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## A High Selective Chemiluminescent Probe Derived from Iso-luminol Enabling High Sensitive Determination of Ferrous Ions in the Environmental Waters

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## Keywords

1 nont

4-amino-5-thiocyanato-phthalylhydrazine | Chemiluminescence | Ferrous ions | Determination | Environmental Waters

## Main observation and conclusion

A new chemiluminescence (CL) reagent named 4-amino-5-thiocyanato-phthalyl-hydrazine (iso-luminol-SCN) is synthesized by the bromide-mediated substitution reaction of iso-luminol with sodium thiocyanate in dimethyl formamide (DMF) at room temperature. Strong CL is observed for iso-luminol-SCN oxidized by hydrogen peroxide ( $H_2O_2$ ) in the presence of Fe<sup>2+</sup>, with the CL intensity strongly depending on the concentration of Fe<sup>2+</sup> and hardly interfering by other common metal ions. Based on this fact, a flow injection CL method is established for the high sensitive and selective determination of Fe<sup>2+</sup>. The CL intensity was linearly correlated with the concentration of Fe<sup>2+</sup> in the range of  $1.0 \times 10^{-8}$  mol/L to  $1.0 \times 10^{-5}$  mol/L following the equations to  $CL_{net}=166.3C+57.7$  (C,  $\times 10^{-6}$  mol/L; n=7; R<sup>2</sup>= 0.9988). The detec-<sup>+:</sup> on limit is calculated to be  $3.2 \times 10^{-9}$  mol/L Fe<sup>2+</sup> based on the  $3S_0/K$  principle. The relative standard deviation (RSD) was 3.7% for  $1.0 \times 10^{-6}$ mol/L Fe<sup>2+</sup> (*n*=11), a support for the precision of Fe<sup>2+</sup> measurement. The recoveries of Fe<sup>2+</sup> were obtained in the range of 9.88-101.6% in the real water samples, showing the applicability and reliability by the proposed method. The possible CL mechanism is proposed based on the kinetic characteristic of the CL reaction and the CL spectrum.

Comprehensive Graphic Content

The magic n	nirror	H <sub>2</sub> O <sub>2</sub>		
* Corresponding authors. E-mail: <u>zhshhai512@1</u>	<u>63.com</u> (S. Zhang)		View HTML Article	Supporting Information
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## **Background and Originality Content**

As the most abundant transition metal for both plants and animals,<sup>[1]</sup> iron is essential for sustaining life and ubiquitous in the environment and in biology,<sup>[2]</sup> plays crucial roles in many physiological and pathological processes such as oxygen transportation,<sup>[3]</sup> participates in DNA and RNA synthesis,[4] increases the synthesis r tes of some proteins,<sup>[5]</sup> acts as the cofactor in many enzymatic eactions,<sup>[6]</sup> etc. Previous studies have shown that the one-electron transfer between the two principal oxidation states (1) arrous ions and ferric ions, Fe<sup>2+</sup> and Fe<sup>3+</sup>) of iron is one of the important redox pairs<sup>[7]</sup> and that it is easily accomplished in bio-<sup>1</sup> gical systems.<sup>[8]</sup> The total iron content in a well-nourished people is about 4 g (70% in Hgb, 25% in storage),<sup>[9]</sup> with either iron deficiency or overload leading to the occurrence of many diseases<sup>[10]</sup>. The decreased synthesis of hemoglobin and the consequent cellular oxygen deficiency are the major effects of iron deficiency in animals, known as the most common cause of anemia.<sup>[11]</sup> The accretion of free iron ions, particularly Fe<sup>2+</sup> has been observed in a number of serious central nervous system diseases such as Parkinson's, Huntington's and Alzheimer's diseases,<sup>[9,12]</sup> with the high levels of iron associated with an increased risk for arthritis, diabees, liver disease, heart disease and cancer, etc.<sup>[13]</sup>

Drinking water is an important source of iron for the human body.<sup>[14]</sup> A security limit of iron in a sanitary standard for drinking water was restricted to 2.0 mg/L ( $3.57 \times 10^{-5}$  mol/L) by World Health Organization (WHO), and European Legislation has established a maximum contaminant level (MCL) at 200 µg/L for iron ( $5.6 \times 10^{-6}$  mol/L).<sup>[13]</sup> In addition to its roles in physiological and thological processes mentioned above, evidence shows that iron can also control the mobility, bioavailability and toxicity of other trace metals in the natural water system.<sup>[15]</sup> Therefore, to evelop rapid, sensitive and efficient methods for measuring the amount of iron in environmental waters is of great importance to sure the public health.

