Fluorescence Spectral Studies on the Interaction of Alanine and Valine with Resorcinol-Based Acrinedione Dyes in Aqueous Solution: A Comparative Study with Glycine

Krishnan Anju,† Anupurath Sumita,‡ Somasundaram Gayathri,† Rajaraman Vasanth,‡ and Kumaran Rajendran*†‡

†Department of Chemistry, Dwaraka Doss, Goverdhan Doss, Vaishnav College (Autonomous), 833, Gokul Bagh, E.V.R. Periyar Road, Arumbakkam, Chennai 600106, Tamil Nadu, India
‡Department of Chemistry, Anna Adarsh College for Women College, Anna Nagar, Chennai 600040, Tamil Nadu, India

Supporting Information

ABSTRACT: Photophysical studies were carried out for simple amino acids like alanine and valine with resorcinol-based aqueous acridinedione (ADDR) dyes. ADDR dyes exhibit interesting excited-state characteristics on altering the substituents at the 9th and 10th sites (Scheme 1). The longest-wavelength absorption maxima remain the same on adding the amino acids to the fluorophore, whereas the excited-state behavior varies significantly mostly based on the nature of the substituent at the 9th position. The absence of fluorescence enhancement was observed with addition of β-alanine, L-alanine, and L-valine to ADDR1 dye (photoinduced electron transfer, PET), whereas addition of glycine exhibits enhancement accompanied with a shift toward a longer-wavelength region. Interestingly, the addition of amino acids to non-PET dyes results in a fluorescence quenching accompanied with a larger shift toward the shorter-wavelength region. The properties of fluorophore and nonfluorophore dyes in the presence of alanine or valine are found to be entirely different from those of glycine. The interaction of alanine with ADDR dyes is predominantly through H-bonding, but the structural aspects of H-bonding interactions of alanine and water are completely different from those of glycine and water. The time-correlated single-photon counting method portrays the existence of fluorophore in two distinguishable microenvironments in the presence of amino acids. The fluorescence spectral technique used as a tool in elucidating the mode of interaction of dye with neutral amino acids in aqueous solution is illustrated in the present study.

INTRODUCTION

Amino acids are broadly classified into three groups, neutral, acidic, and basic, wherein neutral amino acids have a better solubility in water compared to other amino acids. Several studies on the H-bonding characteristics of neutral amino acids have been well established with respect to glycine and alanine. The H-bonding properties and the orientation of amino acids with a water molecule in the gaseous state largely differ from those in the aqueous phase. These amino acids exist in the neutral form (gaseous) and zwitterionic form in water. The amino acid possesses two functional groups involved in the H-bonding interaction, wherein it acts as a H-bond donor as well as an acceptor. In water, they exist as COO− and NH3+, possessing both electrostatic and H-bonding interactions. The orientation of water molecules surrounding amino acids depends upon the nature of the amino acid. Interestingly, the mode of H-bonding of alanine widely differs from that of glycine, which is significant in the field of organized molecular assemblies with water molecules. There are reports that postulate the nature of H-bonding, which is based on the number of water molecules surrounding glycine, alanine, or valine. Even though the alkyl chain length increases by methyl substitution only, a large variation in the H-bonding arrangement is visualized.

There are several reports on the role of glycine—water H-bonding interactions, but the interaction of simple amino acids with fluorophores based on charge transfer (CT) and electron transfer (ET) is found to be very less confined to the field of photophysics.1–7 Glycine acts as a H-bond donor as well as an acceptor and further behaves like an excellent guest molecule with water, forming various types of H-bonding patterns. Our earlier report6 reveals that a nonfluorescent amino acid (glycine) interaction with photoinduced electron transfer (PET) and non-PET-based fluorophore in aqueous solution have established that the ground-state and excited-state characteristics of acridinedione dyes are largely influenced and governed by the H-bonding between amino acids and fluorophore.

Received: April 9, 2019
Accepted: June 26, 2019
Published: July 18, 2019
Compared to glycine, alanine is a nonpolar hydrophobic amino acid and L-alanine is the smallest chiral R-amino acid with a nonreactive methyl group as the side chain. L-alanine interacts favorably with water molecules compared to glycine, which interacts with water molecules. The structural properties of alanine in aqueous solution differ significantly from those in the crystalline phase. The structural variation mainly attributed to H-bonding and the number of water molecules involved in it. The H-bonding properties of neutral amino acids in water illustrate that alanine is entirely different from glycine even though there is no large variation in the structure. In the hydrophobicity scale, alanine is placed after glycine, leucine, isoleucine, and valine. The α-carbon atom of alanine is bound to a methyl group, making it one of the simplest α-amino acids. Compared to glycine, alanine participates in several metabolic functions like in sugar and acid metabolism in our body. Alanine enhances the immune system by producing antibodies; provides energy for muscle tissues, brain, and central nervous system; and acts as a stimulant for glucagon secretion. It is also used in pharmaceutical preparations for injection or infusion, in dietary supplements, and as a flavor compound, which makes alanine more important than glycine.

In spite of the fact that there are a number of studies on the H-bonding properties of alanine, a majority of such known reports are confined and related to theoretical studies only. Quantum mechanical treatment, molecular modeling, and density functional theory studies have been carried out in depth regarding the parameters confined to H-bonding interactions of alanine with water molecules in a gaseous phase, and the structural parameters are obtained from computational studies only. These parameters and information could not be directly applied to an aqueous phase due to the change in the structural conformation of amino acids. The widely used experimental zwitterionic structure of alanine is normally assumed to be the structure in aqueous media, and this is derived from solid-state crystallographic data. However, the nature and origin of the zwitterionic form of alanine in the solid phase and in water are found to be entirely different as ascertained by spectroscopic techniques like infrared, Raman, and neutron scattering techniques.

To obtain a clear idea on the H-bonding properties of alanine with resorcinol-based aqueous acridinedione (ADDR) dyes by fluorescence spectral studies, we compared the photophysical properties of PET- and non-PET-based acridinedione dyes with valine and glycine in water. ADDR dyes have been widely studied mainly as a sensor since they possess a unique advantage such that both PET and internal charge transfer (ICT) phenomena exist, which is of significant importance in a single molecule. Further, according to the literature, an acridine-based fluorophore acts as a bifunctional molecule such that it behaves like an electron donor as well as an acceptor. The acridine-based fluorophore undergoes various interesting excited-state reactions in the presence of several H-bonding solutes. Metal ions sensors, studies related to anion sensing, and with polymer matrices. These dyes possess a unique advantage over other PET- and ICT-based dyes, which is due to the variation in the substituent present in the 9th and 10th positions of the basic acridinedione ring structure. The fluorescence quantum yield, emission, and lifetime of the ADDR1 dye differ largely from those of ADDR2 and ADDR3 dyes. Further, the nature of the solvent and solutes considerably influences the excited-state characteristics and microenvironment of the ADDR1 dye in aqueous solution. We have reported extensively on the variation of the PET process in the ADDR1 dye such that urea, amides, and proteins suppress the PET process, while gum arabic (GA) promotes the PET process. The electron transfer (ET) phenomenon varies with the nature and type of nonfluorophoric solute employed. Being water-soluble, ADDR dyes have a clear advantage over other organic dyes (insoluble in water), and the solvent-based variation in the photophysical nature of ADDR dyes in the presence of solute plays an important role in the field of fluorescence spectroscopy concerned with host–guest systems.

