Capping Structure of Ligand—Cysteine on CdSe Magic-Sized Clusters

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ABSTRACT: Ligand molecules capping on clusters largely affect the formation and stabilization mechanism and the property of clusters. In semiconductor CdSe clusters, cysteine is used as one of the ligands and allows the formation of ultrastable (CdSe)34 magic-sized clusters. Cysteine has sulfhydryl, amine, and carboxylate groups, all of which have coordination ability to the CdSe surface, and the bonding states of the three functional groups of ligand—cysteine on the CdSe core have not been determined. In this work, the capping structure of ligand—cysteine is examined by performing Fourier transform infrared (FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and multinuclear solid-state nuclear magnetic resonance (NMR) spectroscopy. FT-IR, XPS, and 1H, 13C, and 23Na magic-angle spinning NMR show that the sulfhydryl group of ligand—cysteine forms a sulfur–cadmium bond with a cadmium atom at the CdSe surface, while the carboxylate group does not contribute to the protection of the CdSe core and binds to a sodium ion contained as a counterion. 15N-(77Se) through-bond J-single quantum filtered NMR experiment reveals that the amine group of ligand—cysteine has no coordination to selenium atoms. By considering the N–Cd bond forming ratio (~43%) revealed in our previous work, which is confirmed in this work by analyzing 13C signal intensity (~42%), we concluded that cysteine capping on (CdSe)34 occurs in two ways: one involves both the sulfur–cadmium and nitrogen–cadmium bonds, and the other bears only the sulfur–cadmium bond.

1. INTRODUCTION

Clusters are composed of up to several hundreds of atoms and have different structures from their bulk states. Clusters can be synthesized in a gas phase by, for a few examples, gas aggregation and laser vaporization, and their structures and stabilities have been studied. It has recently been shown that clusters can also be prepared in a solvent with organic molecules working as ligands. Unique structures and electronic stabilities have been studied. It has recently been shown that ligands affect the formation and stabilization of clusters and frequently lead particularly stable clusters called magic-sized clusters (MSCs). Hence, examination of the structure of a ligand on a cluster is essential for understanding the stabilization mechanism of the cluster. For example, in thiolate-capped gold MSCs, thiolate ligands form S–Au–S staple-like units on the surface of gold MSCs, which protect the cluster core consisting of Au atoms. The ligands can also influence physical/chemical properties and functions of clusters. In semiconductor nanoparticles (NPs) and clusters, it has been shown that the ligand–surface interaction largely affects the optical properties. For example, postsynthetic secondary ligand modification for CdSe NPs and MSCs affects the quantum yield of photoluminescence. Ligand treatment for CdS and CdSe MSCs affects exciton peak positions, which was ascribed to structural modification of MSCs induced by additional ligands. Unlike NPs intrinsically having size and shape distributions at the atomic level, MSCs have well-defined structures and thus are suitable for examination of the correlation between ligand–surface interactions and their properties and structures.

II–IV semiconductor CdSe MSCs can be synthesized in a solvent with narrow size distribution. In 2004, Kasuya et al. reported that stoichiometric CdSe MSCs, e.g., (CdSe)13 and (CdSe)34, were detected by laser desorption/ionization–mass spectrometry (LDI-MS) and suggested that they may have a core–cage structure as predicted by first-principles calculation. Since then, CdSe MSCs have been prepared with various ligands such as thiolates, amines, and carboxylates, and versatile studies on their structures and properties have been investigated both experimentally and theoretically. Cysteine, which is used as one of the ligands for (CdSe)34 MSCs, has three functional groups, sulfhydryl, amine, and carboxylate groups, all of which have the ability to bond to the surface atoms of the CdSe core. Park et al. synthesized cysteine-capped (CdSe)34 (CdSe-Cys) for the first time and examined the influence of ligands on the MSC formation using cysteine and its derivatives as ligands. They showed that the sulfhydryl and the amine groups of cysteine play an important role for the growth and stabilization of the (CdSe)34 structure. Nevins et al. found that electron transfer from CdSe-Cys to TiO2 substrate through ligand—cysteine is more efficient than that from CdSe NPs capped with mercaptocarboxylates having...
sulphhydryl and carboxylate groups and suggested that the efficient transfer for the former is caused by the amine–surface interaction in CdSe-Cys. These studies indicate that investigation of the protecting structure of ligand–cysteine in CdSe-Cys is important for both fundamental and applied studies of (CdSe)$_{34}$ MSCs. However, the capping structure of ligand–cysteine has not been determined fully. So far, the studies described above indicate that both sulfur and nitrogen of the sulphhydryl and amine groups bind to the cadmium atom at the CdSe surface. For primary amine-capped CdSe NPs, it has been suggested that the amine groups coordinate to both the surface cadmium and selenium. However, for CdSe-Cys, whether or not the amine group coordinates to selenium has not yet been clarified. As for the carboxylate group, Nevins et al. proposed that a fraction of the carboxylate group as well as the sulphhydryl and amine groups bind to cadmium at the CdSe surface, while Park et al. suggested no coordination of the carboxylate group to the surface cadmium. Furthermore, for semiconductor NPs, it has been suggested that cysteine capping on CdSe and CdTe NPs occurs with the sulphur–cadmium bond and the sulfur–cadmium and carboxylate–surface interactions, respectively. Hence, involvement of the carboxylate group in the capping is also one of the questions addressed in this work. Unfortunately, the single crystals of (CdSe)$_{34}$ MSCs including CdSe-Cys have not been prepared, and thus, the overall structure including the ligands has not yet been experimentally determined by single-crystal XRD analysis.

