Microbial Growth Regulation Using Photoresponsive Siderophore

Takeshi Masuda, Masayuki Azuma, Hiroshi Ooshima, Seizo Tamagaki, and Takeshi Nagasaki*
Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585

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A new trihydroxamate siderophore containing a reversibly *cis* / *trans*-controllable azobenzene framework has been examined for biological activity with *Aureobacterium flavescens*. The *cis*-Fe(III)-complex in monomeric form shows a remarkable activity comparable to ferrioxamine B, a natural siderophore, but no activity is observed with the *trans*-Fe(III)-complex in dimeric form.

Over the past few decades numerous attempts have been made to artificially construct models of biological photo-regulation systems such as photosynthesis,[1] vision,[2] phototropism,[3] and phototaxis;[4] thus, the incorporation of a photochromic moiety into synthetic architecture, for example, ionophore,[5] host-molecule,[6] enzyme inhibitor,[7] enzyme,[8] chemical catalyst,[9] nucleic acid,[10] and peptide[11] has enabled us to control various chemical processes by the photoisomerism. However, there have been few or no applications of such a concept to biological *in vivo* processes. Although iron is an essential element for a living cell, iron is difficult to be acquired by most organisms in spite of the high abundance of iron ion in nature, because the concentration of free ferric ions existing in equilibrium with ferric hydroxide polymers is of the order of 10^{-12} μM in an aerobic environment.[12] Bacteria can grow either when they secrete an iron-specific chelator called a siderophore and take up its ferric complex into the cells via highly efficient transport systems, or when they find an iron chelator in their environment. There are considerable variations among the structures of siderophores; however, the siderophores usually fall into two types according to their binding groups: hydroxamate and catecholate.[13] Gram-positive *Aureobacterium flavescens* JG-9 (ATCC No. 25091) is a siderophore-auxotroph requiring a hydroxamate siderophore.[14] The surface receptor in the iron transport system owns the high specificity for the ferrisiderophore; namely, the affinity of the receptor for the ferrisiderophore is expected to be largely dependent on its chemical structural features. Quite recently, we have reported that the structure of the ferric complex of the trihydroxamate siderophore (Azo–Gly (**1**)) containing an azobenzene framework is reversibly regulated by irradiating it with either visible or UV light, as shown in Figure 1.[15] Therefore the purpose of this work is to explore a photo-regulation system for the microbial growth using the photoresponsive siderophore (**1**).

The growth response of *Aureobacterium flavescens* JG-9 to *cis*- and *trans*-**1** was examined as compared with deferriferrioxamine B (DFB), a native linear trihydroxamate siderophore,[16] where the *cis*/*trans* (86/14) photostationary mixture attained by UV (365 nm) irradiation and the pure *trans* isomer thermally isomerized in the dark were utilized as *cis*-**1** and *trans*-**1**, respectively, and an adventitious ferric ion in the medium was the sole iron source. All samples were incubated in the dark in order to minimize the photoisomerization; a spectroscopic study

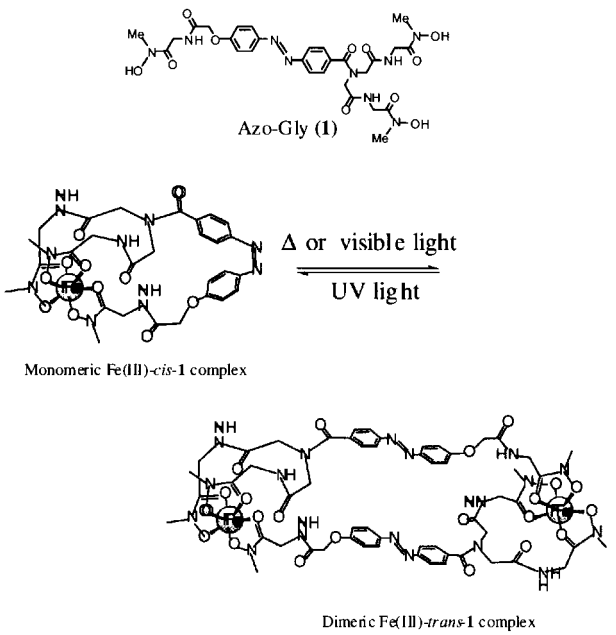

Figure 1. Photoisomerization of Fe(III)-**1** complex.

indicated that 72% of the *cis*-form remained in the *cis*-**1** solution during the incubation. Aliquots (100 μL) were withdrawn at specific intervals to determine spectroscopically the culture turbidity (OD_{660}) indicating the bacteria density. The optical density readings were each corrected for those of control experiments without a siderophore. The results are given in Figure 2. Interestingly, *cis*-**1** has a remarkable bioactivity comparable to the natural siderophore DFB in the concentration range higher