Fluorescence spectroscopic techniques are employed as an ideal tool to probe the host–guest systems, since no significant quantum of photophysical properties of alanine with fluorescent probes are reported in the literature concerned with PET- or ICT-based dyes to the best of our knowledge. The present investigation focuses mainly on the large-scale variation in the fluorescence properties (lifetime and emission intensity) of the ADDR1 dye (PET) with alanine and valine in aqueous solution in comparison with non-PET-based ADDR in aqueous medium. The basic structures of the ADDR dyes used in the present study and the amino acids (glycine, β-alanine, l-alanine, and l-valine) are provided in Schemes 1 and 2, respectively.

**Scheme 1. ADDR Dyes Are Classified into Two Types: PET- and Non-PET-Based Dyes Based on Substitution at the 9th and 10th Positions of the Acridinedione Dye Ring Structure**

```
"ADDR1 (PET), ADDR2 and ADDR3 (non-PET)."
```

**RESULTS AND DISCUSSION**

**Absorption Spectral Studies.** The ADDR1 dye exhibits a broad absorption peak at around 375 ± 5 nm. This peak maximum at 375 ± 5 nm is referred to as the ICT absorption maximum. This is assigned to the charge transfer (CT) from the ring nitrogen (nitrogen at 9th position) to the carbonyl oxygen in the acridinedione ring moiety. The absorption spectra of the ADDR1 dye with β-alanine and l-alanine in water are shown in Figure 1a,b, respectively.

The ICT absorption maximum of the ADDR1 dye remains the same with an increase in the concentration of β-alanine and l-alanine (0.6 and 1.2 M), and this clearly reveals that there is no change in the absorption maximum on addition of alanine and that this also does not influence the ICT absorption maximum. A similar behavioral pattern was also found on addition of glycine to the ADDR1 dye. Also, no characteristic change in the ICT absorption maximum of non-PET-based ADDR dyes was observed on addition of β-alanine or L-alanine, and the absorption spectra of non-PET dyes with L-alanine are shown in Supporting Information Figures S1 and S2, respectively. In our earlier studies on several H-bonding solutes with either PET- or non-PET-based ADDR dyes, the ICT absorption maximum remained unperturbed.
This signiﬁes that the ground-state properties of ADDR dyes are not inﬂuenced by the presence of H-bonding solutes and the nature of interaction between dye and amino acid could not be clearly established.

Interaction of ADDR dyes with alanine or valine signiﬁes that the longest-wavelength absorption maximum remains unperturbed and least inﬂuenced even in the presence of very high concentration of amino acids (>1.0 M). Several studies based on the H-bonding interaction and hydrophobic inﬂuences of nonﬂuorophoric guest systems containing H-bond donor and acceptor moieties with acridinedione dyes were also carried out in aqueous solutions.19,22−25 It has been well established that the ICT absorption peak maximum remains the same in the presence of H-bonding solutes and the ground-state spectral characteristics of both PET- and non-PET-based ADDR dyes are not inﬂuenced by these solutes (urea derivatives, amide derivatives, and GuHCl). In our present study, the absorption and emission spectral behavior of the ADDR1 dye with alanine is thoroughly studied and compared with that with valine and glycine in water. The precise nature of interaction of PET- or non-PET-based ADDR dyes with neutral amino acids could not be completely established from absorption spectral studies. To ascertain the most probable mode of interaction and functional groups involved in direct interaction of ADDR dyes with alanine, we carried out steady-state and time-resolved ﬂuorescence spectral studies by ﬁxing the concentration of the ADDR dye and varying the concentration of amino acids.

Emission Spectral Studies. The ADDR1 dye exhibits emission maximum at 436 ± 2 nm when excited at the ICT absorption maximum. Addition of β-alanine and L-alanine to the ADDR1 dye results in a gradual decrease in the ﬂuorescence (not signiﬁcant) with no considerable shift in the emission maximum. Emission spectra of ADDR1 with β-alanine and L-alanine are shown in Figures 2 and 3, respectively. Interestingly, the decrease in the ﬂuorescence intensity of ADDR1 dye is negligibly small based on the phenomenon of ﬂuorescence quenching such that the excited-state behavior of ADDR1 dye is inﬂuenced to some extent in the presence of alanine. Although both glycine and alanine belong to the class of simple amino acids, their role in the ﬂuorescence emission characteristics of ADDR1 dye is found to be strikingly different. Addition of glycine to the ADDR1 dye results in a larger extent of ﬂuorescence enhancement accompanied with a red shift in the emission maximum, which is not observed in the case of β-alanine or L-alanine. Studies related to the ADDR1 dye in the presence of urea, amides, GuHCl, and bovine serum albumin in water resulted in a ﬂuorescence

Figure 1. (a) Absorption spectra of ADDR1 dye + β-alanine: (1) 0.60 M β-alanine, (2) 1.2 M β-alanine, (3) ADDR1 dye alone, (4) ADDR1 dye + 0.60 M β-alanine, and (5) ADDR1 dye + 1.2 M β-alanine. (b) Absorption spectra of ADDR1 dye + l-alanine: (1) 0.60 M l-alanine, (2) 1.2 M l-alanine, (3) ADDR1 dye alone, (4) ADDR1 dye + 0.60 M l-alanine, and (5) ADDR1 dye + 1.2 M l-alanine.

This signiﬁes that the ground-state properties of ADDR dyes are not inﬂuenced by the presence of H-bonding solutes and the nature of interaction between dye and amino acid could not be clearly established.