For characterization of the surface and interface structures of MSCs and NPs, whose single crystals are difficult to obtain, Fourier transform infrared (FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and solid-state nuclear magnetic resonance (NMR) spectroscopy are effective methods. FT-IR can analyze vibrational states of functional groups of a ligand and thus has been used as a powerful method for characterization of the ligand–surface bond in NPs and MSCs. XPS spectrum shows the binding energies of the ligand and surface atoms, which also gives insight into the ligand–surface bonding state. Solid-state NMR can examine local structures, such as the electronic state around a nuclear spin, internuclear distances, and the presence of a chemical bond, through nuclear spin interactions (chemical shift, dipolar, $J$, and quadrupolar interactions). For example, through-space dipolar-correlated experiments have unraveled the ligand–surface interactions and the surface structures in InP, CdS, and CdSe NPs. Analysis of the chemical shift interaction has led to a unique surface structural model of a CdSe MSC. Recently, we presented a through-bond $J$ interaction-based heteronuclear correlation solid-state NMR experiment allows quantitative analysis for formation ratio of ligand–surface chemical bonds in semiconductor NPs and clusters. Moreover, we showed that combination of a through-bond dipolar-based heteronuclear correlation experiment with the $J$-correlated experiment is a powerful tool for detailed investigation of the surface and interface interactions of NPs. In fact, we revealed the presence of nitrogen–cadmium chemical bonds and determined the ratio of the bond formation to be $\sim$43% and the $^{11}$N–$^{113}$Cd $J$ coupling constant to be 58.5 Hz for $^{113}$Cd- and $^{15}$N-enriched CdSe-Cys. Further, the N–Cd bond length was estimated to be $\sim$0.24 nm using the $^{15}$N–$^{113}$Cd dipolar correlation experiments. However, the bonding states of the remaining functional groups in ligand–cysteine are still unknown.

In this work, by performing multinuclear ($^1$H, $^{13}$C, $^{77}$Se, $^{15}$N, $^{113}$Cd, and $^{23}$Na) solid-state NMR as well as FT-IR and XPS, we examine the protection mechanism of ligand–cysteine in CdSe-Cys through elucidating the bonding states of the three functional groups to the CdSe surface. FT-IR and $^1$H and $^{13}$C NMR suggest that sulfurs of the sulphhydryl group coordinates to the surface cadmium. FT-IR, XPS 0 1s, and $^{13}$C and $^{23}$Na NMR indicate that the carboxylate group does not coordinate to the surface cadmium and binds to a sodium ion that exists in solidified CdSe-Cys as a counterion. From FT-IR and $^1$H NMR, we show that, in ligand–cysteine, ammonium ion structure ($\sim$NH$_3^+$) in the zwitterionic form in bulk L-cysteine is deprotonated and changes to the primary amine structure ($\sim$NH$_2$). By performing $^{13}$C NMR, we indicate that the amine groups take two states, and by performing through-bond $^{15}$N–$^{113}$Cd and $^{15}$N–$^{77}$Se $J$-single quantum filtered (J-1QF) experiments, we show that the amine groups have nitrogen–cadmium chemical bonds and do not form chemical bonding with selenium atoms.

