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Estimation of *i*-Urobilin Movement in an Aquatic Environment

YUICHI MIYABARA, JUNZO SUZUKI and SHIZUO SUZUKI

Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12,
Ichigaya Funagawara-machi, Shinjuku,
Tokyo 162, Japan

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A large amount of *i*-urobilin is present in the bottom sediment of urban rivers, and the value of distribution ratio (sediment/water) increase along the river, being higher at the river mouth. The adsorption equilibrium of *i*-urobilin is important to estimate the movement of *i*-urobilin as a fecal pollution indicator in the aquatic environment.

A model adsorption experiment was carried out to determine the factors affecting the uptake of *i*-urobilin by the bottom sediment. The particle size of sediment affected the adsorption of *i*-urobilin. Furthermore, the content of *i*-urobilin in sediment agreed with the model adsorption experiment. It was reported previously that the content of *i*-urobilin and suspended solids in river water increased after rainfall. These indicated that *i*-urobilin moved with suspended solids, which would precipitate at the river mouth. These results suggested that sediment samples should be collected at the mouth of the river, where *i*-urobilin was concentrated.

Keywords — *i*-urobilin; movement; adsorption; particle size; suspended solid

Introduction

The water quality of urban rivers in Japan, where the population is concentrated around large cities, is worsening due to pollution by a large amount of discharge from sewage treatment plants. Despite this situation, some of the urban rivers are used and some actually must be used as a water supply source. From a public health standpoint, it is important to determine whether an urban river is polluted with fecal matter.

Indicators currently used to estimate fecal pollution of water are the concentration of ammonia nitrogen, the amount of total coliform, fecal coliform and coliphage, and the concentration of coprostanol. However, these indicators suffer from the following disadvantages: they increase or decrease after being discharged into the aquatic environment, and the methods required to deter-

mine their quantity are complex.¹⁻⁴⁾

Urobilinogens are tetrapyrrole compounds produced in the human spleen and secreted into the duodenum through the gall bladder. Urobilins, oxidized products of urobilinogens, are originated from fecal matter and urine, and can therefore serve as a direct indicator of fecal contamination. At present, urobilins in urine are quantified colorimetrically and the amount is used clinically as an indicator to estimate hepatic function.⁵⁾ However, they have never been used to detect of fecal pollution in an aquatic environment. In a previous paper, we developed a highly sensitive method for detecting urobilins by high performance liquid chromatography (HPLC) with fluorescence detection.⁶⁾

During an investigation of the distribution of urobilins in an aquatic environment, it became apparent that the content of *i*-urobilin in the bottom sediment was higher at the

mouth of river, and its content in river water was higher after a rainfall.⁷⁻⁹⁾ Only *i*-urobilin was detected in environmental samples, suggesting that *i*-urobilin might be more stable than *l*-stercobilin. Therefore, we decided that *i*-urobilin could be used as a new fecal pollution indicator.⁷⁻⁹⁾ In order to use it as an indicator of this contamination, it was desirable to determine the mechanism of *i*-urobilin movement in the river. The adsorption of urobilin in the sediment is considered to be one factor in this mechanism.

This work was carried out using bottom sediment, suspended solids (SS) and water samples from urban rivers. The factors affecting distribution of *i*-urobilin in the sediment were estimated from the *i*-urobilin distribution ratio (*K_d*) between the sediment and liquid phase.

Experiments

Sampling of Water and Sediment — Surface water and bottom sediment samples were collected in October, 1991, from the lower reaches of rivers which are not greatly polluted by waste water from the Miura peninsula, and from rivers in Tokyo which are typical urban rivers.

