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Chiral recognition of dextran sulfate with D- and L-cystine studied by multiwavelength surface plasmon resonance

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Abstract—A multiwavelength surface plasmon resonance (mwSPR) approach has been developed to study the chiral discrimination between D- and L-cystine (Cys). A monolayer of the two enantiomers was separately assembled on a pair of gold films of about 50 nm in thickness and their resonance wavelength shifts, $\Delta\lambda$, were measured under a continuous flow of an identical chiral probe solution. Dextran sulfate (DS) was found to be an excellent chiral probe because it has rich chiral centers and is large enough to produce sensitive mwSPR response. The chiral discrimination was investigated either by $\Delta\lambda_{max}$, the maximum resonance wavelength shift in recognition equilibrium, or by recognition kinetics ($\Delta\lambda$ vs time). The equilibrium data showed that D-Cys yielded always the smaller $\Delta\lambda_{max}$ as compared to L-Cys at pH 5.0 or above. This differentiation was enlarged by raising the probe content and became naught at pH <4.5. The kinetic results showed that, as pH increased from 5.0 to 7.5, the non-equilibrium $\Delta\lambda$ for D-Cys rose above the level for L-Cys at the first 30 s of recognition but came back gradually to its equilibrium position after about 150 s, with crossing at 50–150 s depending on DS concentration. This phenomenon was thought to be the result of molecular orientation adjustment after DS binding to D-Cys. Both kinetic and thermodynamic mechanisms were thus considered to be deeply involved in the investigated chiral recognition system.
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1. Introduction

Chirality is a determinant property of most biological molecules and is involved in many physiological processes such as metabolism, and intra- and intercellular communication. A particular enantiomer may serve as an irreplaceable building block in the formation of a functional biomacromolecule. Amino acids are just such a type of chiral compounds used to build up peptides and proteins, and are of utmost importance in chemical, pharmaceutical, and food industries. The predominance of L-amino acids in life forms is a known phenomenon, but recent studies have revealed that their D-forms also played some undesired roles in the human body, causing serious disorders such as Alzheimer disease.[1–3] All these have pointed at the necessity to well inspect the chiral recognition and its kinetics, but this remains a serious challenge due to shortage of methodology.

To address the problem, various techniques and methods have been developed,[4–7] of which HPLC and CE (capillary electrophoresis) are the most powerful tools but labeling the solutes without any chromophore[8,9] is commonly required for sensitive detection. Electrochemical methods[10,11] may allow to eliminate the labeling step but suffer from losing selectivity and interference due to the presence of other components. Scanning probe methods such as STM[12] (scanning tunnel microscopy) allow to directly measure a chiral structure or complex, but it will take time for them to become dynamic methods since they commonly provide only the final topographical picture.

More recently, a new method of surface plasmon resonance (SPR)[13–15] has been explored, featuring label-free and able to perform in situ and real-time

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measurements of molecular interaction events happening nearby or on a metal sensing surface. Hofstetter et al. were the first to develop SPR immunosensors for the detection of native amino acid enantiomers.[16] Later, Kieser et al. tried the SPR measurement of halodiethers with modified cyclodextrins.[17] Last year, Corradini et al. reported a method to probe DNA by SPR with chiral nucleic acid conjugates.[18] One can thus anticipate that SPR could become a promising tool in the investigation of chiral recognition and this was tried in our laboratory, resulting in the elaboration of a multiwavelength SPR (mwSPR) system.

In this paper, an approach will be discussed to get thermodynamic and/or kinetic insights into the chiral discrimination between a pair of amino acid enantiomers using our laboratory-made mwSPR system. D- And L-cystine (Cys) were selected as model compounds and were separately immobilized on gold surfaces through their disulfide functionality to form a monolayer of sensing films. Their chiral recognition was differentiated with another chiral reagent, dextran sulfate (DS) which proved to be an excellent chiral probe, with rich chiral centers and being large enough to generate sufficient mwSPR signals. The results revealed that the chiral interaction was largely dependent on DS concentration and pH as anticipated. But unexpectedly, D-Cys exhibited a somewhat abnormal recognition kinetics: In equilibrium, its maximum mwSPR wavelength shifts were always lower than that of L-Cys, but its dynamic wavelength shifts could climb above those for L-Cys at the first 30 s of interaction especially when pH increased from 5.0 to 7.5. The dynamic shifts went then down gradually to equilibrium after about 50–150 s depending on the concentration of DS. This phenomenon was ascribed to DS adjusting its molecular conformation after interacting with the D-Cys surface.