Figure 1. (a) FT-IR spectra of bulk L-cysteine (black solid line) and CdSe-Cys (red solid line). The blue, green, and purple dashed lines indicate the positions of SH, NH$_3^+$, and COO$^-$ peaks in the spectrum of L-cysteine, respectively. (b) Zwitterionic structure of L-cysteine.
2. RESULTS AND DISCUSSION

2.1. FT-IR. Figure 1 shows the FT-IR spectra of bulk L-cysteine taking the zwitterionic form (black solid line) and CdSe-Cys (red solid line), together with the chemical formula of the L-cysteine zwitterionic form. In the spectrum of L-cysteine, the peak observed at 943 and 2552 cm\(^{-1}\) is assigned to SH bending and stretching, respectively. The peaks at 806, 823, and 1391 cm\(^{-1}\) are ascribed to COO\(^{-}\) bending, wagging, and symmetric stretching, respectively, and the shoulder peak at 1610 cm\(^{-1}\) is assigned to COO\(^{-}\) asymmetric stretching. The peak at 1064 cm\(^{-1}\) is attributed to NH\(^3\)\(^+\) rocking, the peaks at 1525, 1541, and 1577 cm\(^{-1}\) are attributed to NH\(^3\)\(^+\) bending, and the peaks at 2638, 2960, and 3165 cm\(^{-1}\) are attributed to NH\(^3\)\(^+\) stretching. All assignment for the peaks in the spectrum of L-cysteine and referenced articles are shown in Table S1, Supporting Information.

In the spectrum of CdSe-Cys, no SH peaks were observed at 943 and 2552 cm\(^{-1}\), which indicates that the sulphydryl group of ligand—cysteine is deprotonated. The peaks at 801 and 1397 cm\(^{-1}\) are ascribed to COO\(^{-}\) bending and symmetric stretching, respectively, while the COO\(^{-}\) wagging and asymmetric stretching peaks were not observed at around 823 and 1610 cm\(^{-1}\). The COO\(^{-}\) wagging and asymmetric stretching peaks may move from the 823 and 1610 cm\(^{-1}\) region and hide under other peaks. The shift of the COO\(^{-}\) peaks in CdSe-Cys may be ascribed to an interaction with a metal element. In fact, a carboxylate group binding to the metal ion frequently forms the O==C—O\(^-\) structure with a strong C==O stretching peak at the region of ~1640—1740 cm\(^{-1}\).\(^{25,30}\) In the spectrum of CdSe-Cys, however, no such peak was observed. Thus, we tentatively postulate that the carboxylate group of ligand—cysteine has the (O—C—O\(^-\)) structure, where an electron is delocalized equally over the two oxygen atoms. In the CdSe-Cys spectrum, the NH\(^3\)\(^+\) rocking (1064 cm\(^{-1}\)) and stretching peaks (2638, 2960, and 3165 cm\(^{-1}\)) appeared in L-cysteine were not observed, and two broad peaks were found at 1565 and ~2520 cm\(^{-1}\). We attribute the former broad peaks to NH\(^3\)\(^+\) bending, which appears at 1547—1583 and 1541—1639 cm\(^{-1}\) for amines binding to a metal ion or the surface of metal chalcogenide NPs.\(^{35,29}\) The latter broad peak can also be assigned to the stretching of NH\(_2\), which was reported\(^{15,29}\) to appear at 3300 cm\(^{-1}\) for NH\(_2\)/metal ion or metal chalcogenide NPs. The chemical state of the amine group of CdSe-Cys being primary amine (—NH\(_2\)) and not the ammonium ion form (—NH\(_3\)\(^+\)) is consistent with our previous NMR study for the N—Cd chemical bond in CdSe-Cys.\(^{28}\) The assignments of the COO\(^{-}\) and NH\(^3\)\(^+\) peaks of CdSe-Cys are shown in Table S2, Supporting Information.