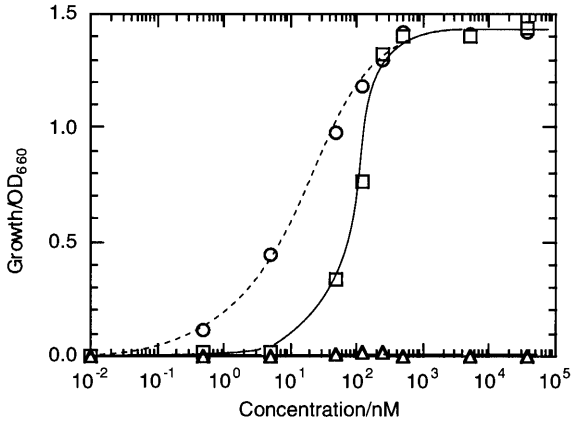

Figure 2. Growth response of *Aureobacterium flavescens* JG-9 to *cis*-**1** (□), *trans*-**1** (Δ) and DFB (○) after 12 hour incubation in ATCC Medium 424[17] (3.15 ml) at 30 °C.

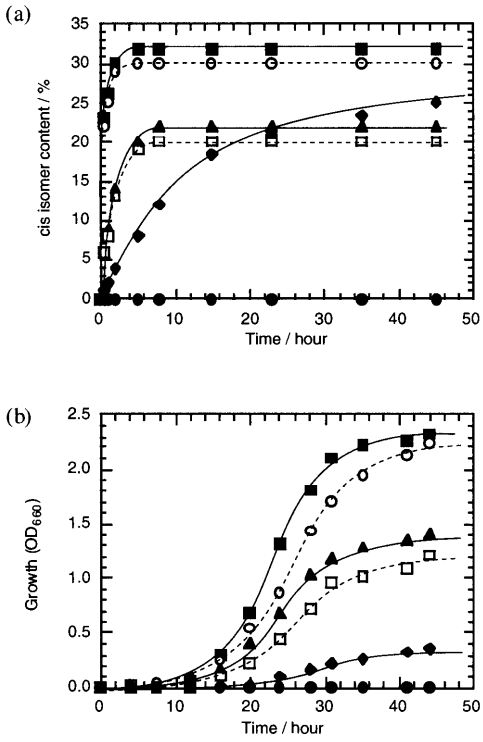

Figure 3. (a) Time courses of *trans*-to-*cis* photoisomerization of **1** in 1 mM phosphate buffer (pH 7.5) by transmitted light of different wavelengths at 30 ℃. The desired light was obtained by wrapping the culture-tubes with blue (■), green (▲), yellow (□), red (◆), and black (●) cellophane film-filters, and without a filter (○); [**1**] = [Fe^{3+}] = 47.5 μM. (b) Growth curves of *Aureobacterium flavescens* JG-9 in the presence of 367 nM **1** in ATCC Medium 424 (3.15 ml) under the same irradiating conditions as described above.

than 300 nM, although the half-maximal growth of *cis*-**1** occurred near 100 nM that is ten-fold higher than that of DFB. Meanwhile, *trans*-**1** exhibits no growth-promotion activity even at its high concentrations, although the previous work demonstrates that the stability of the Fe^{III}-*cis*-**1** complex is almost equal to that of the *trans*-**1** complex. We may safely say, therefore, that the shape and size of the siderophore rather than the iron binding or iron releasing ability restricts the bio-availability. It is not strictly clear at the present time how the iron-transport system discriminate between the monomer and dimer complexes.

Figures 3a and 3b show the *trans*-to-*cis* isomerizing behavior of the *trans*-**1** and the effect of wavelengths of light on the microbial growth, respectively. As direct irradiation with UV or visible light caused the cell-death because of the strong stress, irradiation light is subtly adjusted with a variety of colored film–filters, which decrease not only the initial rate of *trans*-to-*cis* isomerization but also the microbial growth in the same order: blue ≈ transparent > green ≈ yellow > red > black. It is obvious that the microbial growth increases with the proportion of the *cis*- over *trans*-isomer in the initial phase of the incubation rather than that in the photostationary phase, strongly indicating that the growth is primarily determined by the availability of ferric ion in the initial phase rather than in the logarithmically vegetative phase. The opposite situation was observed when *cis*-**1** was incubated at the start; that is, the growth decreased with increasing initial rates of the *cis*-to-*trans* isomerization .

In conclusion, we have now succeeded in developing, for the first time, the photoresponsive siderophore, which works well with excellent bioactivity for the *Aureobacterium flavescens* JG-9 when the UV light is switched on. Furthermore, this work suggests that the iron uptake by *Aureobacterium flavescens* JG-9 receptor follow a exclusion-by-size mechanism.

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17 The medium contains 10 g peptone, 10 g yeast extract, 2 g K_2HPO_4 in 1 litter sterile water.