2. Result and discussion

2.1. Design of chiral recognition model

It is expected to be a very difficult task to monitor the chiral recognition process due to the fact that the effective window in chiral CE is often very narrow and greatly affected by pH.[19,20] To look at such chiral issues by mwSPR, an effective recognition model should be carefully designed. Since amino acids are too small to be sensed directly in a mobile phase by SPR, especially at low concentrations,[21] they are immobilized on gold surfaces[22] and their chiral recognition may hence be differentiated by another large chiral molecule as a mobile probe. As expected, the assembled D- and L-Cys exhibited an evident chiral discrimination in contact with appropriate chiral macromolecules and were demonstrated to be regenerated for at least 20 cycles.

To find a suitable chiral probe, different macromolecules have been tried including proteins (like albumins from various sera) and polysaccharides with and without charges. DS was finally adopted due to its excellent performance, stability, and low viscosity in aqueous solutions. DS is a linear α-(1→6)-linked glucan, with three sulfate groups and four chiral carbons per D-glucose unit. As an effective chiral selector in capillary electrophoresis,[22–25] DS was also shown to be chiral selective and mwSPR sensitive in our experiments.

2.2. Critical factors

With the designed chiral recognition model, some factors were investigated, including DS concentration, pH, the incident angle of source light beam and its intensity, reference for measuring mwSPR spectra, and ways for regenerating the sensing films, of which pH and DS concentration were found to be the most critical.

In recognition equilibrium, a higher content of DS always produced a larger mwSPR wavelength shift as shown in Figure 1, which is preferred for sensitive measurements. Quantitative calculation gave a linear equation between the DS concentration C_{DS} and the maximum resonance wavelength shift, $\Delta\lambda_{max}$ (calculated by referring to a DS-free buffer):

$$\Delta\lambda_{max} = 0.4010 + 0.2059C_{DS}. \quad (1)$$

The linear regression coefficient was 0.9995, which suits to perform quantitative determination of DS content.

The pH of the solution was a more complex parameter and will be discussed later.

To find the resonance wavelength position more precisely, mwSPR spectra for air and reflection spectra produced by horizontally polarized light were recorded

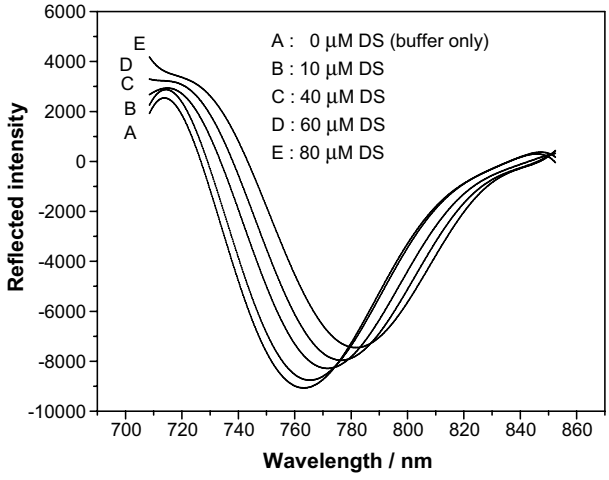

Figure 1. mwSPR absorption spectra obtained from a D-Cys sensing film probed with (A) 0, (B) 10, (C) 40, (D) 60, and (E) 80 μM dextran sulfate at pH 4.50.

and used as background subtrahends, respectively, for the calculation of sample spectra (see Experimental section). No evident difference has been found in the resulting spectra but we preferred to use the air spectra as the subtrahends because their measurement was simple and no hardware adjustment was required. On the other hand, the reflection spectra had to be recorded by changing the polarization of the incident light (normally from a vertical direction to an horizontal one modulated by a polarizer).

Theoretically, the sensitivity of mwSPR is dependent on the incident angle and should be optimized. With our setup, the most sensitive position was found to be at around 62°, independent of the incident light intensity. The incident light source was selected according to the wavelength range required and its intensity was determined by the limitation of an optical electro-transducer, namely ICCD in our case. Its allowable upper limitation is 6 W (before passing through a fiber).