Interaction of ADDR dyes with alanine or valine signiﬁes that the longest-wavelength absorption maximum remains unperturbed and least inﬂuenced even in the presence of very high concentration of amino acids (>1.0 M). Several studies based on the H-bonding interaction and hydrophobic inﬂuences of nonﬂuorophoric guest systems containing H-bond donor and acceptor moieties with acridinedione dyes were also carried out in aqueous solutions.19,22−25 It has been well established that the ICT absorption peak maximum remains the same in the presence of H-bonding solutes and the ground-state spectral characteristics of both PET- and non-PET-based ADDR dyes are not inﬂuenced by these solutes (urea derivatives, amide derivatives, and GuHCl). In our present study, the absorption and emission spectral behavior of the ADDR1 dye with alanine is thoroughly studied and compared with that with valine and glycine in water. The precise nature of interaction of PET- or non-PET-based ADDR dyes with neutral amino acids could not be completely established from absorption spectral studies. To ascertain the most probable mode of interaction and functional groups involved in direct interaction of ADDR dyes with alanine, we carried out steady-state and time-resolved ﬂuorescence spectral studies by ﬁxing the concentration of the ADDR dye and varying the concentration of amino acids.

Emission Spectral Studies. The ADDR1 dye exhibits emission maximum at 436 ± 2 nm when excited at the ICT absorption maximum. Addition of β-alanine and L-alanine to the ADDR1 dye results in a gradual decrease in the ﬂuorescence (not signiﬁcant) with no considerable shift in the emission maximum. Emission spectra of ADDR1 with β-alanine and L-alanine are shown in Figures 2 and 3, respectively. Interestingly, the decrease in the ﬂuorescence intensity of ADDR1 dye is negligibly small based on the phenomenon of ﬂuorescence quenching such that the excited-state behavior of ADDR1 dye is inﬂuenced to some extent in the presence of alanine. Although both glycine and alanine belong to the class of simple amino acids, their role in the ﬂuorescence emission characteristics of ADDR1 dye is found to be strikingly different. Addition of glycine to the ADDR1 dye results in a larger extent of ﬂuorescence enhancement accompanied with a red shift in the emission maximum, which is not observed in the case of β-alanine or L-alanine. Studies related to the ADDR1 dye in the presence of urea, amides, GuHCl, and bovine serum albumin in water resulted in a ﬂuorescence

Figure 1. (a) Absorption spectra of ADDR1 dye + β-alanine: (1) 0.60 M β-alanine, (2) 1.2 M β-alanine, (3) ADDR1 dye alone, (4) ADDR1 dye + 0.60 M β-alanine, and (5) ADDR1 dye + 1.2 M β-alanine. (b) Absorption spectra of ADDR1 dye + l-alanine: (1) 0.60 M l-alanine, (2) 1.2 M l-alanine, (3) ADDR1 dye alone, (4) ADDR1 dye + 0.60 M l-alanine, and (5) ADDR1 dye + 1.2 M l-alanine.

This signiﬁes that the ground-state properties of ADDR dyes are not inﬂuenced by the presence of H-bonding solutes and the nature of interaction between dye and amino acid could not be cleanly established.

Interaction of ADDR dyes with alanine or valine signiﬁes that the longest-wavelength absorption maximum remains unperturbed and least inﬂuenced even in the presence of very high concentration of amino acids (>1.0 M). Several studies based on the H-bonding interaction and hydrophobic inﬂuences of nonﬂuorophoric guest systems containing H-bond donor and acceptor moieties with acridinedione dyes were also carried out in aqueous solutions.19,22−25 It has been well established that the ICT absorption peak maximum remains the same in the presence of H-bonding solutes and the ground-state spectral characteristics of both PET- and non-PET-based ADDR dyes are not inﬂuenced by these solutes (urea derivatives, amide derivatives, and GuHCl). In our present study, the absorption and emission spectral behavior of the ADDR1 dye with alanine is thoroughly studied and compared with that with valine and glycine in water. The precise nature of interaction of PET- or non-PET-based ADDR dyes with neutral amino acids could not be completely established from absorption spectral studies. To ascertain the most probable mode of interaction and functional groups involved in direct interaction of ADDR dyes with alanine, we carried out steady-state and time-resolved ﬂuorescence spectral studies by ﬁxing the concentration of the ADDR dye and varying the concentration of amino acids.

Emission Spectral Studies. The ADDR1 dye exhibits emission maximum at 436 ± 2 nm when excited at the ICT absorption maximum. Addition of β-alanine and L-alanine to the ADDR1 dye results in a gradual decrease in the ﬂuorescence (not signiﬁcant) with no considerable shift in the emission maximum. Emission spectra of ADDR1 with β-alanine and L-alanine are shown in Figures 2 and 3, respectively. Interestingly, the decrease in the ﬂuorescence intensity of ADDR1 dye is negligibly small based on the phenomenon of ﬂuorescence quenching such that the excited-state behavior of ADDR1 dye is inﬂuenced to some extent in the presence of alanine. Although both glycine and alanine belong to the class of simple amino acids, their role in the ﬂuorescence emission characteristics of ADDR1 dye is found to be strikingly different. Addition of glycine to the ADDR1 dye results in a larger extent of ﬂuorescence enhancement accompanied with a red shift in the emission maximum, which is not observed in the case of β-alanine or L-alanine. Studies related to the ADDR1 dye in the presence of urea, amides, GuHCl, and bovine serum albumin in water resulted in a ﬂuorescence
enhancement except in the case of GA. This clearly implies that the excited-state nature of the ADDR1 dye is governed by the nature of the solute, even though all of the solutes belong to the same class of well-structured H-bonding self-assemblies. The addition of valine to the ADDR1 dye also resulted in a pattern of decrease in the fluorescence intensity similar to that observed in alanine. Interestingly, the local-excited (LE)-state emission maximum of the ADDR dye on addition of valine follows a pattern similar to that observed with alanine but differs completely from that in glycine interaction with the ADDR1 dye. The emission spectrum of ADDR1 dye with valine is shown in Supporting Information Figure S3. From emission spectral studies of the ADDR1 dye with alanine or valine, a clear illustration on the role of H-bonding properties and their influence on the LE-state emission are visualized from the variation in the emission intensity. Glycine results in a larger extent of fluorescence enhancement, whereas an increase in the alkyl chain length of amino acids (alanine and valine) results in neither a considerable shift in the position maxima nor a change in the fluorescence intensity. The extent of decrease in the fluorescence intensity of ADDR1 dye in the presence of alanine and valine is less than 5%, which was not observed in any of the H-bonding solutes used so far. The difference on the decrease in the fluorescence quenching, was found to be very less compared to conventional fluorescence quenching phenomenon and in other words based on the fluorescence emission intensity values, we can conveniently ascertain that alanine or valine does not influence the PET process through space in ADDR1 dye like glycine. The variation in the fluorescence intensity of the ADDR1 dye with various amino acids is shown in Figure 4.