Metabolic iron is Fe<sup>2+</sup>,<sup>[5]</sup> with many methods established, such colorimetry,<sup>[9,16]</sup> spectrophotometry,<sup>[15,17]</sup> atomic absorption sr ectrometry (AAS),<sup>[18]</sup> high performance liquid chromatog-<sup>[19]</sup> ion chromatography,<sup>[20]</sup> inductively coupled plasma-mass spectrometry (ICP-MS),<sup>[21]</sup> inductively coupled plasma optical e nission spectrometry (ICP-OES),<sup>[22]</sup> fluorimetry,<sup>[1, 2a, 4, 23]</sup> chemiminescence<sup>[24]</sup> and electrochemical,<sup>[25]</sup> etc., which have been developed for Fe<sup>2+</sup> determination. However, most of the methods n entioned above either lack sufficient sensitivity or require the omplex procedure and expensive analytical equipment, a limit to their potential application. The chemiluminescence method such a luminol-based and other chemiluminescence methods with the dvantages of simple equipment, low cost, high sensitivity, fast analysis and easily automated detection gives its full play for the <sup>2+</sup> determination in water samples<sup>[24]</sup>, but the selectivity for Fe<sup>2+</sup> measuring over other metal ions still needs to be improved.

In this paper, we synthesized a new chemiluminescence (CL) reagent named 4-amino-5-thiocyanato-phthalylhydrazine (iso-luminol-SCN), thus enriching the CL reagent family members. Iso-luminol-SCN oxidized by hydrogen peroxide ( $H_2O_2$ ) in the presence of Fe<sup>2+</sup>, and a strong Fe<sup>2+</sup> concentration-dependent CL was observed. However, other common metal ions can hardly enhance the CL reaction of iso-luminol-SCN and  $H_2O_2$ . Based on this, a new, rapid and simple method with high selectivity and high sensitivity is proposed for the determination of Fe<sup>2+</sup>. Satisfactory results were obtained by using this method for determining Fe<sup>2+</sup> in environmental water samples. Furthermore, the CL reaction mechanism is discussed for iso-luminol-SCN oxidized by  $H_2O_2$  in the presence of Fe<sup>2+</sup>.



**Scheme 1** The key technical step of iso-luminol-SCN synthesis and it is used as the chemiluminescence probe for ferrous ion detection.

## **Results and Discussion**

#### CL selectivity and responsiveness study

**CL** selectivity for ferrous ions Considering the complexity of the actual samples, the iso-luminol-SCN the selective CL response for analyte is of top importance. We examined the CL response of iso-luminol-SCN to Fe<sup>2+</sup> against common metal ions. The CL measurements were conducted with the flow setups as illustrated in **Figure 3**. It can be found in **Figure 1** that Fe<sup>2+</sup> takes overwhelming CL response against the rest metal ions. That is, the obtained iso-luminol-SCN is specific to Fe<sup>2+</sup>.



**Figure 1** Chemiluminescence selectivity of iso-luminol-SCN for Fe<sup>2+</sup> measuring. 1.0×10<sup>-3</sup> mol/L H<sub>2</sub>O<sub>2</sub> and 5.0×10<sup>-6</sup> mol/L iso-luminol-SCN (in 0.1 mol/L pH 7.4 PBS); Metal ions (nitrate, aqueous solution), left to right-blank, 1.0×10<sup>-4</sup> mol/L of NH<sub>4</sub><sup>+</sup>, Li<sup>+</sup>, K<sup>+</sup>, Ag<sup>+</sup>, Mg<sup>2+</sup>, Al<sup>3+</sup>, Ca<sup>2+</sup>, Ba<sup>2+</sup>, Fe<sup>3+</sup>, Fe<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cr<sup>3+</sup>, Sn<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, and Hg<sup>2+</sup>; PMT voltage, -800V.