Regeneration of sensing films is commonly required for cyclic or continuous measurements. Three types of desorption solutions were investigated, that is, 0.1 M phosphoric acid, 0.1 M sodium hydroxide, and 0.5 M potassium chloride buffered with 0.5 M phosphate at pH 7.0. All the solutions worked well but the final one was adopted because it allowed the maximum cycles while minimizing the damage to the modified sensing surface.

2.3. Equilibrium chiral discrimination

By the selected system, chiral discrimination between D- and L-Cys was investigated in reaction equilibrium state. The L-Cys sensors yielded always a larger $\Delta\lambda_{max}$ as compared to D-Cys (Figs. 2 and 3) using solutions of DS at a concentration between 20 and 80 μM and pH $\geqslant$5.0. As pH decreased below 4.5, the chiral discrimination faded to a negligible level or zero, independent of the DS concentration. This implies that a strong electrostatic attraction between the positively charged Cys (pI 5.0) and the negatively charged DS is harmful to the chiral recognition. This study also shows that pH is a factor more important than DS concentration, much similar to what is found in chiral CE.

2.4. Chiral recognition kinetics

2.4.1. General. It has been expected to get some insight into the kinetics of chiral interactions by mwSPR, according to the literature.[26] In order to demonstrate this assumption, the Cys-DS recognition model was studied at various pH. The resulting signal, $\Delta\lambda$, was found to increase first sharply and then slowly with time until reaching a plateau (after about 30 s) as a DS solution at pH 5.0 flowed on the sensor surfaces. Figure 4 shows that the kinetic curves for D-Cys are normally

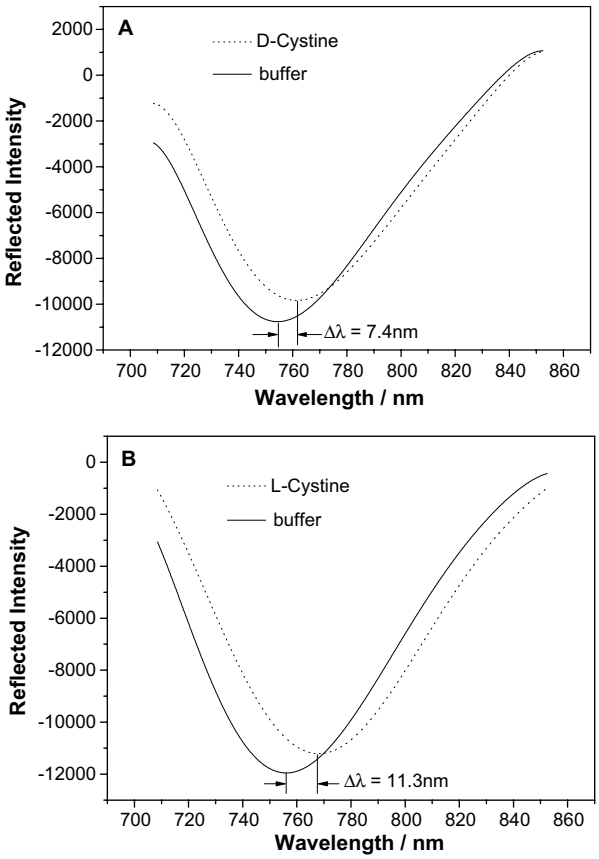

Figure 2. Resonance wavelength shifts of immobilized (A) D-Cys and (B) L-Cys probed with 60 μM dextran sulfate at pH 7.5 by using a pure buffer as reference.

below those for L-Cys, which is in agreement with the equilibrium data. An exception was found with 40 μM DS, where the $\Delta\lambda$ for D-Cys is higher than for L-Cys at the first 20 s. This abnormal variation was enhanced as pH increased and became predominant at pH 7.50. Figure 5 shows that the resonance wavelength shifts for D-Cys increase always faster than for L-Cys at the beginning. This change is strengthened by increasing the concentrations of DS. After about 30–50 s, the signals decrease gradually to their normal position, that is, lower than those for L-Cys. The cross point is located at the time between 50 and 150 s, depending on DS concentration.