Determination of *i*-Urobilin — A mixture of 100 wet g of river bottom sediment and 1000 ml of 0.1 M Tris-HCl buffer (pH 10.0) was shaken at 80 rpm for 1 h at room temperature. The supernatant, after being centrifuged at 5000 rpm for 15 min, was extracted with chloroform at pH 1.0 and evaporated. The residue was dissolved in methanol and filtered through a 0.45 μm membrane filter, then the *i*-urobilin in the filtrate was measured by HPLC.⁸⁾

i-Urobilin in river water was extracted with chloroform at pH 1.0, and its content was determined by the above method.

i-Urobilin adsorbed by SS was determined as follows: 2000 ml of river water was collected, 1000 ml was extracted directly with chloroform, and the other 1000 ml was filtered with No. 5C filter paper and extracted with chloroform. The contents of *i*-urobilin in the two segments was

then determined. From the difference between *i*-urobilin content before (C1) and after (C2) filtration, the amount in SS was calculated as follows:

$$i\text{-Urobilin in SS (ng/mg)} = (C1 - C2) / SS$$

The HPLC conditions for determination of *i*-urobilin was as follows: A Shiseido CAPCELL PAK C18 (250 × 4.6 mm i.d., 5 μm) column packed with ODS-Hypersil was used. The excitation and emission wavelengths for fluorescence detection were 485 nm and 513 nm, respectively, and the flow-rate was 1.0 ml/min. The eluent consisted of 0.1% zinc acetate, 75 mM boric acid, 15 mM sodium chloride, and 75% methanol, and was adjusted to pH 6.0 with hydrochloric acid.⁶⁾ *i*-Urobilin used as a standard was purchased from Porphyrin Products Inc. (Logan, UT, USA).

Water and Sediment Analyses — Chemical oxygen demand (COD), ignition loss (IL) and ammonia nitrogen (NH₄-N) were determined according to the Standard Method of Analysis for Hygienic Chemists.¹⁰⁾

SS in river water was determined as follows: The water was filtered through a No. 5C filter paper, which was dried in an air oven at 105°C until a constant weight was reached, and the filter paper was then dried again under the same conditions. The increment was defined as the suspended solid.

Adsorption Studies — The river bottom sediment of Sakai River (Kanagawa pref.) was fractionated on the basis of grain size by passing it through 12 (1.25 mm), 150 (0.105 mm) and 400 (0.037 mm) mesh sieves successively. The suspension of <0.037 mm fraction was centrifuged at 3000 rpm for 15 min, and the sediment was defined as the particles <0.037 mm. The fractionated sediment was dried in an air oven at 105°C until a constant weight was reached. Varying amounts of the fractionated sediment were added to 4 ml of the buffer solution (pH 7.0) containing 1 mg/l *i*-urobilin, and shaken for 3 h at room temperature. Under these conditions, the *i*-urobilin adsorption attained equilibrium within 1 h.⁸⁾ The equilibrium *i*-urobilin concentration in the liquid phase was detected as follows: the mixture was

centrifuged at 3000 rpm for 5 min, and the supernatant was filtered through a 0.45 μ m membrane filter, then the *i*-urobilin in the filtrate was measured by HPLC. The value of K and n were estimated from the plots of $\log S$ v.s. $\log C$ according to Freundlich's equation as follows:

$$S = KC^n: \text{Freundlich's equation}$$

$$\log S = \log K + n \log C$$

C is the equilibrium *i*-urobilin concentration in the liquid phase (mg/l);

S is the content of *i*-urobilin adsorbed by the sediment (mg/kg);

$$S = (1 - C) (\text{mg/l}) / \text{sediment (kg)} \times 0.004 \text{ (l)}$$

K is the equilibrium constant indicative of the strength of adsorption;

n is the degree of non-linearity.

