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Synthesis of a novel azobenzene-based trihydroxamate siderophore and photoregulation of its ferric complex structure

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Abstract

In order to develop an artificial photoresponsive siderophore by utilizing the photo-isomerization of an azobenzene group, a novel trihydroxamate siderophore based on an azobenzene skeleton was synthesized. The 1:1 and 2:2 stoichiometry of the ferric complex can be almost perfectly regulated reversibly by irradiation with UV and visible light, respectively. © 2000 Elsevier Science Ltd. All rights reserved.

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Photoresponsive systems play important roles in biological process, such as photosynthesis,[1] vision,[2] phototropism,[3] and phototaxis.[4] One common attribute of every system is the regulation of activity of a biologically active macromolecule by a low molecular weight photochromic molecule capable of assuming at least two states, whose relative concentrations are determined by light of specific wavelengths. To apply this concept of naturally occurring processes to chemical processes, a great deal of effort has been made on the development of artificial photoregulation systems such as ionophores,[5] sugar-receptors,[6] macrocyclic host-molecules,[7] oligonucleotides,[8] enzyme-inhibitors,[9] enzymes themselves,[10] and catalysts for chemical reactions.[11] On the other hand, a variety of microorganisms produce low molecular weight iron carrier molecules called siderophores, which solubilize iron and make it available to iron-deficient cells. Herein we report the synthesis and properties of a novel and photoresponsive artificial siderophore based on an azobenzene skeleton which changes the structure of the ferric complex in response to photo-irradiation.

The synthetic procedure for Azo-Gly is depicted in Scheme 1. Since azobenzene is easily reduced to the hydrazine under the reductive conditions for deprotection of a benzyl group (a useful protecting group for a hydroxamic acid), direct acylation of an amino group involved in a hydroxamate derivative was chosen for linking of hydroxamate units to an azobenzene skeleton. The photoresponsive siderophore, Azo-Gly, was finally prepared in moderate yield (52%) by a coupling reaction between azobenzene-based tricarboxylic acid (IV) and glycine-*N*-hydroxyl-*N*-methylamide in the presence of DCC and HOBt

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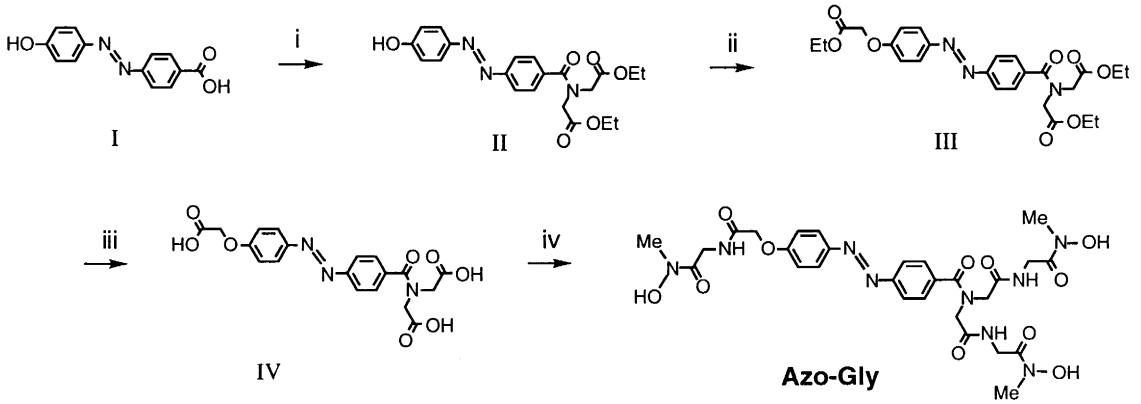
followed by HPLC purification (ODS column; eluents: H_2O/CH_3CN=50/50). The product was identified by IR, ^1H NMR, and high resolution-positive FAB-mass spectroscopies and elemental analysis.† The absorption spectrum of *trans*-Azo-Gly in DMF had an absorption peak at 354 nm with a molar extinction coefficient of 31 000. Exposure to UV light by a high-pressure Hg-lamp (400 W) through a visible filter ($300<\lambda<400$ nm) converted 86% of *trans*-isomer to *cis*-Azo-Gly. This photostationary state was achieved in 90 s. The *cis*-isomer exhibits an absorption maxima at 442 nm (ε=2 440). For the experiment described below, we used the *cis*/*trans* (86/14) mixture as *cis*-Azo-Gly induced by UV irradiation. When the mixture was further irradiated with visible light (λ>400 nm), the *cis*-isomer isomerized back to the *trans* form instantly. In contrast, *cis–trans* thermal isomerization of Azo-Gly was very slow (half-life 20 h) at 30°C. Thus, the decrease in the content of the *cis*-isomer by the thermal isomerization was negligible during the measurement of spectroscopies of *cis*-Azo-Gly.



