severely inhibited than that of the wild-type. These results provide evidence that SakR1 is involved in the detoxification of methylglyoxal in Synechococcus sp. PCC 7002. This suggestion is in agreement with other reports that AKRs are involved in methylglyoxal reduction in other organisms (Wermuth et al., 1977;
Compared with other AKRs (Ellis, 2002), recombinant SakR1 has a high affinity for methylglyoxal and its catalytic efficiency in reducing methylglyoxal is moderately high, supporting the proposal that it plays an important role in methylglyoxal detoxification in vivo.

One interesting observation is that AKm1 has a slower rate of growth on solid medium than the wild-type, while it has a similar growth rate to that of the wild-type in liquid medium. We speculate that there is some methylglyoxal production in Synechococcus sp. PCC 7002 even in the absence of exogenous glycerol, and that excretion of methylglyoxal, which is also a detoxification mechanism in other bacteria (Tempest & Neijssel 1984; Baskaran et al., 1989; Russel, 1993), is not as efficient on solid medium as in liquid medium.

The transient increase of methylglyoxal in the wild-type after addition of glycerol to the growth medium (Fig. 5) suggests that the detoxification system is regulated. Because the amount of SakR1 remains unchanged by the increased methylglyoxal concentration (Fig. 5B), we speculate that there exists a mechanism of regulating enzyme activity. The profile of enzyme activities at different pH values (Fig. 7B) shows that SakR1 activity increases nearly fivefold from pH 8 to 7. This suggests that cells could increase SakR1 activity by lowering cytosolic pH when cellular methylglyoxal concentration increases, a strategy adopted by many organisms (Ferguson et al., 1998).

The function of many putative AKRs in various organisms is unclear. This is largely due to their broad substrate specificity and to the difficulty in performing genetic analysis, because many organisms have multiple genes encoding putative AKRs that could be functionally redundant. For example, E. coli has six AKR genes (Blattner et al., 1997), while yeast has 14 (Goffeau et al., 1996). Our survey shows that the number of AKR genes in cyanobacteria varies greatly. Synechocystis sp. PCC 6803, Synechococcus sp. PCC 6301 and Nostoc punctiforme have four, two and 21 potential AKR genes, respectively. There are four potential AKR genes in Synechococcus sp. PCC 7002, based on its genomic
sequence. Besides recombinant SakR1, we have obtained a soluble recombinant protein encoded by another potential AKR gene. However, we did not detect any enzyme activity with this recombinant protein using NADPH and the substrates listed in Table 1 (Xu and Zhao, unpublished results).

The detoxification of methylglyoxal by SakR1 in *Synechococcus* sp. PCC 7002 is therefore at present unknown.


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**REFERENCES**


**Table 2.** Apparent enzymic kinetic constants of recombinant SakR1

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$k_{cat}$ (min$^{-1}$)</th>
<th>$K_m$ (mM)</th>
<th>$k_{cat}/K_m$ (M$^{-1}$ min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylglyoxal</td>
<td>50.8 ± 1.7</td>
<td>0.08 ± 0.011</td>
<td>5.9 × 10$^5$</td>
</tr>
<tr>
<td>2-Nitrobenzaldehyde</td>
<td>78.6 ± 3.6</td>
<td>0.31 ± 0.03</td>
<td>2.6 × 10$^5$</td>
</tr>
<tr>
<td>3-Nitrobenzaldehyde</td>
<td>66.9 ± 1.6</td>
<td>0.91 ± 0.05</td>
<td>2.2 × 10$^5$</td>
</tr>
<tr>
<td>4-Nitrobenzaldehyde</td>
<td>74.6 ± 5.9</td>
<td>0.85 ± 0.12</td>
<td>8.8 × 10$^4$</td>
</tr>
<tr>
<td>DL-Glyceraldehyde</td>
<td>31.1 ± 3.2</td>
<td>0.56 ± 0.12</td>
<td>5.5 × 10$^4$</td>
</tr>
<tr>
<td>Benzoaldehyde</td>
<td>62.7 ± 15.5</td>
<td>1.69 ± 0.70</td>
<td>3.7 × 10$^4$</td>
</tr>
<tr>
<td>Isoamylaldehyde</td>
<td>38.9 ± 3.2</td>
<td>1.33 ± 0.32</td>
<td>2.9 × 10$^4$</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>72.9 ± 6.0</td>
<td>104 ± 14</td>
<td>7.0 × 10$^5$</td>
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<tr>
<td>L-Arabinose</td>
<td>76.4 ± 3.2</td>
<td>234 ± 13</td>
<td>3.3 × 10$^5$</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>40.8 ± 15.3</td>
<td>160 ± 24</td>
<td>2.6 × 10$^5$</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>36.9 ± 14.5</td>
<td>445 ± 102</td>
<td>8.0 × 10$^4$</td>
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