The emission spectrum of non-PET-based ADDR dyes also exhibit emission maximum above 440 nm. The LE state emission is at around 440 ± 2 nm, and a broad LE-state-promoted CT state emission occurs in the emission range of 470 ± 5 nm. Addition of alanine (β-alanine and i-alanine) or valine to the ADDR2 dye results in a fluorescence quenching concerned with the emission intensity corresponding to the CT state, and a gradual quenching of emission intensity at 470 ± 5 nm results. Further, a clear blue shift results and the emission maxima almost correspond to the LE state emission (440 ± 2 nm) (Figures 5 and 6). Quite interestingly, the addition of alanine or valine to the ADDR3 dye results in no significant change in the fluorescence intensity, as observed in

![Figure 2](image2.png)

**Figure 2.** Emission spectra of ADDR1 dye + β-alanine: (1) ADDR1 dye alone, (2) ADDR1 dye + 0.075 M β-alanine, (3) ADDR1 dye + 0.15 M β-alanine, (4) ADDR1 dye + 0.30 M β-alanine, (5) ADDR1 dye + 0.45 M β-alanine, and (6) ADDR1 dye + 0.60 M β-alanine.

![Figure 3](image3.png)

**Figure 3.** Emission spectra of ADDR1 dye + i-alanine: (1) ADDR1 dye alone, (2) ADDR1 dye + 0.075 M i-alanine, (3) ADDR1 dye + 0.15 M i-alanine, (4) ADDR1 dye + 0.30 M i-alanine, (5) ADDR1 dye + 0.45 M i-alanine, and (6) ADDR1 dye + 0.60 M i-alanine.

![Figure 4](image4.png)

**Figure 4.** Extent of fluorescence enhancement/quenching of ADDR1 dye with various amino acids: (black circle, solid), glycine; (red circle, solid), β-alanine; (green circle, solid), i-alanine; and (blue circle, solid), i-valine.

![Figure 5](image5.png)

**Figure 5.** Emission spectra of ADDR2 dye + β-alanine: (1) ADDR2 dye alone, (2) ADDR2 dye + 0.075 M β-alanine, (3) ADDR2 dye + 0.15 M β-alanine, (4) ADDR2 dye + 0.30 M β-alanine, (5) ADDR2 dye + 0.45 M β-alanine, and (6) ADDR2 dye + 0.60 M β-alanine.
the case of other H-bonding solutes. Although both ADDR2 and ADDR3 dyes belong to the class of non-PET nature, their excited-state properties are found to be different in the presence of alanine or valine. In our report on the interaction of glycine with the ADDR2 or ADDR3 dye, the extent of fluorescence quenching was less than 10%, which is contrary to our present results. Interaction of urea derivatives, amide derivatives, and GuHCl with the ADDR3 dye does not result in any significant change in the fluorescence intensity values, and this was attributed to the absence of an electron-donating group (OCH$_3$) at the para position of the phenyl ring attached to the 9th carbon of the basic dye structure. The emission spectra of ADDR2 dye with $\beta$-alanine are shown in Figure 5 and those of $\iota$-alanine are shown in Figure 6.

A variation in the fluorescence intensity and a shift in the emission maxima are visualized when the solute is changed from glycine to valine. Addition of valine to the ADDR2 dye also exhibits a similar behavior to that of alanine. A fluorescence enhancement was observed on addition of glycine, urea, amide derivatives, and GuHCl to the ADDR3 dye. The increase in the fluorescence intensity illustrates that these H-bonding solutes effectively suppress the PET process through space, thereby increasing the quantum yield. On the contrary, a drastic decrease in the fluorescence intensity of ADDR1 dye resulted on addition of GA, which is a well-known food hydrocolloid soluble in water that contains several H-bonding sugar moieties.\textsuperscript{34} This quenching phenomenon is completely attributed to the promotion of PET process through space. This variation in the fluorescence behavior clearly shows that the PET processes through space are influenced by the nature of the solute, solute–solvent interactions, and presence of hydrophobic or hydrophilic moieties. We observe that alanine or valine does not suppress nor promotes the PET process effectively compared with glycine\textsuperscript{7} and other hydrogen-bonding solutes.\textsuperscript{19,22,23,35} It is well known that the solute concentration and the type of solute also play a major role in the microenvironment, such that the addition of glycine results in a bathochromic shift, whereas alanine or valine (hydrophobic in nature compared to glycine) results in no significant shift. Further, urea or amides hardly influence the excited-state nature of non-PET dyes in water, whereas the LE-state nature of these dyes is completely quenched in the presence of alanine or valine and to a certain extent in glycine. The extent of fluorescence quenching of ADDR2 dye with various amino acids is shown in Figure 7.

From emission spectral studies, it is evident that $\beta$-alanine or $\iota$-alanine interaction with ADDR dyes is entirely different from that of glycine, and this presumably is attributed to the variation in the H-bonding arrangement of the amino acid–dye–water system.

To establish the existence of dye located in distinguishable microenvironments of aqueous amino acids, we carried out fluorescence lifetime studies.

**Time-Correlated Fluorescence Spectral Properties.** The ADDR1 dye exhibits a lifetime of 500 ± 10 ps in aqueous solution.\textsuperscript{19} On addition of $\beta$-alanine or $\iota$-alanine to the ADDR1 dye, the fluorescence lifetime of ADDR dye becomes biexponential. The PET lifetime component increases, and the long-lifetime component decreases substantially. The lifetime decay profiles of the ADDR1 dye with $\beta$-alanine and $\iota$-alanine are shown in Figures 8 and 9, respectively, and their lifetime components along with the relative amplitude are provided in Tables 1 and 2, respectively.

**Figure 6.** Emission spectra of ADDR2 dye + $\iota$-alanine: (1) ADDR2 dye alone, (2) ADDR2 dye + 0.075 M $\iota$-alanine, (3) ADDR2 dye + 0.15 M $\iota$-alanine, (4) ADDR2 dye + 0.30 M $\iota$-alanine, (5) ADDR2 dye + 0.45 M $\iota$-alanine, and (6) ADDR2 dye + 0.60 M $\iota$-alanine.

**Figure 7.** Extent of fluorescence quenching of ADDR2 dye with various amino acids: (black circle, solid), glycine; (red circle, solid), $\beta$-alanine; (green circle, solid), $\iota$-alanine; and (blue circle, solid), $\iota$-valine.