**Kinetic characteristic of CL reaction** Kinetic characteristics of the CL reactions were examined. The CL intensity–time curve is shown in **Figure 2**. When H<sub>2</sub>O<sub>2</sub> solution was injected into the mixture of iso-luminol-SCN and Fe<sup>2+</sup> (0.1 mL), a strong CL reaction was initiated immediately. The maximum CL intensity was obtained at about 27 s after H<sub>2</sub>O<sub>2</sub> addition. Thereafter, the CL signal declined to the baseline in 300 s approximately. So, the iso-luminol-SCN~ H<sub>2</sub>O<sub>2</sub>~ Fe<sup>2+</sup> CL reaction is a fast CL reaction and our experiment results suggest iso-luminol-SCN a potential application for Fe<sup>2+</sup> detection.



**F** ;ure 2 CL intensity–time curve.  $0.1 \text{ mL } 1.0 \times 10^{-2} \text{ mol/L } H_2O_2$  (in 0.1 mol/L H 7.4 PBS) was injected into the mixture of 0.8 mL  $1.0 \times 10^{-4}$  mol/L iso-luminol-SCN (in 0.1 mol/L pH 7.4 PBS) and 0.1 mL  $1.0 \times 10^{-3}$  mol/L of Fe<sup>2+</sup> v queous solution); PMT voltage, -800V.

#### ptimization of CL conditions

The initial test showed that a high CL selectivity to  $Fe^{2+}$  was observed in 0.1 mol/L pH 7.4 PBS; therefore, 0.1 mol/L pH 7.4 PBS was used to control the reaction medium in the following experiments. In order to obtain the optimum analytical conditions of  $e^{2+}$ , the parameters for flow system, flow rate, iso-luminol-SCN concentration and  $H_2O_2$  concentration were optimized using the single factor rotation method.

Effect of flow systems In order to obtain the maximal CL signal, several flow systems were designed. When Fe<sup>2+</sup> solution is carried hy air to react with the mixing stream of iso-luminol-SCN and H<sub>2</sub>O<sub>2</sub>, the weak CL signal was detected (Fig. S2). When employing the stem shown schematically in Figure 3 and Fe<sup>2+</sup> is carried by H<sub>2</sub>O<sub>2</sub> solution to react with iso-luminol-SCN, no CL signal was detected. If stead, a strong CL signal with good repeatability was captured imediately by adopting iso-luminol-SCN to carry Fe<sup>2+</sup> solution to react with H<sub>2</sub>O<sub>2</sub>. So, the flow system shown in Figure 3 was se-<sup>1</sup> cted.



gure **3** Schematic diagram of CL flow system: (a)  $H_2O_2$  solution; (b)  $Fe^{2*}$  standard or sample solution; (c) iso-luminol-SCN solution. P: peristaltic imp; V: injection valve; F: flow cell; W: waste; HV: high voltage; PMT: photomultiplier tube; PC: personal computer.

Effect of flow rate on CL intensity The flow rate influences the analytical sensitivity in adopting the flow-injection chemiluminescence analysis system shown schematically in Figure 3. The effect of the flow rate of pump on CL intensity was examined in the range of 1.5~4.5 mL/min. The results showed that the CL signal increased with the increasing of flow rate due to this rapid CL reaction. Accordingly, the maximal flow rate 4.5 mL/min was selected in the following experiments (Figure4).



Figure 4 Effect of flow rate of pump on CL intensity.  $H_2O_2$ ,  $1.0 \times 10^{-3}$  mol/L (in 0.1 mol/L pH 7.4 PBS); Fe<sup>2+</sup>,  $8.0 \times 10^{-5}$  mol/L (aqueous solution); iso-luminol-SCN,  $5.0 \times 10^{-6}$  mol/L (in 0.1 mol/L pH 7.4 PBS); PMT voltage, -600V.