Distribution of *i*-Urobilin in Sediment Fractions—Two bottom sediments from Sakai River were prepared, which were fractionated on the basis of grain size by passing through 12 (1.25 mm), 150 (0.105 mm) and 400 (0.037 mm) mesh sieves under a wet condition to prevent *i*-urobilin degradation. The suspension of fraction <0.037 mm was centrifuged at 3000 rpm for 15 min, and the sediment was used as the <0.037 mm particles. We previously reported the adsorption equilibrium of *i*-urobilin was affected by the pH value.⁸⁾ The pH value of this sediment was 7.67, and hence the amount of *i*-urobilin in the water phase was negligible compared to that adsorbed in sediment. Fractions from one of these sediments were dried in an air oven at 105°C until a constant weight was reached to estimate the distribution of grain size. The other sediment fractions were mixed with 0.1 M Tris-HCl buffer (pH 10.0) and shaken at 80 rpm for 1 h at room temperature. The supernatant after centrifugation at 3000 rpm for 15 min was extracted with chloroform at pH 1.0 and evaporated. The residue was dissolved in methanol and filtered through a 0.45 μ m membrane filter, and the *i*-urobilin in the filtrate was measured by HPLC.

Results

The Adsorption Equilibrium of *i*-Urobilin

Distributions of *i*-urobilin, IL, COD and

NH₄-N in aquatic environments in the Tokyo area and the Miura peninsula are shown in Table I. Larger quantities of *i*-urobilin were detected in Tokyo rivers, which are believed to be polluted by fecal matter, than in the Miura rivers, which are relatively clean. The volume of the other water pollution indicators in the Tokyo area were also larger indicating that these urban rivers were more heavily polluted. The distribution ratio (Kd) of *i*-urobilin in aquatic environments was calculated as follows:

$$Kd = \frac{\text{i-Urobilin in sediment (ng/kg)}}{\text{i-Urobilin in water (ng/l)}}$$

The value of Kd in Tokyo was higher than that of the Miura peninsula. Because the *i*-urobilin adsorption might have been affected by water pollution, it was of interest to lean whether there was a correlation between Kd and IL, COD and NH₄-N; this was checked and no correlation was found. Thus, it was concluded that the adsorption equilibrium was affected by characteristics of the sediment, not the quality of the water.

On the basis of Table I, the correlation between the amounts of *i*-urobilin in river water and bottom sediment is shown in Fig. 1. The logarithm amounts of *i*-urobilin in the two factors were correlated with each other ($r=0.847$, 21 data). When *i*-urobilin content was equilibrated between water and river bottom sediment, the constants of K and n as calculated by Freundlich's equation were 1.33×10^{-4} and 1.21, respectively. The value of *i*-urobilin in the bottom sediment was higher than that in water, indicating that *i*-urobilin tended to be adsorbed in the sediment.

The Effect of Particle Size on Adsorption

Figure 2 shows the effect of particle size of the bottom sediment on *i*-urobilin adsorption. Bottom sediment from the Sakai River was fractionated on the basis of grain size by passing it through 12 (1.25 mm), 150 (0.105 mm), and 400 (0.037 mm) mesh sieves. The values of K and n were estimated from the