**Figure 8.** Lifetime decay of ADDR1 dye + $\beta$-alanine: (1) light-emitting diode (LED) profile, (2) ADDR1 dye alone, (3) ADDR1 dye + 0.60 M $\beta$-alanine, and (4) ADDR1 dye + 1.2 M $\beta$-alanine.
The extent of increase in the PET component 500 ± 10 ps of the ADDR1 dye is more predominant in the case of β-alanine rather in ν-alanine. The increase in the fluorescence lifetime of ADDR1 dye from 500 ps up to 750 ps in the presence of alanine shows that the PET process is completely suppressed, which results in an increase in the lifetime of the fluorophore. The biexponential component of ADDR1 dye with β-alanine reveals that the dye molecule is situated in two distinguishable microenvironments in water. The long-lifetime-component distribution is around 25%, whereas the short-lifetime component (PET) exists in the aqueous phase with 75% amplitude distribution. Even though ν-alanine differs from β-alanine structurally, it also exhibits two lifetime components similar to β-alanine, but the lifetime distribution components are entirely different. Addition of glycine to the ADDR1 dye resulted in two different lifetime components with varying lifetimes and amplitudes, which differ from those of alanine. Fluorescence lifetime studies authenticate that the type of the solute influences the excited-state fluorescence lifetime properties of the PET-based dye, which is clearly distinguishable from glycine or alanine. Herewith, we propose that ADDR1 exists in more than one microenvironment and is correlated to the fluorophore predominantly surrounded by the water molecule and less number of alanine molecules. The relatively long-lifetime species is correlated to dye predominately surrounded by alanine molecules only with more or less number of water molecules. A similar behavior resulted on introduction of glycine to the ADDR1 dye. We visualize an increase in the fluorescent lifetime of the PET component from 500 to 740 ps. A relatively long-lifetime component of 6.00 ns is accompanied with the PET component also. The fluorescent lifetime of 740 ps is assigned to the orientation of ADDR1 dye surrounded mainly by the water molecule only. The long-lifetime component is attributed to the dye surrounded by less number of water molecules, and the dye resides in a constrained environment. The coexistence of short- and long-lifetime components with variation in their lifetime in the presence of alanine illustrates that β-alanine or ν-alanine induces a new microenvironment in the aqueous phase. An entirely predominant hydrophobic environment formed in the aqueous medium coexists with dye surrounded by water molecules. The proportion of hydrophobic environment rapidly changes on addition of amino acids. The ADDR1 dye is categorized as hydrophobic dye, and, in general, it prefers to orient toward a more hydrophobic domain rather than toward the water molecules (hydrophilic environment). This phenomenon is the reason for the increase in the relative amplitude of the long-lifetime component on addition of alanine. A similar pattern of two different lifetime components was also observed in the ADDR1 dye in the presence of valine.

The creation of two distinguishable microenvironments suggests that the presence of hydrophobic influences on the H-bonding interaction is predominant at a higher concentration of amino acid. The creation and stabilization of the hydrophobic environment results in the orientation of dye toward the hydrophobic moieties (alanine). The hydrophobic nature increases with the methyl substitution of the amino acid such that the proportion of dye in an environment surrounded by glycine (2.4 M) is 10%, whereas in the case of alanine (1.2 M), the amplitude distribution is 25%. The gradual increase in the lifetime of PET component and the existence of the long-lifetime component reveal that alanine effectively suppresses the PET process; further, it also paves way for the creation of variation in the microenvironment in the aqueous phase by the introduction of hydrophobic groups. Interestingly, we did not observe any fluorescence enhancement of the ADDR1 dye through steady-state emission spectral studies but a prominent increase in the fluorescence lifetime of the ADDR1 dye (0.5 ns) is observed on addition of alanine. Through time-correlated single-photon counting (TCSPC) techniques, we authenticate that alanine suppresses the PET process similar to H-bonding solutes, which results in an increase in the fluorescence lifetime of the ADDR1 dye. Fluorescence lifetime characteristics of alanine with the ADDR dye are almost similar to those with glycine. Addition of valine results in a decrease in the lifetime of the PET component as well as the non-PET component of the ADDR1 dye, but the decrease in the PET component was found to be less (500 ± 10 to 400 ± 10 ps) (Supporting Information Figure S4). This is in accordance with the decrease in the fluorescence intensity of the ADDR1 dye in the presence of valine through steady-state measurements. A gradual decrease in the PET lifetime component with an increase in the concentration of valine reveals that the fluorescence lifetime behavior of the ADDR1 dye in the presence of valine is completely different from that with alanine.

**Fluorescence Lifetime Studies of ADDR2 Dye with Alanine.** The ADDR2 dye exhibits a long-fluorescence-lifetime component of 8.3 ± 0.2 ns in water. The relatively long lifetime of the ADDR2 dye is attributed to very high fluorescence quantum yield (>0.90), whereas the short lifetime is suppressed, which results in an increase in the lifetime of the fluorophore. The biexponential component of ADDR2 dye with β-alanine or ν-alanine induces a new microenvironment in the aqueous phase.

**Table 1. Lifetime Decay Analysis of ADDR1 Dye with β-Alanine**

| [β-alanine] (M) | τ1 (ns) | τ2 (ns) | B1 (%) | B2 (%) | χ² |
|----------------|--------|--------|--------|--------|----|
| 0              | 0.53   | 6.04   | 97     | 3      | 1.13 |
| 0.6            | 0.65   | 5.36   | 84     | 16     | 1.29 |
| 1.2            | 0.74   | 5.19   | 75     | 25     | 1.23 |

**Table 2. Lifetime Decay Analysis of ADDR1 Dye with ν-Alanine**

| [ν-alanine] (M) | τ1 (ns) | τ2 (ns) | B1 (%) | B2 (%) | χ² |
|----------------|--------|--------|--------|--------|----|
| 0              | 0.52   | 6.29   | 97     | 3      | 1.24 |
| 0.6            | 0.59   | 6.16   | 94     | 6      | 1.24 |
| 1.2            | 0.66   | 6.08   | 91     | 9      | 1.23 |

Figure 9. Lifetime decay of ADDR1 dye + ν-alanine: (1) LED profile, (2) ADDR1 dye alone, (3) ADDR1 dye + 0.60 M ν-alanine, and (4) ADDR1 dye + 1.2 M ν-alanine.
observed in the ADDR1 dye is due to the PET process through space in water. Addition of β-alanine or L-alanine to the ADDR2 dye results in a single exponential decay as observed in the case of glycine. The fluorescence decay profiles of the ADDR2 dye with β-alanine, L-alanine, and L-valine are shown in Supporting Information Figures S5−S7, respectively. A small decrease in fluorescence lifetime from 8.3 to 8.0 ns results at the highest concentration of alanine. Such a small decrease in the fluorescence lifetime does not correlate to the conventional fluorescence quenching behavior. A similar pattern of decrease in the fluorescence lifetime of ADDR2 dye was also observed in glycine. Interestingly, the proportion of decrease in the fluorescence intensity of ADDR2 dye by alanine through steady-state emission spectral measurements was found to be more significant in the context of the fluorescence quenching phenomenon. This observation predicts a larger role of alanine in the local-excited-state emission of the ADDR2 dye, which is apparently seen in the shift in the emission maximum toward the blue region. The hypsochromic shift suggests a hydrophobic influence on the excited-state properties of the ADDR2 dye. This type of spectral behavior was not observed in the case of glycine interaction with ADDR2 dye. Further, no drastic change in the fluorescence intensity or lifetime and shift in the emission maxima toward a red or blue region resulted in the case of glycine, whereas a distinct behavior was obtained in alanine. This confirms that the properties resulting in a large variation in the photophysical behavior of ADDR dyes by glycine or alanine are found to be entirely different and that the structural orientation and mode of H-bonding play a crucial role.