Effect of concentration of hydrogen peroxide  $H_2O_2$  reacts with  $Fe^{2+}$  to produce  $\cdot OH$ , acting as the oxidant in the CL reaction, and hence the concentration of  $H_2O_2$  influences the CL signal. The effect of  $H_2O_2$  concentration on the CL intensity was investigated in the range of  $1.0 \times 10^{-4}$  mol/L to  $5.0 \times 10^{-3}$  mol/L. Experiments showed that the maximal CL signal could be obtained with  $1.0 \times 10^{-3}$  mol/L  $H_2O_2$  (Figure 5).



Figure 5 Effect of  $H_2O_2$  concentration on CL intensity. iso-luminol-SCN,  $5.0 \times 10^{-6}$  mol/L (in 0.1 mol/L pH 7.4 PBS); Fe<sup>2+</sup>, 80.0  $\mu$ M (aqueous solution);  $H_2O_2$ , varying from 0.1 to  $5.0 \times 10^{-3}$  mol/L (in 0.1 mol/L pH 7.4 PBS); PMT voltage, -600V.

Effect of concentration of iso-luminol-SCN The concentration of CL reagent has an effect on the CL intensity, and therefore, the effect was investigated of iso-luminol-SCN concentration on the CL intensity in the range of 1.0  $\mu$ M to 0.1 mM. The results showed that the CL signal increased with the increasing of iso-luminol-SCN till its concentration reached 25.0  $\mu$ M with the concentration selected to obtain the maximal CL signal (Figure 6).



Figure 6 Effect of iso-luminol-SCN concentration on CL intensity.  $H_2O_2$ , 1.0 ×10<sup>-3</sup> mol/L (in 0.1 mol/L pH 7.4 PBS); Fe<sup>2+</sup>, 8.0×10<sup>-5</sup> mol/L (aqueous solu-

## Report

tion); iso-luminol-SCN, varying from 1.0×10<sup>-6</sup> mol/L to 1.0×10<sup>-4</sup> mol/L (in 0.1 mol/L pH 7.4 PBS); PMT voltage, –500V.

#### Dynamic range, detection limit and RSD

In the optimized conditions, the CL intensity was linear to Fe<sup>2+</sup> concentration in the range of  $1.0 \times 10^8$  mol/L to  $10.0~\mu M$  following equations to CL<sub>net</sub>=166.3C+57.7 (C, $\times 10^6$  mol/L; n=7; R<sup>2</sup>= 0.9988) (Figure 7). The detection limit is calculated to be  $3.2 \times 10^9$  mol/L Fe<sup>2+</sup> according to the IUPAC principle. The RSD was found to be 3.7% by 11 replicate determinations of  $1.0 \times 10^6$  mol/L Fe<sup>2+</sup>.



...gure 7 Linear relationship between reaction of CL intensity and Fe<sup>2+</sup> (1.0×10<sup>-8</sup> mol/L ~1.0×10<sup>-5</sup> mol/L) (aqueous solution). H<sub>2</sub>O<sub>2</sub>, 1.0×10<sup>-3</sup> mol/L (in 0.1 mol/L pH 7.4 PBS); iso-luminol-SCN, 2.5×10<sup>-5</sup> mol/L (in 0.1 mol/L pH 7.4 PBS); PMT voltage, -900V.

#### Interference study

The influence of foreign species was investigated by measuring a standard solution to  $1.0 \times 10^{-6}$  mol/L Fe<sup>2+</sup> to which increasing amounts of adscititious species were added. A substance was c nsidered to be of no interference for the variation of the CL intensity to be within ±5%. It was found that the tolerable concentration ratios of foreign species to 1.0  $\mu$ M of Fe<sup>2+</sup> were over D-fold for Cu<sup>2+</sup>, 60-fold for Co<sup>2+</sup>, Hg<sup>2+</sup>, 100-fold Fe<sup>3+</sup>, Ca<sup>2+</sup>, >200-fold for NH<sub>4</sub><sup>+</sup>, Li<sup>+</sup>, Ag<sup>+</sup>, Mg<sup>2+</sup>, Ba<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Sn<sup>2+</sup>, Pb<sup>2+</sup>, C<sup>3+</sup>, Al<sup>3+</sup>; 10-fold for S<sup>2-</sup>, 25-fold SO<sub>3</sub><sup>2-</sup>, 20-fold NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, CO<sub>2</sub><sup>2-</sup> and >100-fold for F<sup>-</sup>, Cl<sup>-</sup>, Br, l<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>. Therefore, the proposed method has adequate selectivity for the determination of Fe<sup>2+</sup> in real water samples.