TABLE I. Distribution of Water Pollution Indicators in Urban Rivers

River	Sediment		Water			<i>Kd</i> of <i>i</i> -Urobilin (S/W)
	<i>i</i> -Urobilin ng/dry-kg	IL %	<i>i</i> -Urobilin ng/l	COD mg/l	NH ₄ -N mg/l	
(Tokyo)						
Ichinoesakai	800	8.63	75.6	10.8	8.32	10.6
Shin	2 340	15.1	39.3	0.80	5.84	59.5
Kyunaka	43 200	26.2	6 990	52.9	14.4	6.18
Kitajukken	531	8.63	4.0	9.62	12.7	133
Yokojukken	1 780	11.4	7.5	44.9	15.4	237
Ayase	639	13.5	25.3	13.2	4.63	25.3
Kenaga	1 850	6.55	76.2	6.81	4.75	24.3
Kanda	16 300	5.91	128	30.9	8.74	127
Shibuya	78 300	0.69	304	27.7	2.58	258
Meguro	6 890	1.65	835	26.4	30.7	8.25
Zenpukuji	6 550	1.24	105	35.3	6.44	62.4
(Miura peninsula)						
Miya	193.0	11.6	N.D.	27.3	4.03	—
Jiju	60.5	15.0	N.D.	16.6	7.74	—
Hirasaku	1 270	14.7	26.8	15.6	9.88	47.4
Nobi	N.D.	0.43	N.D.	8.61	3.85	—
Nagasawa	2.5	0.51	N.D.	39.7	10.37	—
Tsukui	N.D.	5.82	N.D.	27.7	7.99	—
Ichiban	N.D.	10.2	6.54	17.6	1.36	—
Kawama	12.0	8.67	N.D.	19.6	3.57	—
Take	10.8	1.78	47.7	21.6	10.18	0.23
Matsukoe	210.0	1.26	10.3	22.6	3.83	20.4
Maeda	2.1	0.57	1.61	24.0	0.46	1.30
Shimoyama	1.7	4.23	3.46	34.1	0.66	0.49
Morito	17.9	3.33	6.91	14.0	3.27	2.59
Tagoe	2.5	1.30	0.61	26.0	0.83	4.10
Nameri	3.9	2.23	0.74	17.0	0.29	5.27
Sakai	1 070	2.20	N.D.	16.0	11.98	—
Hikiji	3.5	2.01	1.12	18.0	6.70	3.13
Tsujido	12.5	2.01	1.24	23.0	16.86	10.1

K_d; distribution ratio, N. D.: not detected.

plots of $\log S$ v.s. $\log C$ according to Freundlich's equation (Table II). Results showed that the value of K was smaller in sediment with larger grain size, while, the value of n increased with grain size. This indicated that the value of K might be affected by the surface area, and that the value of n might be reflected by the *i*-urobilin adsorption style to the particle, e.g. characteristics and surface shape of the particle.

The amount of *i*-urobilin in each fractionated portion of Sakai River bottom sediment is shown in Table III. The distribution of >1.25 mm, >0.105 mm, >0.037 mm

and <0.037 mm fraction decreased in that order. Content in the smallest grain size fraction was greater than in the other fractions, so that the highest distribution of *i*-urobilin was found in the smallest grain size fraction. The content of *i*-urobilin in the sediment thus could depend on the distribution of sediment particle size.

The Amount of *i*-Urobilin in Suspended Solid

Table IV shows the distribution of *i*-urobilin in the liquid phase and suspended solids in the river water. Both the average content of *i*-urobilin and SS in river water

Fig. 1. Relationship between the Amount of *i*-Urobilin in River Waters and River Sediments: (Freundlich's Eq. Plot)

Fig. 2. Freundlich's Equation Plots in an Adsorption Equilibrium of *i*-Urobilin

(○) <0.037 mm; (△) >0.037 mm; (□) >0.105 mm; (■) >1.25 mm.

after a rain were higher than with no rainfall. The previous paper indicated that both the amounts of *i*-urobilin and SS in river water were higher after a rain,^{7,9)} suggesting that the increase in river water by rainfall could stir up the river bottom sediment, and that *i*-urobilin was transported from upper to lower reaches. If *i*-urobilin contents were equilibrated between suspended solids and water, the constants of *K* and *n* calculated by Freundlich's equation would be 0.251 and 0.71, respectively. This *K* value between the suspended solid and the liquid phase was higher than that between the bottom sediment and the liquid

TABLE II. Effects of Grain Size on Freundlich's Equation Constants of *K* and *n*

Grain size mm	<i>K</i>	<i>n</i>
>1.25	5.01×10^{-3}	4.85
1.25–0.105	1.48×10^{-3}	4.07
0.105–0.037	2.88×10^{-3}	2.58
<0.037	2.57×10^{-2}	1.34