Scheme 1. (i) $SOCl_2$, diethyl iminodiacetate, Et_3N (91%); (ii) Cs_2CO_3, ethyl bromoacetate (87%); (iii) H_2O, OH^- then H^+ (96%); (iv) DCC, HOBt, glycine-*N*-hydroxyl-*N*-methylamide hydrochloride, Et_3N (52%)

As the solubility of Azo-Gly in water was too low, its Fe^{III}-chelating properties were investigated in DMF in the presence of triethylamine. In the UV–vis spectrum of a 1:1 molar mixture of Fe^{III} and *cis*-Azo-Gly in the presence of fourfold excess of triethylamine, the characteristic LMCT (ligand to metal charge transfer) band was observed at 420 nm (ε=2 750). The λ_{max} and ε value suggested the intramolecular formation of the 1:3 complex of Fe^{III} to the hydroxamate group of *cis*-Azo-Gly. Formation of the 1:3 complex was also confirmed by the molar ratio plot (data not shown). Contrary to our inference, *trans*-Azo-Gly also formed the 1:3 complex (λ_{max}=425 nm, ε=2 800), although the three hydroxamate groups can not get close to each other to form an intramolecular 1:3 complex with a Fe^{III} ion on the basis of an examination of molecular models. As the intramolecular 1:3 complex is impossible, an intermolecular cluster structure consisting of two or more molecules is suggested. In order to characterize the structures of the Fe^{III} complexes, electrospray ionization (ESI) mass spectroscopy was used. The mass spectrum of the ferric complex with *cis*-Azo-Gly (Fig. 1a) exhibits a strong sodiated molecular ion peak (*m/z* 749.7, 749.2 calculated for $[C_{28}H_{32}N_9O_{11}Fe\cdot Na]^+$). Since *cis*-Azo-Gly is the *cis*/*trans* (86/14) mixture, peaks (*m/z* 1454.7 and 1475.9, 1453.3 and 1475.3 calculated for $[(C_{28}H_{32}N_9O_{11}Fe)_2\cdot H]^+$ and $[(C_{28}H_{32}N_9O_{11}Fe)_2\cdot Na]^+$, respectively) due to a dimeric complex comprised of two *trans*-Azo-Glys also appear. On the other hand, relatively strong peaks (*m/z* 1453.9 and 1476.1) due to the dimeric complex are

† Selected data for Azo-Gly: mp 123.5–125°C, IR (KBr)/cm^{-1}: 3600–2700, 1685–1640; δ_H[$(CD_3)_2SO$, 100°C]: 3.13 (s, 9H, -CH_3), 4.04–4.10 (m, 10H, -NCH_2-), 4.67 (s, 2H, -OCH_2-), 7.20 (d, *J*=8.8 Hz, 2H, Ar-3*H*), 7.66 (d, *J*=8.8 Hz, 2H, Ar-3′*H*), 7.84 (d, *J*=8.8 Hz, 2H, Ar-2*H*), 7.89 (d, *J*=8.8 Hz, 2H, Ar-2′*H*); mass spectrum (FAB, thioglycerol matrix) *m/z* 674.2522 [$(M+H)^+$; calcd for $C_{28}H_{36}N_9O_{11}$, 674.2534]. Anal. calcd for ($C_{28}H_{35}N_9O_{11}\cdot 2H_2O$): C, 47.39; H, 5.54; N, 17.76. Found: C, 47.68; H, 5.73; N, 17.69%.