This is an interesting phenomenon, not yet clearly addressed in the literature. Sota and Hasegawa suggested that conformational changes of a molecule might lead to changes in the SPR signal.[27] There is no doubt that both DS and Cys may change their molecular conformation after forming transient complexes. It seems that the molecular conformations of DS and D-Cys are ready in solutions to quickly form an unstable transient complex. It will then adjust gradually to a thermodynamically stable state which takes some time (about 100 s as estimated from Fig. 5). Differently, this was not

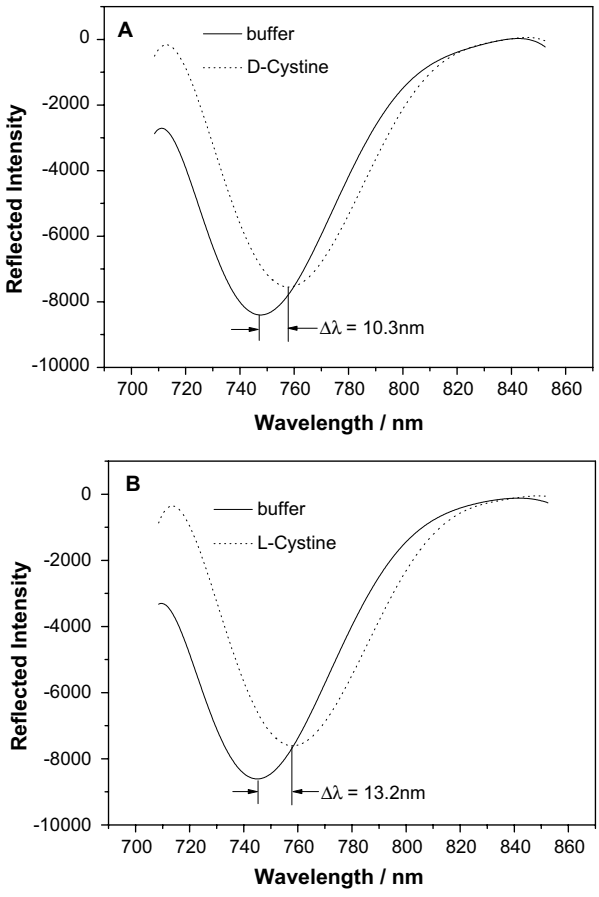

Figure 3. Resonance wavelength shifts of immobilized (A) D-Cys and (B) L-Cys probed with 60 μM dextran sulfate at pH 5.0 by using a pure buffer as reference.

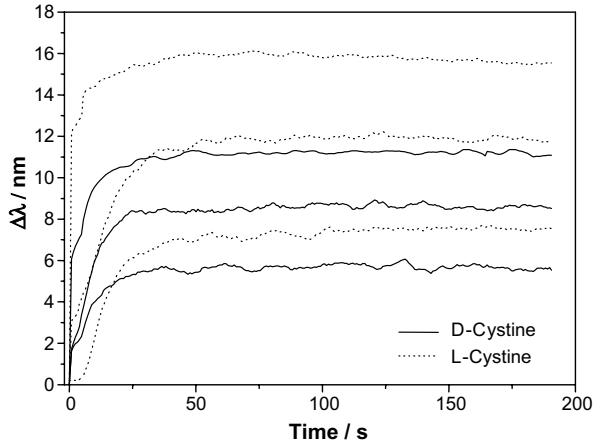

Figure 4. mwSPR kinetic curves for immobilized D-Cys (solid line) and L-Cys (dot line) probed with (from bottom to top) 40, 60, and 80 μM dextran sulfate at pH 5.0.

observed between DS and L-Cys, meaning that their complexes are stable and further adjustment is not required.

