Novel Absorption and Fluorescence Characteristics of L-Lysine

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L-Lysine displays new absorption and fluorescence features at high concentrations (~ 0.5 M) in aqueous medium. A new absorption peak was detected around ~ 270 nm (ε = 0.34 M^{-1} cm^{-1}). Blue fluorescence (~ 435 nm) was visible on excitation at 355 nm. The above features which were concentration dependent are attributed to likely aggregates of L-lysine.

Several peptides and many proteins are known to form aggregates in the solution phase.[1] The presence of protein aggregates (amyloid) is known to be associated with diseases like Alzheimer's.[2] Consequently investigations of these complexes have acquired importance in the recent years. Among the amino acids, lysine has been identified as a key residue causing protein aggregation.[3] Several reports on crystalline aggregates involving lysine have also appeared.[4] However, to the best of our knowledge, there are no reports of amino acid lysine forming aggregates in the fluid phase.

In this work, we report our observations on L-lysine using absorption and steady state fluorescence techniques, which indicate that lysine is likely to be aggregated in the aqueous phase.

Figure 1 shows the absorption spectrum of L-lysine monohydrochloride (henceforth referred to as lysine) at pH 7.0[5] for different concentrations from 0.1 to 1.0 M. Starting from 0.1 M, we observe a gradual emergence of absorption with increasing concentration. A significant shoulder at ~ 270 nm is noticeable at 1.0 M. The figure also displays the absorption recorded for 0.5 M glycine under the same condition. Clearly lysine at 0.5 M has appreciably more (about five fold higher at 270 nm) absorbance than glycine. Thus the side chain of lysine has a definite role to play in the absorption highlighted above and importantly the α–carboxyl and α-amino groups of lysine are much less involved.

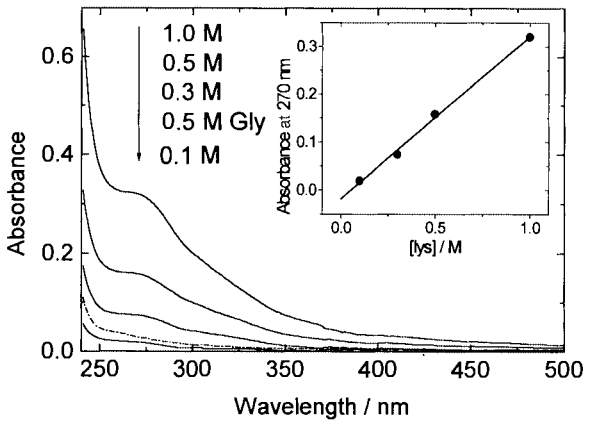

Figure 1. Absorption spectra of L-lysine monohydrochloride (——) from 0.1 to 1.0 M in 0.1 M NaH_2PO_4, pH 7.0 at room temperature. Same is also shown for 0.5 M glycine (–·–·–). INSET: The absorbance at 270 nm is plotted against lysine concentration.

The inset in Figure 1 shows the absorbance of lysine at 270 nm plotted against concentration. A linear dependence is observed. From the slope the molar extinction coefficient was calculated to be 0.34 M^{-1} cm^{-1} at 270 nm. Both the wavelength of absorption and the extinction coefficient are difficult to explain in terms of known electronic transitions expected from lysine side chain. An interaction between individual lysine molecules seems likely as origin for the observations above.

Figure 2 shows the fluorescence emission spectrum of lysine at different concentrations in aqueous medium at pH 7.0.[5] On excitation at 290 nm[6] with low concentrations (~ 0.01 M) of lysine, we observe tiny fluorescence peaks at ~ 321 nm and ~ 334 nm. Importantly, negligible emission, if any, is observed around ~ 420 nm in this case. With gradual increase in the concentration of lysine until 1 M, we notice a concomitant rise in fluorescence intensity around 420 nm. A broad spectrum devoid of any vibrational features is noticeable. This emission was significantly absent under identical conditions in glycine and a few other amino acids like L-arginine, L-serine, L-glutamate and L-isoleucine, which were randomly chosen (data not shown). Hence, it is apparent that the observed luminescence is unique to lysine alone. From the structure of lysine, it is difficult to account for the observed fluorescence since it lacks a conjugated system or an aromatic moiety.[7]

The inset A in Figure 2 depicts the plot showing the variation of the fluorescence intensity at 435 nm with lysine concen-

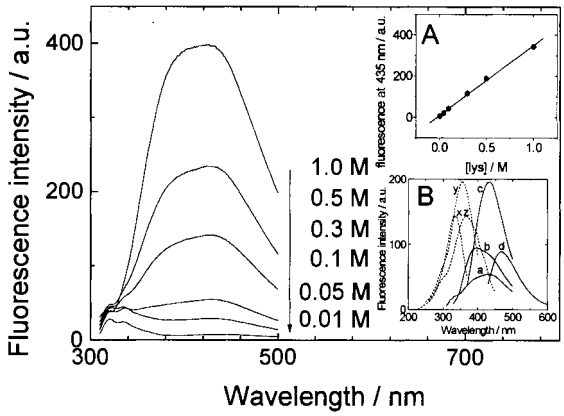

Figure 2. Emission spectra of L-lysine monohydrochloride from 0.01 to 1.0 M (λ_{exc} = 290 nm). Excitation slit width = 5 nm, emission slit width = 10 nm. Other conditions are similar to Figure 1. INSET A: Emission intensity at 435 nm is plotted against lysine concentration, here λ_{exc} = 355 nm. B: Emission spectra (——), the excitation wavelengths are: (a) = 290 nm, (b) = 310 nm, (c) = 355 nm and (d) = 410 nm. Excitation spectra (------), the emission wavelengths are: (x) = 393 nm, (y) = 435 nm and (z) = 471 nm. For all spectra above, excitation and emission slit widths = 5 nm and [lysine] = 0.5 M. Other conditions are similar to Figure 1.