2.2. XPS. Figure 2 shows the XPS O 1s spectra of L-cysteine (black solid line), CdSe-Cys (red solid line), and cadmium acetate dihydrate [Cd(OAc)\(_2\)·2H\(_2\)O] (blue solid line). A background signal derived from carbon tape is also shown (gray). The O 1s peaks of the three samples were observed at 531.1, 532.3, and 533.4 eV. Cd(OAc)\(_2\)·2H\(_2\)O has two kinds of oxygen atoms (the carboxylate group and the water molecule); hence, the peak of Cd(OAc)\(_2\)·2H\(_2\)O would be composed of two O 1s peaks overlapping each other. The peak position of CdSe-Cys is different from those of L-cysteine and Cd(OAc)\(_2\)·2H\(_2\)O, which indicates that the electronic state of the carboxylate group of ligand—cysteine is different from either that of L-cysteine having no coordination or that of Cd(OAc)\(_2\)·2H\(_2\)O binding to a cadmium ion. As the carboxylate structure in Cd(OAc)\(_2\)·2H\(_2\)O is the (O—C—O\(^-\)) form, these XPS results may indicate that the carboxylate group in CdSe-Cys does not bind to cadmium. This will be discussed afterward with the IR and NMR results.

2.3. 1\(^H\) MAS NMR. To gain further insight into the bonding state of ligand—cysteine, we conducted solid-state NMR. Figure 3 shows the 1\(^H\) magic-angle spinning (MAS) spectra of L-cysteine and CdSe-Cys. The spectra of L-cysteine and CdSe-Cys were obtained at spinning speeds of 40 and 20 kHz, respectively.
results of the IR measurement for CdSe-Cys, that is, the sulphydryl groups of ligand–cysteine are deprotonated. For the CH$_2$ peak in the $^1$H spectrum of CdSe-Cys, it appears that the CH$_2$ peak merge into the $\sim$4.8 ppm peak composed of CH and NH$_2$. To confirm, we measured the $^1$H MAS spectrum of CdSe-Cys having a deuterated amine group in ligand–cysteine and observed a narrow major peak at 4.8 ppm and minor peaks at around 1–2 ppm (Figure S2, Supporting Information). Because CdSe-Cys is an intrinsic semiconductor, this high-frequency shift from 2.0 to 4.8 ppm is not ascribed to paramagnetic electrons. Further, the shift difference ($\sim$2.8 ppm) is too large to be attributed to the pH $\approx$ 13 condition in the preparation of CdSe-Cys; the shift difference of the CH$_2$ proton signal of the cysteine residue in a tripeptide between the acid and the conjugate base forms is only 0.09 ppm. Hence, we consider that structural deformation and change in electron delocalization of cysteine upon capping onto the CdSe surface cause the shift of the $^1$H signal. To conclude, the major peak at 4.8 ppm can be ascribed to the sum of the CH$_2$, CH, and NH$_2$ signals. As for the minor peaks at $\sim$0–2 ppm, similar peaks were observed in the $^1$H MAS spectrum of cysteine-capped gold NPs and assigned to SH and CH$_2$ peaks of mobile cysteine molecules existing around ligand–cysteine of gold NPs. We also follow this and assigned the minor peaks to free mobile cysteine present in the solidified CdSe-Cys as an impurity.

2.4. $^{13}$C CP/MAS NMR. $^1$H $\rightarrow$ $^{13}$C cross-polarization (CP)/MAS NMR spectra of l-cysteine and CdSe-Cys are shown in Figure 4a,b, respectively. The C$^\alpha$, C$^\beta$, and COO$^-$ peaks of l-cysteine were observed at 27.5, 55.4, and 172.8 ppm, respectively. In the spectrum of CdSe-Cys, all three peaks broaden, and the C$^\beta$ and COO$^-$ peaks shift to the higher frequency (32.1 and 180.2 ppm, respectively). The C$^\alpha$ peak of CdSe-Cys bears two peaks at 54.4 and 59.4 ppm with some small shoulder peaks. Additionally, a minor peak was observed at 165.1 ppm.

Firstly, we assign the latter minor peak. It is notable that $^{13}$C signals derived from carbonates and carbamates are observed at $\sim$162–170 ppm. This suggests that carbonate or carbamate may be mixed in the CdSe-Cys solid. To examine this, we solidified CdSe-Cys from the synthesis solution with N$_2$ bubbling (more detailed procedures are explained in the Supporting Information). In the $^{13}$C CP/MAS spectrum for this CdSe-Cys sample, no peak was observed at 165.1 ppm (see Figure S3, Supporting Information), which indicates that the $^{13}$C minor peak at 165.1 ppm is not derived from the functional groups of ligand–cysteine but carbonate/carbamate from cysteine reacted with CO$_2$ during the solidification process.