Experiments have been tried to confirm the suggested mechanism by using CE and NMR but failed because

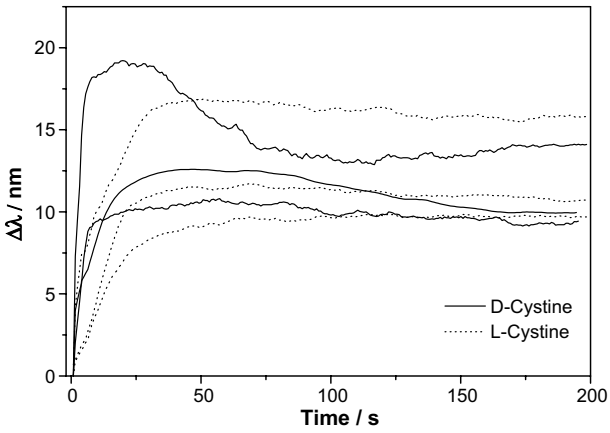

Figure 5. mwSPR kinetic curves for immobilized D-Cys (solid line) and L-Cys (dot line) probed with (from bottom to top) 40, 60, and 80 μM dextran sulfate at pH 7.5.

the reactions are too fast to be followed by tools other than SPR. However, in a slower case, a somewhat similar mechanism has been observed by Simonato et al.[28] They have found, by NMR, that the enantioselectivity ratio of [*R*]/[*S*] varied with the reaction time for a chiral recognition system of cobalt(III) chiroporphyrin and amino alcohol.

2.4.2. Kinetic model. To quantitatively study the chiral recognition process, a reversible reaction was proposed to exist on the sensing surfaces as shown in Eq. 2:

$$\mathrm{DS} + \mathrm{Cys} \underset{k_\mathrm{d}}{\overset{k_\mathrm{a}}{\leftrightarrow}} \mathrm{DS\text{-}Cys}, \tag{2}$$

where k_a is the association rate constant and k_d the dissociation rate constant. The formation rate of DS-Cys complex at time t can thus be written as Eq. 3:

$$\frac{\mathrm{d[DS\text{-}Cys]}}{\mathrm{d}t} = k_\mathrm{a}[\mathrm{DS}][\mathrm{Cys}] - k_\mathrm{d}[\mathrm{DS\text{-}Cys}]. \tag{3}$$

Considering that the concentration of free Cys on a sensing surface, [Cys], can be given by $[\mathrm{Cys}]_\mathrm{o} - [\mathrm{DS\text{-}Cys}]$, where $[\mathrm{Cys}]_\mathrm{o}$ is the total concentration of Cys available on the surface, Eq. 3 can be changed to Eq. 4:

$$\frac{\mathrm{d[DS\text{-}Cys]}}{\mathrm{d}t} = k_\mathrm{a}([\mathrm{Cys}]_\mathrm{o} - [\mathrm{DS\text{-}Cys}])[\mathrm{DS}] - k_\mathrm{d}[\mathrm{DS\text{-}Cys}]. \tag{4}$$

It is clear that only [DS-Cys] will vary if [DS] is maintained by continuous regeneration. The resonance wavelength shift $\Delta\lambda$ measured at any time will then be proportional only to [DS-Cys] and $\Delta\lambda_\mathrm{max}$ to $[\mathrm{Cys}]_\mathrm{o}$. Eq. 4 can thus be replaced by

$$\frac{\mathrm{d}\Delta\lambda}{\mathrm{d}t} = k_\mathrm{a}(\Delta\lambda_\mathrm{max} - \Delta\lambda)C_\mathrm{DS} - k_\mathrm{d}\,\Delta\lambda, \tag{5}$$

where $\mathrm{d}\Delta\lambda/\mathrm{d}t$ represents the formation rate of the surface complex, C_DS is the constant concentration of DS

Table 1. Influence of chiral probe concentration on the apparent rate constant, k_{ap}, of the chiral recognition reaction measured by multi-wavelength SPR

Dextran sulfate concentration (mol/L)	PH 7.5		pH 5.0	
	D-Cystine	L-Cystine	D-Cystine	L-Cystine
8.000×10^{-5}	0.2861	0.1708	0.1554	0.2388
6.000×10^{-5}	0.2161	0.1395	0.1363	0.1869
4.000×10^{-5}	0.1457	0.1081	0.1172	0.1341

in solution, $\Delta\lambda_{max}$ denotes the capacity of the recognition sites on a sensor surface, and $(\Delta\lambda_{max} - \Delta\lambda)$ is equivalent to the number of vacant surface binding sites at time t. Eq. 5 can be rewritten as Eq. 6:

$$\frac{d\Delta\lambda}{dt} = k_a C_{DS}\Delta\lambda_{max} - (k_a C_{DS} + k_d)\Delta\lambda = k' + k_{ap}\Delta\lambda, \quad (6)$$

