Desferrioxamine B, an Inhibitor of *Escherichia coli* Motility, Reversing the Stimulating Effect of Cyclic Adenosine 3',5'-Monophosphate

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A substance inhibiting *Escherichia coli* motility was isolated by "motilometry" assay from the culture broth of *Streptomyces* sp. strain NR9GG8 and was found to be identical with desferrioxamine B. Its inhibitory effect was reversed by cyclic adenosine 3',5'-monophosphate (cAMP), while the motility stimulation by cAMP was diminished by this inhibitor. Its effects on various enzymes involved in cAMP metabolism of function of cAMP were examined.

In an attempt to find compounds specifically influencing bacterial motility, a simple assay method named "motilometry" (11) was developed, which quantifies the so-called "swarming" capacity due to bacterial motility originally pointed out by Adler and by Adler and Templeton (1, 2). During the course of screening for inhibitors of *Escherichia coli* motility from culture broths of actinomycetes by motilometry assay, a substance with this property was obtained and is described in this communication. This microbial product has been identified as desferrioxamine B.

It is reported that cyclic adenosine 3',5'-monophosphate (cAMP), among others, plays a role in bacterial motility through flagellar biosynthesis (1, 8, 17) and that the exogenous addition of cAMP stimulates the motility (4, 8, 11, 17), although the mechanism of its involvement in the motility and flagellar synthesis is not clear. It is therefore postulated that there might be a certain type of motility inhibitor which could antagonize the function or metabolism of cAMP in some respects. Desferrioxamine B presented here was found to reverse the motility-stimulating effect of cAMP in motilometry (4, 11) as well as some of its functions in different systems. There is no previously reported case of an actinomycete metabolite antagonizing cAMP function in any of its aspects.

The motility was determined by the migration distance of *Escherichia coli* S-26 cells from the inoculation spot to the spearhead of migration (Band I) by the method of Maruyama and Azuma (11). A thin glass tube (2 mm [diameter] by 400 mm) was filled with the semi-solid motility medium (GMM, [11]) with or without the supplementation of test broth and was closed by a stopper at the bottom. From the top end, a suspension (5 µl) of 10⁷ bacterial cells grown overnight in N-broth (Eiken Co., Tokyo) was inoculated and incubated at 37°C for 15 to 17 h without shaking. The inhibitory principle was prepared using 5 liters of a 3-day-old culture broth, shaken at 27°C in a medium (11), of *Streptomyces* sp. NR-9GG8, which had been newly isolated from a soil sample collected in Matsumoto, Japan. Taxonomical studies indicated that the strain was closely related to *Streptomyces albaduncus* ISP5478, which produced damonycin (16). The active broth was adsorbed on a column of Dowex 50 (NH₄⁺) and eluted with dilute NH₄OH. After lyophilization, 1.01 g of crude material was obtained in powder form. This was further purified by applying it on a column of Amberlite CG-50 (NH₄⁺), followed by elution with water. The active fractions were lyophilized to yield 503 mg of a white powder. Repeated recrystallization from waterethanol gave 275 mg of colorless, fine crystals, *I*, melting at 134 to 135°C. Analysis gave the following results: C, 52.33; H, 8.74; and N, 12.34.

\[
\begin{align*}
\text{H}_2\text{N} & (\text{CH}_2)\text{NCO} (\text{CH}_2) \text{CONH} (\text{CH}_2)\text{N} \\
\text{OH} & (I) \\
\text{-NCO} (\text{CH}_2) & \text{CONH} (\text{CH}_2)\text{NCOCH}_2 \text{ I} \\
\text{OH} & \text{OH}
\end{align*}
\]

*I* was used throughout the experiment. Identity of *I* as desferrioxamine B (I), a demetalized form of ferrioxamine B (5, 6) which had been reported (5) as an antagonist of the iron-containing antibiotics, sideromycins, was confirmed by directly comparing the iron complex of *I* with authentic ferrioxamine B with regard...
to the chromatographic and electrophoretic behavior of the complex (5) and the proton magnetic resonance spectrum of compound I with that of desferrioxamine B. I exhibited no antimicrobial activity against 24 strains tested except for Proteus vulgaris (minimal inhibitory concentration, 50 μg/ml) and caused no growth inhibition of E. coli S-26 even at 3 mg/ml.

Since cAMP reverses the motility inhibition caused by various substances, it was anticipated that some agents active on motilometry might have a particular relation to the metabolism or function of cAMP (4). Therefore, the motility inhibition by I was analysed with regard to its reversal by cAMP. I inhibits E. coli S-26 motility with 50% inhibitory dose value of 8.2 × 10⁻⁶ M, and blocks the motility-stimulating effect of cAMP (Fig. 1). When the inhibition kinetics of I was studied as the function of cAMP, the inhibition by I was completely reversed by cAMP, according to Type II interaction reported previously (4), similar to the case of colchicine. The mode of the inhibition seems to be of a mixed type with regard to cAMP (apparent $K_a = 2.1 \times 10^{-4}$ M), as revealed by a Lineweaver-Burk plot (Fig. 1). If it can be assumed that classical competitive kinetics is followed, cAMP and I could compete for a binding site, to which I is bound irreversibly once bound. Or, binding of both I and cAMP could take place on different sites and influence the conformation of the same macromolecule or a certain surface structure. In any case, further study is needed for the precise mechanism of inhibition.