detected in the spectrum of *trans*-Azo-Gly (Fig. 1b), whereas no peak corresponding to the monomeric complex is observed. These mass data support that *trans*-Azo-Gly forms the 2:2 complex with Fe^{III}. Furthermore, visible light irradiation of the ferric complex solution of *cis*-Azo-Gly afforded the same ESI mass spectrum as that of the *trans*-Azo-Gly complex. The opposite situation was observed with UV irradiation of the *trans*-Azo-Gly complex leading to the monomeric complex. These results indicate that the structure of monomeric and dimeric complexes is photochemically and reversibly controllable, as shown in Fig. 2.

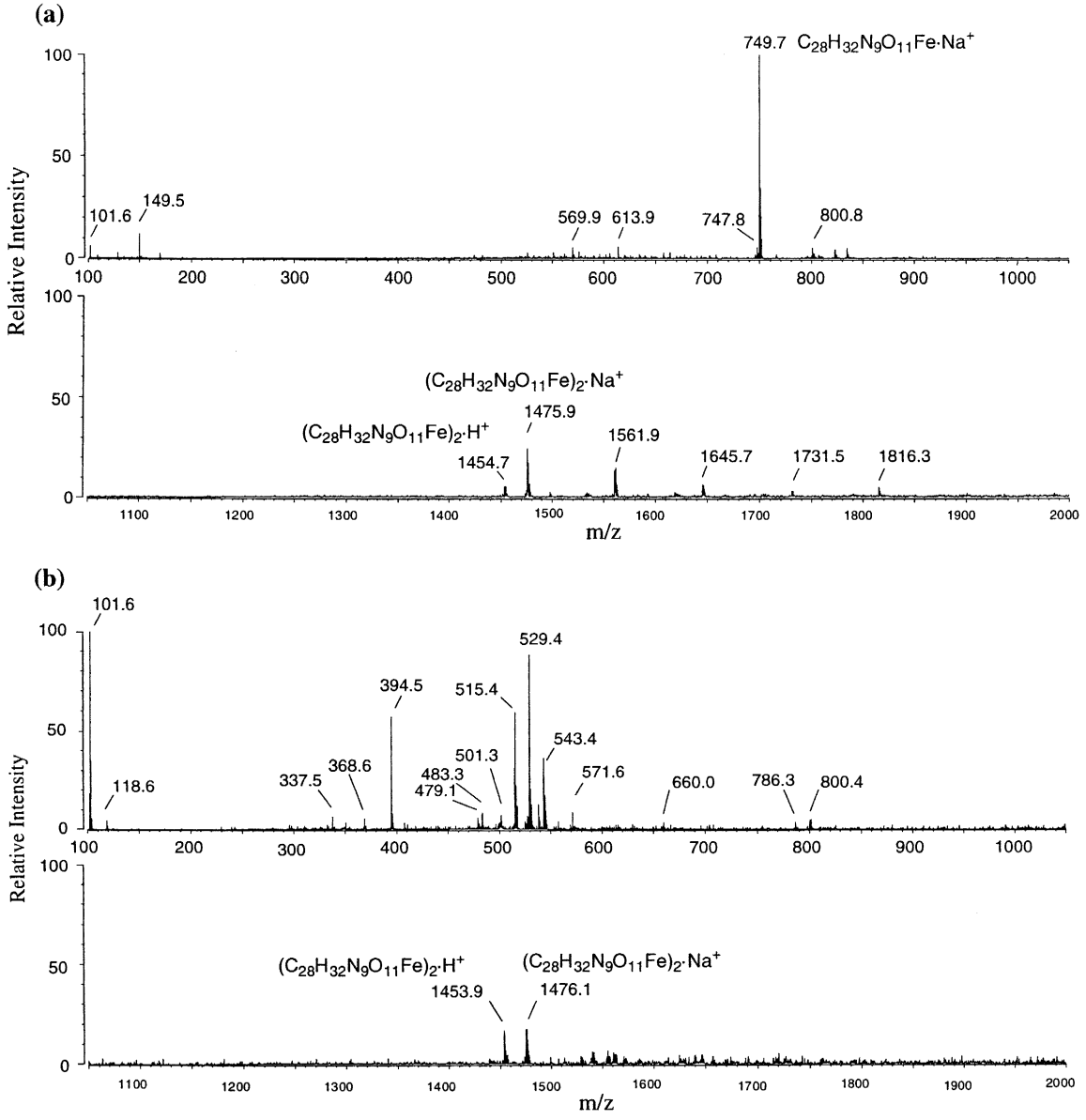
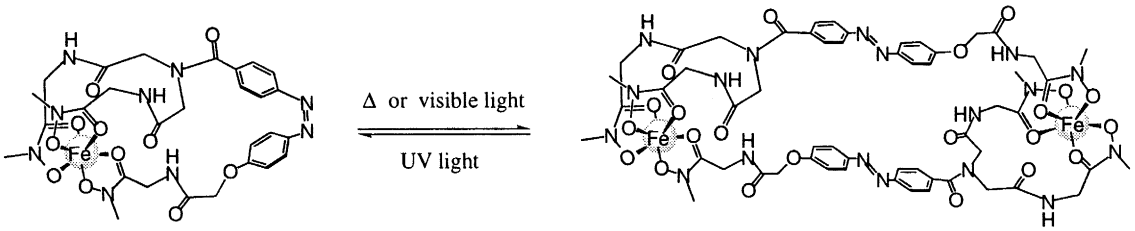