The broadening of other $^{13}$C peaks in CdSe-Cys is attributed to chemical shift distribution of the $^{13}$C peaks due to disorder of the orientation of ligand–cysteine. The frequency shifts of the C$^\beta$ and COO$^-$ peaks imply that the sulphydryl binding to the C$^\alpha$ and carboxylate groups of ligand–cysteine have states different from those of free l-cysteine; in other words, the sulphydryl and carboxylate groups may interact with the CdSe core. The two C$^\alpha$ peaks can be attributed to the two kinds of the amine groups, namely, one binding to Cd and the other is not, as shown by $^{15}$N-$^{[113}$Cd] J-1QF experiment in our previous work. The small shoulder peaks indicate the existence of C$^\beta$ with slightly different coordination geometry. The signal intensity ratio for the two C$^\beta$ peaks was estimated to be the sharper one: the broader one is 42:58 by line-shape analysis, which is consistent with the previous result (43:57). The sharper C$^\beta$ signal for ligand–cysteine with the Cd–N bond is understandable as the Cd–N bond would reduce structural distribution.

2.5. $^{15}$N-$^{[113}$Cd] and $^{15}$N-$^{[77}$Se] J-1QF NMR. To confirm the presence of the nitrogen–cadmium bond in CdSe-Cys newly synthesized in this work and to examine whether the nitrogen–selenium bond exists or not, $^{15}$N-$^{[113}$Cd] and $^{15}$N-$^{[77}$Se] J-1QF NMR experiments were performed. In the $^1$H $\rightarrow$ $^{13}$C CP/MAS NMR spectrum of CdSe-Cys (Figure 5a), two peaks (hereafter peak A and peak B), were observed at $\sim$35 and $\sim$97 ppm, respectively. The $^{15}$N spectrum of the $^{15}$N-$^{[113}$Cd] J-1QF experiment conducted with the echo refocusing time $\tau$ of 6.05 ms (Figure 5b) displays only peak A and that of the $^{15}$N-$^{[77}$Se] experiment with $\tau$ of 5 ms (Figure 5c) bears no peak. In the $^{15}$N-$^{[77}$Se] experiment with $\tau$ of 10 ms, no signal was also observed (data not shown).

![Figure 4](image-url)  
Figure 4. $^{13}$C CP/MAS spectra of (a) l-cysteine and (b) CdSe-Cys. The spectra were acquired with a spinning speed of 20 kHz and contact time of 1 ms.

![Figure 5](image-url)  
Figure 5. (a) $^{15}$N CP/MAS, (b) $^{15}$N-$^{[113}$Cd] J-1QF, and (c) $^{15}$N-$^{[77}$Se] J-1QF spectra for CdSe-Cys. Two peaks at $\sim$35 and $\sim$97 ppm in the CP/MAS spectrum are labeled as peak A and peak B, respectively. The $^{15}$N-$^{[113}$Cd] and $^{15}$N-$^{[77}$Se] J-1QF spectra were obtained with $\tau$ = 6.05 ms and 5 ms, respectively.
The former result indicates that the amine group of peak A forms the nitrogen–cadmium chemical bond with the surface cadmium of the CdSe core, while the amine group of peak B forms no nitrogen–cadmium bond, which is consistent with our previous report. The latter 15N–{77Se} experiment shows that no amine group coordinates to the surface selenium.

It was shown that the amine group of peak B has no chemical bond to either cadmium or selenium. Following the assignment of the 13C minor peak to carbonate or carbamate, we also assign peak B to the amine group binding to a carbonate ion, i.e., carbamate. In fact, the chemical shift value of peak B is contained in the chemical shift range of 13C carbamate signals. It is true that the N2 gas bubbling for an 15N-enriched sample would give conclusive evidence, but we have not done it as the assignment of peak B is not crucial in this work.

### Table 1. 13C Chemical Shift Values (ppm) of CdSe-Cys Prepared with NaOH, RbOH, and CsOH

| Peaks | NaOH | RbOH | CsOH |
|-------|------|------|------|
| C\(^\beta\) | 32.14 ± 0.01 | 32.41 ± 0.01 | 32.18 ± 0.03 |
| C\(^\gamma\) (right) | 54.17 ± 0.01 | 54.25 ± 0.01 | 54.08 ± 0.03 |
| C\(^\gamma\) (left) | 59.10 ± 0.03 | 59.50 ± 0.03 | 59.37 ± 0.09 |
| COO\(^-\) | 180.17 ± 0.02 | 179.49 ± 0.02 | 179.18 ± 0.05 |