Figure 8 Influence of coexisting ions on CL intensity.  $H_2O_2$ ,  $1.0 \times 10^{-3}$  mol/L (in 0.1 mol/L pH 7.4 PBS); iso-luminol-SCN,  $2.5 \times 10^{-5}$  mol/L (in 0.1 mol/L pH 7.4 PBS); Fe<sup>2+</sup>,  $1.0 \times 10^{-6}$  mol/L (aqueous solution); PMT voltage, –900V.

#### Analytical application

In order to evaluate the applicability and reliability by the proposed methodology, it was applied to detect  $Fe^{2+}$  in the environmental water samples. Three water samples from Hanjiang

River, tap water from our laboratory, and commercial available mineral spring water. The sample solutions were prepared in Section 4.4 and analyzed in Section 4.3. To examine the accuracy, the recovery experiments were carried out with all samples spiked with iron standard solutions in two levels. The obtained recoveries were reasonable for  $Fe^{2+}$  in samples in a range of 95%~104%. The results of the determination for sample solution are shown in **Table 1**.

Tab1 Results for determination of Fe <sup>2+</sup> in water	r samples
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Sample	Fe <sup>2+</sup> added(µg/L)	Found(µg/L)	Recovery(%)
Hanjiang River water <sup>a</sup>	0	89.7 ± 1.9	
	100	188.9 ± 1.8	99.1 %
	200	290.7 ± 2.3	101.1 %
Tap water	0	68.1 ± 1.5	
	100	168.7 ± 1.7	100.9 %
	200	269.2 ± 2.1	101.6 %
Mineral spring water	0	163.7 ± 2.2	
	100	265.7 ± 1.6	101.2 %
	200	361.7 ± 2.4	98.8 %

 $^{\it a}$  (Collected from Hanyin County, An'kang city.)  $^{\it b}$  (Mean value ± standard deviation (n = 3) and 95% confidence limit.)

#### Mechanism speculation

When H<sub>2</sub>O<sub>2</sub> was injected into the mixture of iso-luminol-SCN and Fe<sup>2+</sup>, a strong CL signal was observed; however, no CL signal was observed in the absence of Fe2+. It has been confirmed that the inorganic SCN<sup>-</sup> and isomers of -SCN can be fast oxidized by H<sub>2</sub>O<sub>2</sub> in producing a series of complex intermediates.<sup>[26]</sup> We speculated that -SCN substituent also could be reacted with  $H_2O_2$  in generating the corresponding intermediate, with the possible mechanism of iso-luminol-SCN~H<sub>2</sub>O<sub>2</sub>~Fe<sup>2+</sup> CL reaction proposed in the following. H<sub>2</sub>O<sub>2</sub> reacted with Fe<sup>2+</sup> to produce hydroxyl radical (·OH), which cooperated with excess  $H_2O_2$  to oxidize iso-luminol-SCN to produce the excited state 4-amino-5-thio- cyanatophthalate (4-APH-SCN\*). 4-APH-SCN\* came back to the ground state and released its energy in the form of light radiation (λmax=448 nm). The mechanism was assumed to be synergistically oxidized by ·OH and H<sub>2</sub>O<sub>2</sub>, which could be expressed simply as follows (Supporting information, Fig. S15 and S18):

- (1)  $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + \bullet OH$
- (2) OH + iso-luminol-SCN+  $2H_2O_2 \rightarrow 4$ -APH-SCN\* +  $2H_2O$ +  $N_2$
- (3) 4-APH-SCN\*  $\rightarrow$  4-APH-SCN + hv ( $\lambda$ max = 448 nm)

#### Conclusions

In this work, a novel CL reagent iso-luminol-SCN was synthesized, enriching the CL reagent family members. Based on its CL properties, a good selectivity and high sensitivity method for the determination of  $Fe^{2+}$  was established. The method was satisfactorily applied to detect the  $Fe^{2+}$  in environmental water samples. Furthermore, the mechanism for the iso-luminol-SCN~H<sub>2</sub>O<sub>2</sub>~Fe<sup>2+</sup> CL reaction was briefly discussed.