In spite of observing a large variation in the fluorescence emission pattern in the presence of alanine compared to that with glycine, a clear mode of interaction between glycine with non-PET dyes could be established. No concrete or precise information regarding the fluorescence lifetime as compared to that of PET dye was obtained in the case of glycine. A characteristic bathochromic shift was observed in the ADDR1 dye, whereas no significant shift of the emission maximum of ADDR2 or ADDR3 dye resulted on addition of glycine. Furthermore, the nature of interaction is not determined only through H-bonding, but hydrophobic influences also coexist; however, hydrophobic influences have a lesser contribution and are found to be less predominant compared to H-bonding. From the above results, we have provided a schematic representation of the ADDR1 dye with alanine in Scheme 3.

Scheme 3. Schematic Representation of ADDR Dye Interaction with Alanine

H-bonding Arrangement in Water: Alanine versus Glycine. The variation in the photophysical characteristics of various ADDR dyes in the presence of alanine is found to be entirely different from that in the presence of glycine. This is predominantly attributed to the variation in the H-bonding nature of glycine−water from that of alanine−water framework. The nature of interaction varies extensively based on the number of water molecules surrounding the amino acid. Ide et al. reported on the structure of water in solutions containing various amino acids at neutral pH and proposed that the water structure is not influenced by the nature and length of the amino acid side chains.11 If the above statement would have been applied for all neutral amino acids, a similar pattern of behavior would have been resulted for glycine, alanine, or valine irrespective of the nature of the substituent. On the contrary, a difference in the fluorescence emission behavior results on moving from glycine to alanine. Further, it has been well established that the hydration numbers41,42 for the ammonium group largely differ for glycine to alanine. The value being 3.0 refers to glycine, and 2.4 corresponds to alanine such that the amino acid hydration structure depends on the type of the amino acid and the presence of substitution in the R group of the amino acid.42−44 Several other experimental studies on the structural properties of water with amino acids reveal that the existence of a zwitterionic structure and the number of water molecules surrounding the amino acid influence the H-bonding interactions. Even though the structure and dynamic properties of various amino acids in aqueous solutions were obtained through computer simulation studies, no clear experimental proof in aqueous solution has validated those theoretical findings. This is largely attributed to the presence of more number of water molecules, which is not included in many theoretical studies. Fluorescence spectral techniques using probes in aqueous solution containing H-bonding solutes are influenced by the water molecules. In our present study, the fluorescence spectral behavior of alanine varies with that of glycine, which is predominantly attributed to the variation in the number of water molecules surrounding the amino acid. This results in a difference in the number of water molecules present in the hydration sphere surrounding the amino acids and the number of water molecules directly involved in H-bonding with amino acid and dye. On the contrary, the structural parameters of amino acid−water interactions and their vibrational spectra and the stability of the zwitterionic structure in aqueous media have been thoroughly studied using ab initio methods. The studies confirm that the less number of water molecules resulting in a marked difference in the H-bonding properties of amino acids in aqueous and gaseous phase. Both hydrophobic influences and H-bonding interactions along with electrostatic interactions differ from the hydration sphere surrounding the close vicinity of alanine.13−15,45−49 This results in the creation of several microenvironments in the aqueous phase containing alanine, which differ in the H-bonding pattern. The formation and the stabilization of these microenvironments depend upon the concentration of the amino acid.

In the interaction of ADDR1 dye with glycine or alanine, biexponential decay signifies the existence of dye in two different domains that are dependent on the concentration and the nature of the amino acid. Due to less quantum yield of the ADDR1 dye, an increase in the fluorescence lifetime and emission is clearly visualized on addition of amino acids. Since...
ADDR2 and ADDR3 dyes possess a very high quantum yield (0.90), the magnitude of fluorescence quenching on addition of amino acids could not be easily ascertained due to the lesser decrease in the fluorescence lifetime.

**CONCLUSIONS**

Photophysical studies of the ADDR dye with alanine and valine in aqueous solution illustrate that the fluorescence spectral characteristics of both resorcinol-based acridinedione dyes are governed through H-bonding interactions and hydrophobic influences. Introduction of alanine amino acid promotes the creation of a new environment comprising several subdomains, which is heterogeneous and arising several phases. These microenvironments are alanine—water, alanine—alanine, and alanine—dye assemblies in the aqueous phase. The existence of biexponential lifetime components of ADDR1 with alanine illustrates the existence of more than one domain in which the dye resides. The mode of interaction of ADDR1 dye with alanine is mostly by H-bonding, but the dye molecule selectively orients toward the hydrophobic phase when the concentration of the amino acid increases. An increase in the PET component and the formation of a long-lifetime component authenticate that alanine results in suppression of the PET phenomenon but also pushes favorably the dye toward the region where several alanine molecules reside and a fewer number of water molecules are present (hydrophobic domain). Briefly, neutral and essential amino acids like alanine and valine act as excellent molecules to study in depth the fluorescence emission lifetime and quantum yield behavior of both PET- and non-PET-based acridinedione dyes in water. Fluorescence spectral techniques are used as an efficient marker for underlying photophysical properties of ADDR dyes in the presence of amino acids.

**EXPERIMENTAL METHODS**

Absorption spectral studies were performed using an Agilent 8453 diode array spectrophotometer, fluorescence spectral studies (emission spectra) were performed using a Fluoromax-4P spectrometer (Horiba Jobin Yvon), and the spectral data were smoothed using Origin 6.1 software. The emission spectrum was corrected as per the literature reported.32,33 Time-resolved fluorescence decays were obtained by the time-correlated single-photon counting (TCSPC) method32 using a tunable laser source of 377 nm and a nano LED of 370 nm. The experimental setup is provided in the Supporting Information.