2.6. Capping Structure of Ligand–Cysteine. As a sulphydryl group is known to have binding ability to metal elements, especially cadmium, the observation of no SH peaks in the 2H NMR and FT-IR spectra led us to envisage that the sulfur of the sulphydryl group of ligand–cysteine binds to the surface atoms of the CdSe core. It has been reported that by examining the 13C NMR spectra for cadmium–cysteine complexes and cysteine-capped CdSe and CdTe NPs, a cadmium–cysteine bond brings a high-frequency shift to the C\(^\alpha\) peak, which is consistent with the observed high-frequency shift of the C\(^\alpha\) peak of CdSe-Cys. We therefore conclude that, in CdSe-Cys, the sulfur in the sulphydryl group of ligand–cysteine forms the sulfur–cadmium bond with the surface cadmium of the CdSe core.

In the following, we examine binding states of the carboxylate group in CdSe-Cys. The FT-IR results indicate that the carboxylate group of ligand–cysteine takes the (O=C–O)\(^-\) form. Nevins et al. observed a similar peak shift of the C\(^\alpha\) peak of CdSe-Cys, which is consistent with the observed high-frequency shift of the C\(^\alpha\) peak of CdSe-Cys.44 Hence, in cysteine-capped (CdSe)\(_{34}\) nano-clusters (N = 1–10, 13, 16, and 19) shows that the sulfur and oxygen atoms of cysteine in alkaline solution cannot simultaneously coordinate to a cadmium atom at the surface of CdSe nanoclusters because of the structural rigidity of cysteine. It is also unlikely for the carboxylate group to form bridging coordination to sodium and cadmium. To conclude, the carboxylate group of ligand–cysteine binds to the counter Na\(^+\) ions in the solid-state CdSe-Cys and not to the CdSe core surface.

As for the amine group, the 13N–{113Cd} and 13N–{77Se} J-1QF experiments revealed that the nitrogen of the amine group of ligand–cysteine has a chemical bond to the surface cadmium but not to selenium. In our previous work, the ratio of the amine group forming the nitrogen–cadmium bond in CdSe-Cys was estimated to be ∼43% through the quantitative 13N–{113Cd} J-1QF analysis. The rest (∼57%) of the amine group has no chemical bond to the surface and thus does not contribute to the stabilization of the CdSe core. The ratio is consistent with the N–Cd bond forming ratio of the amines obtained from the 13C peak area ratio of the two C\(^\alpha\) peaks (42:58).

Let us summarize the results of the bonding states of the three functional groups of the ligated cysteine. The sulphydryl group and ∼43% of the amine groups form the sulfur–cadmium and nitrogen–cadmium chemical bonds to the CdSe core surface, while the carboxylate group binds to the counter Na\(^+\) ion in the solid-state CdSe-Cys. This leads to the two types of ligand–cysteine complex; ∼43% of the complex has the sulfur–cadmium and nitrogen–cadmium bonds and the rest (∼57%) has only the sulfur–cadmium bond to stabilize the (CdSe)\(_{34}\) structure. The stabilization mechanism by both sulfur and nitrogen of ligand–cysteine binding to the CdSe core surface has also been suggested by comparing the MSC formation ability between i-cysteine and its derivatives as ligands.13 In this work, we could show the existence of the two kinds of the capping structure of ligand–cysteine in CdSe-Cys. The difference of the ratio of the two ligand–surface bonds,
i.e., the sulfur–cadmium (100%) and nitrogen–cadmium (43%), indicates that the sulfur–cadmium bond works dominantly in the stabilization of (CdSe)4. This is consistent with the result of our previous work, that is, the nitrogen–cadmium chemical bond in CdSe is not strong as evidenced by the small 113Cd J coupling constant (58.5 Hz) and the corresponding long N–Cd bond length (~2.4 Å). A theoretical study has shown that cysteine capping on CdSe nanoclusters in alkaline solution forms a strong sulfur–cadmium bond and a weak nitrogen–cadmium bond,44 which is in good agreement with our experimental results. It has been reported that CdSe MSCs can be synthesized with thiolate ligands alone.7,45 However, as compared to the ligands alone,7,45 UV–vis spectra of our CdSe-Cys is sharper. This can also be explained by the additional coordination of a part of the nitrogen to the surface cadmium leading a more stable CdSe core with narrower structural distribution. The two types of ligand–cysteine protecting (CdSe)4 can therefore be essential to understand the stability of CdSe-Cys.