TABLE III. Distribution of *i*-Urobilin in the Sakai River Sediment

Grain size		<i>i</i> -Urobilin		Water content
mm	%	ng/dry-kg	%	%
>1.25	57.0	195	3.5	20.5
1.25–0.105	34.2	1 130	12.0	25.1
0.105–0.037	6.6	13 400	27.5	60.5
<0.037	2.2	82 800	57.0	81.1

phase shown in Table II. Further, the content of *i*-urobilin in the suspended solid was about 3 orders of magnitude higher than that in the river bottom sediment. These results indicate that a considerable amount of *i*-urobilin must be transported in an adsorbed form on suspended solids in addition to that in a solubilized form in an aquatic environment.

Distribution of *i*-Urobilin in Rivers

The distribution of *i*-urobilin in river water and sediment is shown in Fig. 3. The Hirasaku and Take are small rivers running through the Miura peninsula. The content of *i*-urobilin in the sediment increased with proximity to the river mouth, while the content in the water decreased. The *Kd* increased at the river mouth therefore, was higher, suggesting that *i*-urobilin accumulated in the bottom sediment at this location by sedimentation of suspended solids and by adsorption to the particles in the sediment.

Discussion

The smallest particle size of river sediment is found at the river mouth, and SS settle down as a result of a salting out effect

TABLE IV. Amount of *i*-Urobilin Adsorbed by Suspended Solids River Water

River	Sampling point	SS mg/l	Total <i>i</i> -urobilin ng/l	Adsorbed <i>i</i> -urobilin		Weather
				ng/mg	%	
Shingashi	Hasune (down)	0.08	2.40	7.00	23	no rainfall
Shingashi	Hayase (middle)	2.06	14.0	5.21	77	
Shingashi	Shibamiya (up)	8.25	11.3	0.29	21	
Naka	Nakagawa	17.7	4.31	0.13	55	
Hirasaku	Hinode	0.77	224	172	59	
Sakai	Nishihama	14.3	57.2	0.64	16	
Average		7.19	52.2	30.9	42	
Kanda	Omokage (down)	15.0	16 400	136	12	rainy
Kanda	Tatsumi (up)	20.8	5 220	86.6	34	
Shingashi	Hasune (down)	4.63	1 070	220	95	
Shingashi	Tokumaru (middle)	34.8	400	10.3	89	
Shingashi	Shibamiya (up)	419	58.6	0.11	79	
Kyunaka	Hirai	30.5	1.87	0.032	52	
Average		87.5	3 860	75.5	60

Fig. 3. Horizontal Distributions of *i*-Urobilin in River Water and Sediment

(○) Water; (●) Sediment.

and a slower stream.¹¹⁾ The adsorption equilibrium of *i*-urobilin was not affected by sea-water (unpublished data). Therefore, the adsorption of *i*-urobilin in the sediment was affected by sediment particle size, and small size particles, including suspended solids, are viewed as playing an important role in *i*-urobilin movement in an aquatic environment.

In conclusion, the movement of *i*-urobilin in rivers can be described as follows (Fig. 4). Some portions of *i*-urobilin discharged from sewage is adsorbed quickly by SS in river water, and moves along with the stream flow. Some is also easily adsorbed by small-size particles in the sediment, which are apt to be stirred up by the movement of the water and which make up a part of the suspended solids. A large part of *i*-urobilin is therefore transported with SS along the river and SS usually accumulate at the river mouth, leading to the greater amount of *i*-urobilin in the river bottom sediment. The *i*-urobilin in the sediment decomposed slowly under anaerobic conditions. Our preliminary experiments showed that the product is relative stable in sediment more under anaerobic condition for more than two weeks.¹²⁾

The concentration of *i*-urobilin can

Fig. 4. The Movement of *i*-Urobilin in a River

reflect the fecal pollution of a river, and if we want to use it as an indicator of fecal contamination, it is preferable to collect bottom sediment samples at the mouth of rivers, and to determine the content of the small particle fractions.

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