**ASSOCIATED CONTENT**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01011.

Experimental setup of the time-correlated single-photon counting (TCSPC) technique; absorption spectra of ADDR2 dye with L-alanine; absorption spectra of ADDR3 dye with L-alanine; emission spectra of ADDR1 with L-valine dye; fluorescence lifetime decay of ADDR1 dye with L-valine; and fluorescence lifetime decay of ADDR2 dye with β-alanine, L-alanine, and L-valine (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

*E-mail: kumaranwau@rediffmail.com, rajendrankumaran@gmail.com. Mobile: 091-9884207497. Phone: 091-44-2475 6655. Fax: 091-44-2475 4349.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors thank Professor P. Ramamurthy and Dr. C. Selvaraju of National Centre for Ultrafast Processes (NCFUP), University of Madras, Taramani campus, Chennai, for allowing us to use and providing the lab facilities. K.R. thanks Dr. S. G. Srivatsan and Dr. Hazra Partha of IISER Pune for carrying out fluorescence lifetime studies. The authors further acknowledge the help of research scholars, T.S.K. and S. Suresh of NCFUP, University of Madras, Taramani Campus, Chennai, for valuable discussions and suggestions regarding this work. The authors thank Dr. R. Ganesh Principal, D.G. Vaishnav College (Autonomous), Chennai, and Shri. Ashok Kumar Mundra, Secretary, for permitting us to avail the laboratory facilities. K.R. also thanks Dr. S. Ilango, Former Head of the Department, Department of Chemistry, D.G. Vaishnav College, Chennai, for his constant support and encouragement.

**REFERENCES**

(1) Alonso, J. L.; Isabel Peña, M.; Sanz, E.; Vaquero, V.; Mata, S.; Cabezas, C.; Juan López, C. Observation of dihydrated glycine. Chem. Commun. 2013, 49, 3443.

(2) Bachrach, S. M. Microsolvation of glycine: a DFT study. J. Phys. Chem. A 2008, 112, 3722.

(3) Alonso, J. L.; Cocinero, E. J.; Lesarri, A.; Eugenia Sanz, M.; Lopez, J. C. The glycine - water complex. Angew. Chem., Int. Ed. 2006, 45, 3471.

(4) Jensen, J. H.; Mark Gordon, S. On the number of water molecules necessary to stabilize the glycine zwitterions. J. Am. Chem. Soc. 1995, 117, 8159.

(5) Ding, Y.; Jesperson, K. K. The glycine zwitterion does not exist in the gas phase: results from a detailed ab initio electronic structure study. Chem. Phys. Lett. 1992, 199, 261.

(6) Basch, H.; Walter Stevens, J. The structure of glycine-water H-bonded complexes. Chem. Phys. Lett. 1990, 169, 275.

(7) Bonaccorsi, R.; Palla, P.; Tomasi, J. Conformational energy of glycine in aqueous solutions and relative stability of the zwitterionic and neutral forms An ab initi study. J. Am. Chem. Soc. 1984, 106, 1945.

(8) Anju, K.; Gayathri, S.; Sumita, A.; Ramamurthy, P.; Kumaran, R. Photophysical studies on the interaction of PET and non-PET based acridinedione dyes with glycine in water. J. Lumin. 2018, 199, 352–362.

(9) Creighton, T. E. Proteins Structure and Molecular Properties, 2nd ed.; W. H. Freeman & Co.: 1992.

(10) Radzicka, A.; Wollenden, R. comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. Biochemistry 1988, 27, 1664–1670.

(11) Wollenden, R.; Andersson, L.; Cullis, P. M.; Southgate, C. C. Affinities of amino acid side chains for solvent water. Biochemistry 1981, 20, 849–855.

(12) Degtyareno, I. M.; Karl Jalkane, J.; Andrey Gurtovenko, A.; Risto Nieminen, M. L-Alanine in a Droplet of Water: A Density-Functional Molecular Dynamics Study. J. Phys. Chem. B 2007, 111, 4227–4234.

(13) Tajkhorshid, E.; Jalkane, K. J.; Suhai, S. Structure and Vibrational Spectra of the Zwitterion l-Alanine in the Presence of
Explicit Water Molecules: A Density Functional Analysis. J. Phys. Chem. B 1998, 102, 5899–5913.

(14) Frimand, K.; Bohr, H.; Jalkanen, K. J.; Suhai, S. Structures, vibrational absorption and vibrational circular dichroism spectra of alanine in aqueous solution: a density functional theory and RHF study. Chem. Phys. 2000, 255, 165–194.

(15) Kikuchi, O.; Watanabe, T.; Ogawa, Y.; Takase, H.; Takahashi, O. AB initio MO and Monte Carlo simulation study on the conformation of L-alanine zwitterion in aqueous solution. J. Phys. Org. Chem. 1997, 10, 145–151.

(16) Destro, R.; Marsh, R. E. R.; Bianchi, R. A low-temperature (23 K) study of L-alanine. J. Phys. Chem. A 1988, 92, 966–973.

(17) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. Precision neutron diffraction structure determination of protein and nucleic acid components. I. Crystal and molecular structure of the amino acid L-alanine. J. Am. Chem. Soc. 1972, 94, 2657–2660.

(18) Simpson, H., Jr.; Marsh, R. E. The crystal structure of L-alanine. Acta Crystallogr. 1966, 20, 550–555.

(19) Kumaran, R.; Ramamurthy, P. PET suppression of acridinediones by urea derivatives in water and methanol. J. Phys. Chem. B 2006, 110, 23783.

(20) Kumaran, R.; Vanjinathan, M.; Ramamurthy, P. Role of H-bonding and photoinduced electron transfer (PET) on the interaction of resorcinol based acridinedione with Bovine Serum Albumin (BSA) in water. J. Lumin. 2015, 164, 146.

(21) Rajendran, K.; Ramamurthy, P. Photophysical studies of PET based acridinedione dyes with globular protein: Bovine Serum Albumin (BSA). J. Lumin. 2010, 130, 1203.

(22) Kumaran, R.; Varalakshmi, T.; Padma Malar, E. J.; Ramamurthy, P. Photophysical studies on the interaction of acridinedione dyes with universal protein denaturant: guanidine hydrochloride. J. Fluoresc. 2010, 20, 993.

(23) Kumaran, R.; Ramamurthy, P. Photophysical studies on the interaction of formamide and alkyl substituted amides with photoinduced electron transfer (PET) based acridinedione dyes in water. J. Fluoresc. 2011, 21, 2165.