3. CONCLUSIONS

We synthesized 113Cd, 77Se, and 15N-enriched CdSe-Cys and examined the capping structure of ligand–cysteine in CdSe-Cys by performing FT-IR, XPS, and multinuclear solid-state NMR spectroscopy. FT-IR and 1H MAS NMR showed that the sulfhydryl group of ligand–cysteine was deprotonated, and 13C CP/MAS NMR indicated that the sulfhydryl formed the sulfur–cadmium bond with the cadmium atoms at the CdSe surface. XPS O 1s spectra suggested that the electronic state of the carboxylate group of ligand–cysteine is different from that of the carboxylate coordinating to a cadmium ion. By examining 13C CP/MAS NMR, we showed that the chemical shift values of the carboxylate group varied for different counterions (Na+, Rb+, and Cs+). This led us to conclude that the ratio of the two structures obtained by the 15N CP/MAS NMR is consistent with the 13C peak area intensities of the two Cα signals (42.58). The strong stabilization structure does show narrower structural distribution, leading to the sharp UV–vis absorption peak.

4. MATERIALS AND METHODS

4.1. Materials. Cadmium (foil; 113Cd, 93.35%) and selenium (powder; 77Se, 99.20%) were purchased from ISOFLEX USA. l-cysteine (15N, 98%) was obtained from Cambridge Isotope Laboratories. Sodium sulfite (Na2SO3) (97%), sodium hydrate (NaOH) (93%), nitric acid (HNO3) (60–61%), cadmium acetate dihydrate [Cd(OAc)2·2H2O] (98%), and acetone (99.5%) were purchased from Wako Pure Chemical Corporation. All chemical reagents were used as received.

4.2. Synthesis and Solidification of Isotope-Enriched CdSe-Cys. CdSe-Cys was synthesized in an aqueous solution and solidified by using our previous method with some modifications.28 The 77Se precursor, Na2SeSO3(aq), was prepared by stirring (950 rpm) 25 mg of 77Se powder, 118 mg of Na2SO3, and 12.5 mL of distilled H2O in a brown glass vial at ~90 °C overnight. Cadmium hydroxide (Cd(OH)2) was produced by adding 5 M NaOH to a solution of 55.6 mg of 113Cd metal in HNO3, and collected by centrifugation at 10,000 rpm for 5 min. The 113Cd precursor, aqueous Cd–cysteine complex, was obtained by mixing Cd(OH)2, 2.00 mL of 1 M l-[15N] cysteine, 8.9 mL of 1 M NaOH, and 10.9 mL of distilled H2O. The 113Cd precursor was divided into two centrifuge tubes at equal amounts, and CdSe-Cys solution was obtained by adding 4.83 mL of the 77Se precursor to each centrifuge tube. The mixture was kept at room temperature for 7 d, and the UV–vis absorption spectrum of CdSe-Cys was measured (see Figure S1, Supporting Information). The observed first absorption peak at 422 nm with additional peaks at 389 and 360 nm indicates that CdSe-Cys is successfully synthesized.5,10,46 Further, the observed narrow width of the first absorption peak (~15 nm) suggests that the size distribution is not large. Acetone (~7.5 mL) was slowly added for 15 min to each of the two mixture solutions by using a syringe under air to precipitate CdSe-Cys. A yellow CdSe-Cys solid was collected by centrifugation at 10,000 rpm for 5 min and finally obtained after slow vacuum drying (~1.5 d) in a desiccator.

4.3. FT-IR Spectroscopy. FT-IR spectra for l-cysteine and CdSe-Cys were measured by using a Nicolet iS5 FT-IR spectrometer (Thermo Scientific) with an iDS ATR accessory.

4.4. XPS. XPS O 1s spectra of l-cysteine, CdSe-Cys, and Cd(OAc)2·2H2O were recorded on an ESCA-3400 (Shimadzu Corp.) with a Mg anode and an X-ray power of 200 W (acceleration voltage: 10 kV; emission current: 20 mA). In the measurement, the samples were attached on sample holders by using carbon tape. Binding energies were referenced to the C 1s main peak of carbon tape at 284.8 eV.