In order to obtain more information of its functional relation to cAMP, I was further investigated for its effect on adenylate cyclase from E. coli (9), cAMP phosphodiesterase from the rat platelets (7) and from E. coli (9) and cAMP-dependent protein kinase (12). Crude or partially purified enzymes were prepared and assayed by exactly following each method cited as above. It was found that I caused no remarkable effect on E. coli adenylate cyclase. The stimulatory effect of cAMP on the protein kinase (12) was slightly reduced by I in a dose-independent manner. Binding of labeled cAMP to the crude binding protein prepared from E. coli by the method of Anderson et al. (3) was also inhibited by 15 to 20% at a concentration of $10^{-4}$ to $10^{-3}$ M I. Although I showed no inhibition of cAMP phosphodiesterase from rat platelets at a concentration less than $10^{-4}$ M, it exhibited a clear stimulation of cAMP hydrolysis (200 to 320%) at a concentration between $5 \times 10^{-4}$ and $10 \times 10^{-4}$ M when assayed with

![Fig. 1. Inhibition of E. coli S-26 motility by desferrioxamine B (F). Migration percent (250 mm = 100%) for a 15-h incubation was assayed by motilometry in the repression medium (GMM, [8]) in the presence of I alone (●); I + 1 mM cAMP (○); I + 0.1 mM FeCl₃ (▲) and of ferrioxamine B (△).]
crude enzyme (the supernatant fraction of 0.2% Triton homogenate of the rat blood platelets) by the method of Dalton et al. (7). It is noticeable that I had no stimulative effect on the same enzyme purified or on the phosphodiesterase from E. coli (9). Details of its effect on cAMP phosphodiesterases will be reported elsewhere.

Antiviral activity of cAMP on ECHO virus-African green monkey kidney cell system (S. Kuwahata, H. Ishitshuka, and K. Takano, Abstr. 94th Annu. Meet. Jpn. Soc. Pharm. Sci. 94:III-90, 1974) was completely reversed by I at 25 μg/ml, while this dose caused no effect on the virus itself (Kuwahata et al.; H. Azuma, Y. Kotoh, Y. Suhara, and H. B. Maruyama, Abstr. 94th Annu. Meet. Jpn. Soc. Pharm. Sci. 94:III-100, 1974). In this system, however, cAMP did not affect the viral production but inhibited the intercellular release of the progeny (Kuwahata et al.), probably imparting some kinds of the rigidity to the cell membrane. This fact prompted us to examine the effect of I on the membrane stability. I exhibited a remarkable labilization of the rat tritosome membrane (15) which was purified (Y. Takagi, T. Sawada, N. Nozawa, M. Onitsuka, and H. B. Maruyama, Abstr. 94th Annu. Meet. Jpn. Soc. Pharm. Sci. 94:III-101, 1974; Table 1).

Ferrioxamine B, the iron complex of I (5), showed neither motility inhibition (Fig. 1) nor such activities on various systems as mentioned above (Table 1). Furthermore, the simultaneous or prior addition of FeCl₃ to the reaction mixture reduced the activity of I (Fig. 1), indicating the essential role of its chelate-forming property. Although the mechanism of the inhibition of E. coli motility and of cAMP functions remains unknown, results reported here suggest that the interaction with Fe³⁺ ion (14) and the unstabilization of membranous structure would at least partially account for the mechanism.

Desferrioxamine B, known as a potent iron chelating agent (13), has been therapeutically used to facilitate the removal of iron in the treatment of acute iron intoxication (10) and to treat hemochromatosis and acute porphyria (F. Woehler, Intern. Kong. Chemother. Abstr., p. 189, 1963). On the other hand, ferrioxamine B, one of so-called sideramines, is known for its growth promoting activity through iron transport (14). In the E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis that were examined, I showed no growth promotion.

### Table 1. Labilizing effect of I on the rat liver lysosome

<table>
<thead>
<tr>
<th>Addition</th>
<th>Release of β-glucuronidase</th>
<th>Release (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>0.000 ± 0.003</td>
<td>0</td>
</tr>
<tr>
<td>Triton X-100 (3%)</td>
<td>0.718 ± 0.001</td>
<td>100</td>
</tr>
<tr>
<td>I (10⁻⁴ M)</td>
<td>0.384 ± 0.020</td>
<td>53</td>
</tr>
<tr>
<td>I (10⁻⁴ M) + cAMP</td>
<td>0.395 ± 0.004</td>
<td>55</td>
</tr>
<tr>
<td>(3 mM)</td>
<td>0.365 ± 0.028</td>
<td>49</td>
</tr>
<tr>
<td>cAMP (3 mM)</td>
<td>0.003 ± 0.012</td>
<td>0.4</td>
</tr>
<tr>
<td>Ferrioxamine B (10⁻⁴ M)</td>
<td>0.080 ± 0.022</td>
<td>11</td>
</tr>
<tr>
<td>Ferrioxamine B (10⁻⁴ M)</td>
<td>0.090 ± 0.055</td>
<td>12</td>
</tr>
</tbody>
</table>

*The liver lysosome (tritosome) was obtained from Triton WR1339 (Ruger Chemicals, N.J., gift from D. Mizuno, University of Tokyo)-fed male Wistar rats and isolated by the sucrose gradient flotation method of Troet (15). The incubation mixture contained in 650 μl: 0.075 M acetate buffer, pH 5.2, 0.25 M sucrose, freshly prepared tritosome (about 20 mg of protein), and 10 to 100 μl of test sample or distilled water (control). After 1 h of preincubation at 37°C, 50 μl of 10 mM O-nitrophenyl-β-D-glucuronide was added and incubated at 37°C for another 40 min. The liberated O-nitrophenol was assayed at an absorbancy at 420 nm (A₄₂₀) after addition of 2.0 ml of glycine buffer, pH 11.0.

### LITERATURE CITED