Fig. 1. ESI mass spectra of Fe^{III}-*cis*- (a) and *trans*- (b) Azo-Gly complexes in DMF

The relative stability of Fe^{III}-complexes with *cis*-Azo-Gly and *trans*-Azo-Gly was estimated by the competition reaction with EDTA.[12] Strangely, the stability of Fe^{III}-*cis*-Azo-Gly was almost equal to that of *trans*-Azo-Gly, suggesting that the entropic disadvantage of the 2:2 complex formation with the *trans*



Monomeric Fe(III)-*cis*-**Azo-Gly** complex

Dimeric Fe(III)-*trans*-**Azo-Gly** complex

Fig. 2. Photoregulation of structure of Fe^{III} complex with Azo-Gly

form be cancelled by the enthalpically unstable *cis* azobenzene structure. However, their stability is below that of ferrioxamine B, a native trihydroxamate siderophore. Interestingly, Fe^{III} influenced *cis–trans* thermal isomerization of Azo-Gly; the thermal isomerization in the presence of Fe^{III} was pronouncedly slower (half-life 45 h). However, the *cis*% at the photostationary state remains the same. The first-order rate constants were determined spectrophotometrically by following the increase in the absorption band of *trans*-isomers to be 3.1×10^{-5} and 4.5×10^{-5} s^{-1} at 30°C in the presence and absence of Fe^{III}, respectively. The fact that Fe^{III} suppresses the rate of thermal *cis–trans* isomerization also supports the intramolecular formation of the 1:3 complex of Fe^{III} with the hydroxamate group of *cis*-Azo-Gly.

In conclusion, the present study demonstrated for the first time that the structure of ferric complex with the photoresponsive trihydroxamate siderophore containing an azobenzene framework can be regulated reversibly by irradiating with either visible or UV light. The application of the present findings to the photoregulation of bacterial growth in which a siderophore is required is currently under way.

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