4.5. Solid-State NMR Spectroscopy. Solid-state NMR experiments for l-cysteine and isotope-enriched CdSe-Cys were conducted in a 9.4 T magnet (1H, 400.23 MHz; 13C, 100.65 MHz; 113Cd, 88.81 MHz; 77Se, 76.29 MHz; and 15N, 40.56 MHz) on an OPENCORE spectrometer with a homemade MAS probe for 1H using a 1.6 mm rotor or a T3 homemade MAS probe for 1H using a 1.6 mm rotor or a T3 HXY MAS probe (3.2 mm rotor, Chemagnetics). For 1H–113Cd–15N and 1H–77Se–15N tuning, homemade plug-in units were made for the T3 HXY MAS probe. 1H MAS spectra for l-cysteine and CdSe-Cys were measured at spinning speeds of 40 and 20 kHz with 90° pulse lengths of 2.3 and 2.5 μs, recycling delays of 20 and 1 s, corresponding to five-fold of each 1H spin-lattice relaxation time, and 16 and 32 scans, respectively. For background suppression, the DEPTH sequence was used for the acquisition of the 1H spectra. 1H→13C and 1H→15N CP/MAS spectra were acquired at a spinning speed of 20 kHz under 1H heteronuclear TPPM decoupling with a radio-frequency (rf) pulse strength of 90 kHz. The contact time for CP/MAS was set to 1 ms (13C) and 0.4 ms (15N) with rf strengths of 70 kHz for 1H and 50 kHz ramped in the range of ±2 kHz for 13C and 15N.
experiments for l-cysteine and CdSe-Cys, the recycling delays of 4 s and the accumulation numbers of 3600 and 100000 scans were used, respectively. In the $^{15}$N measurement for CdSe-Cys, the recycling delay of 1 s and 15000 scans were used. $^{13}$N-$^{\{113}$Cd$\}$ and $^{15}$N-$^{\{77}$Se$\}$ J-1QF experiments$^{28}$ were done using the pulse sequence shown in Figure 6 under a spinning speed of 20 kHz and $^1$H TPPM decoupling with an rf strength of 90 kHz. In the $^{13}$N-$^{\{113}$Cd$\}$ J-1QF experiment, the $^{15}$N 180° pulse length and $^{13}$Cd 90° pulse length were 9.4 and 2.8 $\mu$s, respectively. In the $^{13}$N-$^{\{77}$Se$\}$ J-1QF experiment, the $^{15}$N 180° pulse length and $^{77}$Se 90° pulse length were 10.1 and 3.6 $\mu$s, respectively. The $^1$H $\rightarrow$ $^{13}$N ramped CP contact time was 0.4 ms with rf strengths of 70 and 50 ± 7 kHz for $^1$H and $^{15}$N, respectively. In the $^{15}$N-$^{\{113}$Cd$\}$ and $^{15}$N-$^{\{77}$Se$\}$ J-1QF experiments, the recycling delay of 1 s and the accumulation number of 100,000 and 10,000 scans were used, respectively.

The chemical shifts of $^1$H, $^{13}$C, and $^{15}$N were referenced to TMS at 0 ppm, CH$_2$ of adamantane at 37.85 ppm relative to TMS, and NH$_4$Cl at 39.3 ppm to NH$_3$ liquid,$^{50}$ respectively.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free on the ACS Publications website at DOI: 10.1021/acsomega.8b02752.

Synthesis of CdSe-Cys with RBH and CsOH; synthesis of CdSe-Cys with N$_2$ bubbling; synthesis of partially deuterated CdSe-Cys; experimental conditions for UV–vis; experimental conditions for $^1$H $\rightarrow$ $^{13}$C CP/MAS and $^{23}$Na MAS NMR; $^1$H–$^1$H MAS spectrum for CdSe-Cys; $^1$H MAS NMR spectrum for partially deuterated CdSe-Cys; $^{13}$C CP/CPMAS spectrum and $^{23}$Na MAS NMR spectrum for CdSe-Cys solidified with N$_2$ bubbling; $^{23}$Na MAS NMR spectrum for isotopically enriched CdSe-Cys; $^{13}$C CP/CPMAS spectra for CdSe-Cys prepared with RBH and CsOH; assignment of IR peaks for the zwitterionic form of l-cysteine; and assignments of IR peaks for the COO$^-$ and NH$_2$ group of CdSe-Cys (PDF)

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### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

This work was supported by JSPS Grants-in-Aid for Scientific Research on Innovation Areas “Mixed anion” (grant number 16H06440) and Grants-in-Aid for JSPS Fellows (grant number 18J11973).

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