tration (λ_{exc} = 355 nm). An almost linear dependence is seen for the fluorescence intensity with concentration. Based on the concentration dependence, a vibrationally featureless spectrum and arguments above, the origin for the observed fluorescence is likely to be molecular complexes or aggregates of lysine. The inset B in Figure 2 shows the emission spectra (solid curve) of lysine upon excitation at different wavelengths. The emission was only faintly visible with 290 nm excitation (curve a). However, an almost four fold increase in peak intensity was observed with respect to curve 'a' when exciting at 355 nm (curve c). The blue fluorescence (λ_{max} = 435 nm) was clearly visible to the naked eye under this condition. The excitation spectra (dashed curve) for curves b, c and d are shown in curves x, y and z, respectively. Evidently each emission spectrum shown has its characteristic excitation band. The presence of multiple excitation and emission bands indicates that multiple states and/or multiple species are likely to be involved. The latter would imply a heterogeneous population.

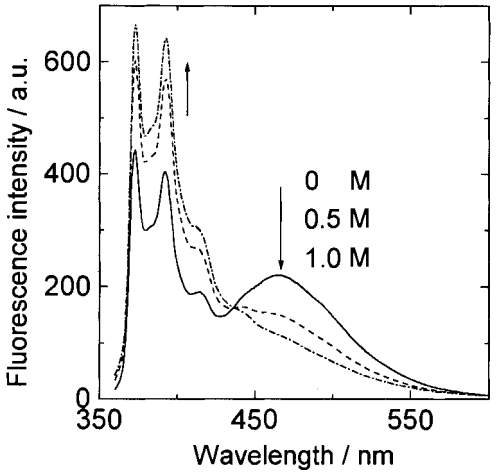

Figure 3. Fluorescence emission spectrum of pyrene in the presence of 0 (solid line), 0.5 (dashed line) and 1.0 M (dots and dashes) L-lysine monohydrochloride. λ_{exc} = 340 nm. [pyrene] = 2.5×10^{-5} M for all curves. Excitation slit width[8b] = 3 nm, emission slit width = 5 nm. Other conditions are similar to Figure 1.

If aggregates of lysine do exist in solution, they probably might have the methylene groups of the side chain forming an hydrophobic core. To check this possibility we added pyrene to lysine solutions. Figure 3 depicts the pyrene fluorescence in the presence of 0, 0.5 and 1.0 M lysine. A marked decrease in the pyrene excimer fluorescence (around ~ 467 nm) coupled with an increase in the monomer fluorescence (peaks at 373 and 392 nm) is observed in the presence of 0.5 M lysine. Further increase in the lysine to 1.0 M results in a similar trend. Pyrene, being a strongly hydrophobic probe with low solubility in water (2–3 μM)[8a], interacts more with lysine molecules or aggregates compared to pyrene monomers, when both lysine and pyrene are present. To ascertain the presence of aggregates, we compared the peak intensity ratio of vibronic bands (I/III) in pyrene emission (data not shown).[8a] The ratio dropped from 1.87[8c,8d] in aqueous buffer (pH 7) in the absence of lysine to 1.65 in the presence of 0.5 M lysine under similar conditions. A value of 1.65 indicates that pyrene is most likely bound to small clusters of lysine molecules in a mildly non-polar environment[8c,8d].

The possibility of water soluble impurities[7b] accounting for the spectral properties described here appears unlikely since a) the spectra are broad and structureless, similar to those observed in molecular complexes and b) the observations were consistent among samples procured from three different sources.

Taken together the studies above establish a likelihood for the presence of aggregates in concentrated solutions of lysine. These molecular complexes are likely to have a heterogeneous population based on the fluorescence data. Indeed, the spectroscopic methods described above offer a direct, yet simple and convenient tool to track these self-assembling aggregates which are normally difficult to detect. More evidences from light scattering, equilibrium ultracentrifugation and other techniques are required to conclusively prove the existence of these aggregates apart from determining their size(s). The nature of electronic transitions from aggregate-like species also needs to be thoroughly probed. These investigations are presently being carried out.

References and Notes

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5 Similar results were obtained in deionised water and 0.1 M acetate buffer, pH 4.6. Absorptions measurements were performed on a Hitachi spectrophotometer (Model U2001). Fluorescence data were acquired using a Shimadzu steady state fluorimeter (Model RF-5301PC).

6 The excitation at 290 nm was chosen to highlight the observation that emission peaks at 321 and 334 nm remain unaffected by concentration changes. There is a slight decrease in the intensity at 420 nm at concentrations higher than 0.3 M due to inner filter effect.

7 a) N. J. Turro, "Modern Molecular Photochemistry," University Science Books, Mill Valley, California (1991). b) We experimented with L-lysine obtained from multiple sources including the Sigma Aldrich Company to rule out impurities. c) Also extracts with ether from the L-lysine employed, failed to yield any extraneous component that could account for the observations.

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