#### Experimental

## Apparatus

The flow injection CL analyzer (IFFM-E, Xi'an Remex analysis Instrument Co., Ltd., China) was equipped with an automatic injection system and a detection system. The flow cell was a coil of glass tube positioned in front of the detection window of the PMT. CL dynamic profile and brightness are measured with the above-mentioned CL analyzer; CL spectra were measured with a fluorescence spectrometer (RF-5301PC, Shimadzu Co., Ltd., Japan).

#### **Reagents and solutions**

Unless otherwise stated, all the chemical reagents are used directly without further purification. Hydrogen peroxide ( $H_2O_2$ , 30%) is purchased from Tianjin Tianli Chemical Reagents Ltd. (Tianjin, China), sodium hypochlorite(NaClO, active chlorine $\geq$ 10%) is purchased from Tianjin Fuchen Reagents Ltd. (Tianjin, China), and All other AR grade chemicals, including sodium hydrox-ide(NaOH), disodium hydrogen phosphate(Na<sub>2</sub>HPO<sub>4</sub>), sodium di-hydrogen phosphate dihydrate(NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O), ferrous sulfate heptahydrate (FeSO<sub>4</sub>·7H<sub>2</sub>O), etc. are purchased from Sinopharm C mical Reagent Co., Ltd. (Xi'an, China).

The standard solution to  $Fe^{2+}$  (1.0×10<sup>-2</sup> mol/L) was prepared '  $\prime$  dissolving 0.278 g FeSO<sub>4</sub>·7H<sub>2</sub>O in water and diluting to 100 mL. The 1.0×10<sup>-4</sup> mol/L stock solution to iso-luminol-SCN was prepared b  $\prime$  dissolving 23.5 mg iso-luminol-SCN (The synthetic processes e detailed in the *Supporting information*) in 20 mL 1.0×10<sup>-2</sup> mol/L NaOH solution, and then by diluting them to 1000 mL with 1 mol/L pH 7.4 PBS. The 1.0×10<sup>-3</sup> mol/L H<sub>2</sub>O<sub>2</sub> solution was prepared by diluting the H<sub>2</sub>O<sub>2</sub> with 0.1 mol/L pH 7.4 PBS directly.

Water is purified by using a Millipore water purification system (18.2 M $\Omega$ ·cm, Direct-Q 3UV, Millipore, FR) and used throughout the whole experiment.

#### Sample preparation

All samples including the river water collected from Hanjiang River, tap water obtained from our laboratory and bottled mineral water purchased from local market were filtered through 0.22  $\mu$ m filter paper and saturated with nitrogen at room temperature in order to minimize the influence of the dissolved oxygen in the samples, and they are used for the chemiluminescence determination as soon as possible.

## CL signal measurement

Investigations of CL behaviors were performed using the system shown schematically in Figure 1. Flow tubes (a, b and c) were connected with H<sub>2</sub>O<sub>2</sub>, Fe<sup>2+</sup> standard or sample solution and p-luminol-SCN, respectively. The sample or Fe<sup>2+</sup> standard solution was injected into iso-luminol-SCN solution by a six-way injector valve, and then merged with H<sub>2</sub>O<sub>2</sub> solution through a Y-piece to produce CL in the flow cell. The CL intensity produced by H<sub>2</sub>O<sub>2</sub> concorn was considered as the blank. The concentration of Fe<sup>2+</sup> was quantified via the peak height (CL intensity), which was obtained by subtracting the blank CL intensity from that of the sample or Fe<sup>2+</sup> standard solution.

## upporting Information

The supporting information for this article is available on the WW under <u>https://doi.org/10.1002/cjoc.2021xxxxx</u> or from the author.

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## **Entry for the Table of Contents**

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