where k' is the intercept of the equation and k_{ap}, the apparent rate constant, is the slope of curve $d\Delta\lambda/dt \sim \Delta\lambda$. Similarly, k_a and k_d can be calculated from curves of k_{ap} versus C_{DS}. Unfortunately, such measurements were tedious and time consuming. To reduce the workload, Eq. 6 was integrated to give Eq. 7:

$$\Delta\lambda = \frac{k'}{k_{ap}}[1 - \exp(-k_{ap}t)]. \quad (7)$$

Constant k_{ap} could now be calculated by performing curve fitting of $\Delta\lambda$ versus t. Table 1 shows a set of fitting results obtained in the experiment. Obviously, k_{ap} varies with pH and DS concentration, and the k_{ap} for D-Cys is in all cases smaller than for L-Cys at pH 5.0 but larger at pH 7.5. This is parallel to the observation discussed above, meaning that the kinetic difference is indeed an important cause responsible for the chiral discrimination. In addition, k_{ap} increases steadily with C_{DS}, independent of pH.

In conclusion, immobilized D- and L-Cys on gold films can well be differentiated by mwSPR using DS as a chiral probe. The recognition kinetics was shown to be largely involved in chiral discrimination in addition to thermodynamics. D-Cys produced smaller equilibrium resonance wavelength shifts but larger kinetic shifts than L-Cys. Kinetic study by mwSPR seems to be a valuable new way to elucidate some fast chiral recognition mechanism at the scale of seconds.

3. Experimental

3.1. Reagents and solutions

DS (MW 500,000), D-, and L-Cys were purchased from Sigma (St. Louis, USA) and used as received. Other reagents were all of analytical grade.

DS solns were prepared in 0.1 M sodium acetate adjusted to a required pH with acetic acid. All aqueous solns were prepared in doubly distilled water.

3.2. Apparatus

The construction of the mwSPR system has been reported in previous papers.[29,30] Briefly, an outspreading light from a halogen bulb (12 V, 5 W, from Germany) was collimated and polarized through two optical lenses coupled with a polarizer to yield a vertically polarized parallel light beam. It was then directed into an adjustable glass prism from its one side to illuminate a sensing film on the bottom. The film was sealed in an analytical cell with its sensing surface outward so that a probe soln could interact with it by injection or flowing through. After interaction, the light reflected from the film was collected from the other side of the prism and introduced into a monochromator by an optic fiber. All the wavelengths covering the resonance range (about 140 nm in width) were simultaneously recorded by an ICCD (model ICCD-576/G1, Princeton Instruments, Inc., USA) and saved for producing the resonance absorption spectra later.

3.3. mwSPR measurements

To measure a resonance spectrum, the incident angle was adjusted until total internal reflection appeared (around 61.5°). An mwSPR spectrum of air was first recorded and served as a background.[29,30] A target soln was then injected or pumped continuously into the analytical cell, the reflected spectrum was collected and from it the air background was subtracted to produce a valley-shaped mwSPR absorption spectrum. By such spectra, the resonance wavelength shift for a sample could be calculated according to Eq. 8:

$$\Delta\lambda = \lambda_s - \lambda_0, \quad (8)$$

where λ_s is the maximum resonance absorption wavelength of the sample while λ_0 is that of a DS-free buffer.

3.4. Preparation of sensing film

Gold films were prepared by vapor deposition of about 2 nm of chromium and 48 nm of gold on microscope slides. Immediately before use, the gold films were cleaned by dipping in a Piranha solution (3:1 H_2SO_4/30% H_2O_2, *Caution*: It is highly oxidative to organic compounds and should be used under protection!) at room temperature for 2 min. The slides were rinsed copiously with water, then with abs EtOH, and dried with N_2. A cleaned gold film was immersed in 1 mM D- or L-Cys soln at room temperature for 12 h, followed by rinsing with water. The gold film was dried under N_2 stream and brought in close contact with the prism

($n = 1.516$) using a refractive-index-matching oil (n 1.51). In continuous measurements, the sensing films were generated using 0.5 M potassium chloride buffered with 0.5 M phosphate soln at pH 7.0.

Acknowledgements

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