(24) Thiagarajan, V.; Indirapriyadharshini, V. K.; Ramamurthy, P. Fencing of photoinduced electron transfer in non-conjugated bicycromophoric system by B-Cyclodextrin nanocavity. J. Inclusion Phenom. Macrocyclic Chem. 2006, 56, 309.

(25) Indirapriyadharshini, V. K.; Karunanithi, P.; Ramamurthy, P. Inclusion of resorcinol based acridinedione dyes in cyclodextrins: fluorescence enhancement. Langmuir 2001, 17, 4056.

(26) Ashok Kumar, P.; Ramakrishnan, V. T.; Ramamurthy, P. Photoinduced electron transfer (PET) Based Zn2+ fluorescent probe: transformation of turn-on sensors into ratiometric ones with dual emission in acetone. J. Phys. Chem. A 2011, 115, 14292.

(27) Ashok Kumar, P.; Ramakrishnan, V. T.; Ramamurthy, P. Solvent-controlled metal ion binding selectivity and anion interaction of the acridinedione-based heteroditopic host. J. Phys. Chem. B 2011, 115, 84–92.

(28) Ashok Kumar, P.; Thiagarajan, V.; Vasanthi, S.; Ramamurthy, P. Triple fluorescence of acridinedione: locally excited, photoinduced electron transfer promoted charge transfer and anion induced charge transfer states. J. Photochem. Photobiol., A 2009, 208, 117.

(29) Koteswari, R.; Ashok Kumar, P.; Ramakrishnan, V. T.; Padma Malar, E. J.; Ramamurthy, P. Unprecedented formation of an N-benzamidobisthiourea derivative and its role in the formation of a new CT state specific towards fluoride ion. Chem. Commun. 2010, 46, 3268.

(30) Koteswari, R.; Ashok Kumar, P.; Padma Malar, E. J.; Ramakrishnan, V. T.; Ramamurthy, P. Highly selective, sensitive and quantitative detection of Hg 2+ in aqueous medium under broad pH range. Chem. Commun. 2011, 47, 7695.

(31) Thiagarajan, V.; Selvaraju, C.; Ramamurthy, P. Excited state behavior of acridinedione dyes in PMMA matrix: inhomogeneous broadening and enhancement of triplet. J. Photochem. Photobiol., A 2003, 157, 23.

(32) Chi, Z.; Liu, R.; Zhang, H. Non-covalent interaction of oxytetracycline with the enzyme trypsin. Biomacromolecules 2010, 11, 2454.

(33) Zong, W.; Liu, R.; Sun, Y.; Teng, X.; Fang, J.; Chai, J. A new strategy to identify and eliminate the inner filter effects by outer filter technique. J. Fluoresc. 2011, 21, 1249.

(34) Sowontharya, C.; Gayathri, S.; Dhenadayalan, N.; Vasanthi, R.; Vanjinathan, M.; Kumaran, R. Photophysical studies of a food hydrocolloid, Gum Arabic with resorcinol based acridinedione dyes in water. J. Photochem. Photobiol., A 2017, 341, 78–86.

(35) Selvaraju, C.; Thiagarajan, V.; Ramamurthy, P. Interaction of 1,8-acridinedione dye with urea dimer in methanol. Chem. Phys. Lett. 2003, 379, 437.

(36) Srividya, N.; Ramamurthy, P.; Ramakrishnan, V. T. Solvent effects on the absorption and fluorescence spectra of some acridinedione dyes: determination of ground and excited state dipole moments. Spectrochim. Acta, Part A 1997, 53, 249–257.

(37) Srividya, N.; Ramamurthy, P.; Ramakrishnan, V. T. Photo-physical studies of acridine (1,8) dine dyes: a new class of laser dyes. Spectrochim. Acta, Part A 1998, 54, 245.

(38) Srividya, N.; Ramamurthy, P.; Shanmugasundaram, P.; Ramakrishnan, V. T. Synthesis, characterization, and electrochemistry of some acridine1,8-dione dyes. J. Org. Chem. 1996, 61, 5083.

(39) Srividya, N.; Ramamurthy, P.; Ramakrishnan, V. T. Synthesis, characterization and electrochemistry of some acridine-1,8-dione dyes. Phys. Chem. Chem. Phys. 2000, 2, 5120.

(40) Ide, M.; Maeda, Y.; Kitano, H. Effect of hydrophobicity of amino acids on the structure of water. J. Phys. Chem. B 1997, 101, 7022–7026.

(41) Kameda, Y.; Ugawara, K.; Usuki, T.; Uemura, O. Hydration Structure of Alanine Molecule in Concentrated Aqueous Solutions. Bull. Chem. Soc. Jpn. 2003, 76, 935–943.

(42) Kameda, Y.; Ebata, H.; Usuki, T.; Uemura, O.; Misawa Bull, M. Hydration structure of glycine molecules in concentrated aqueous solutions. Bull. Chem. Soc. Jpn. 1994, 67, 3159–3164.

(43) Fischer, W. B.; Eysel, H. H. Raman and FTIR spectroscopic study on water structural changes in aqueous solutions of amino acids and related compounds. J. Mol. Struct. 1997, 393, 249–257.

(44) Suzuki, M.; Shigematsu, Y.; Fukunishi, Y.; Kodama, T. Hydrophobic hydration analysis on amino acid solutions by the microwave dielectric method. J. Phys. Chem. B 1997, 101, 3839–3845.

(45) Ellzy, M. W.; Jensen, J. O.; Hameka, H. F.; Kay, J. G. Correlation of structure and vibrational spectra of the zwitterion L-alanine in the presence of water: an experimental and density functional analysis. Spectrochim. Acta, Part A 2003, 59, 2619–2633.

(46) Park, S. W.; Ahn, D. S.; Lee, S. Dynamic paths between neutral alanine–water and zwitterionic alanine–water clusters: single, double and triple proton transfer. Chem. Phys. Lett. 2003, 371, 74–79.

(47) Selvarengan, P.; Kolandaivel, P. Potential energy surface study on glycine, alanine and their zwitterionic forms. J. Mol. Struct.: THEOCHEM 2004, 671, 77–86.

(48) Ahn, D. S.; Park, S. W.; Jeon, I. S.; Lee, M. K.; Kim, N. H.; Han, Y. H.; Lee, S. Effects of microsolvation on the structures and reactions of neutral and zwitterion alanine: computational study. J. Phys. Chem. B 2003, 107, 14109–14118.

(49) Jalkanen, K. J.; Nieminen, R. M.; Frimand, K.; Bohr, H.; Bohr, H.; Wade, R. C.; Tajkhorshid, E.; Suhai, S. A comparison of aqueous solvent models used in the calculation of the Raman and ROA spectra of L-alanine. Chem. Phys. 2001, 265, 125–151.