Unusual aggregation properties of single amino acid L-Lysine hydrochloride

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Abstract: The self-assembly of single amino acids is very important topic of research since there are plethora of in-born errors of metabolisms (IEM's) which are caused by the accumulation or excess of amino acids. Hence, it is very pertinent to understand the fate of these excess amino acids in the body and their self-assembling behaviour at molecular level. In this manuscript we report for the very first time self-assembly of lysine hydrocholride to fiber like structures in deionized water. It could be observed that lysine assemble to globular structures in fresh condition and then gradually changes to fiber like morphologies by self-association over time after 24 hours. These fibers gradually change to tubular morphologies after 3 day followed by fractal irregular morphologies in 10 and 15 days respectively. Notably, lysine exists as positively charged amino acid at physiological pH and the amine groups in lysine remain protonated.

Hence, the self-assembling properties of lysine hydrochloride in deionized water is also pertinent and give insights into the fate of this amino acid in body in case it remains unmetabolized.

Introduction

The accumulation or excess of amino acids leads to various inborn errors of metabolisms like maple syrup urine disease,[1, 2] phenylketonuria,[3-5] cystinuria[6] to name a few. The pioneering research done by Gazit and coworkers, for the very first time illustrated formation of amyloid like toxic fibrils caused by the self-assembly of single amino acid phenylalanine.[3] The results observed in this report were very striking since phenylalanine fibrils were toxic to both neural and hepatic cell lines indicating the etiology of phenylketonuria which is associated with hepatic failure and neurodegeneration may be attributed to these toxic fibre formations. Following this pathbreaking research, the group further reported apoptosis inducing fibrils formed by the self-assembly of tryptophan[7] and also the formation of cytotoxic self-assemblies of tyrosine which also induce formation.[8] Following their results, Gazit research group proposed a generic amyloid hypothesis which suggest excess amino acid present in body assemble to amyloid like toxic structures which cause pathological symptoms in IEM's and a common etiology between amyloid associated diseases and single amino acid disorder can be traced.[9] Following this, several other research groups pursued research in single amino acid self-assembly to ascertain its implications in metabolite disorders.[9, 10]

Our group has been interested in studying the self-assembly of single amino acids,[11, 12] peptides,[13-15] oligonucleotides[13] and heterocyclic molecules.[16-24] Following the work of Gazit and our own research interests we have reported formation of amyloid like toxic fibers by the self-assembly of cysteine and methionine.[11] We have also recently reported the formation of toxic aggregates formed by the self-assembly of proline and hydroxyproline.[12]

In the present manuscript, we report for the very first time self-assembled aggregates formed by single amino acid Lysine (Lys) which is present as lysine hydrochloride salt (Lys.HCl) in neutral conditions. The aggregates appear globular and tends to appear to fibres gradually with time. The aggregate also shows morphological changes with time from globules to fibres to tubes and then irregular thick tapes. These aggregates also revealed amyloid nature as they bind with Congo Red (CR) and Thioflavin T (ThT) dye. The MTT assay of Lys assemblies on SH-SY5Y neural cell lines indicates decrease in cell viability which also indicate important implications of these aggregates in rare genetic in-born errors of metabolisms like Hyperlysinemia and also relate it to amyloid associated diseases.

Result and Discussion

The aggregation properties of Lys.HCl were studied in deionized water at various concentrations and time intervals. The morphologies of the aggregates were studied first by optical microscopy (ESI) and then extensively via atomic force microscopy (AFM). The morphologies were studied at 1 mM, 3 mM and 10 mM to assess the optimal concentration which was found to be 1 mM as suggested by AFM (Figure S1, S2). The AFM images of Lys.HCl at 1 mM reveal it first

assembles to globules which aggregate to give thin fibres gradually. (Figure 1). Similar observations were noted in case of optical microscopy as well as SEM (Figure S3, ESI). Notably, the SEM images of Lys could only be recorded without gold coating and at lower magnifications since the assemblies were extremely soft in nature and starts melting with higher energy electron beam (Figure S3, ESI). Phase contrast microscopy also supported this observation amorphous nature of assemblies confirmed amorphous non crystalline nature of aggregates confirming they are formed by the process of self-assembly and not crystallization (ESI).

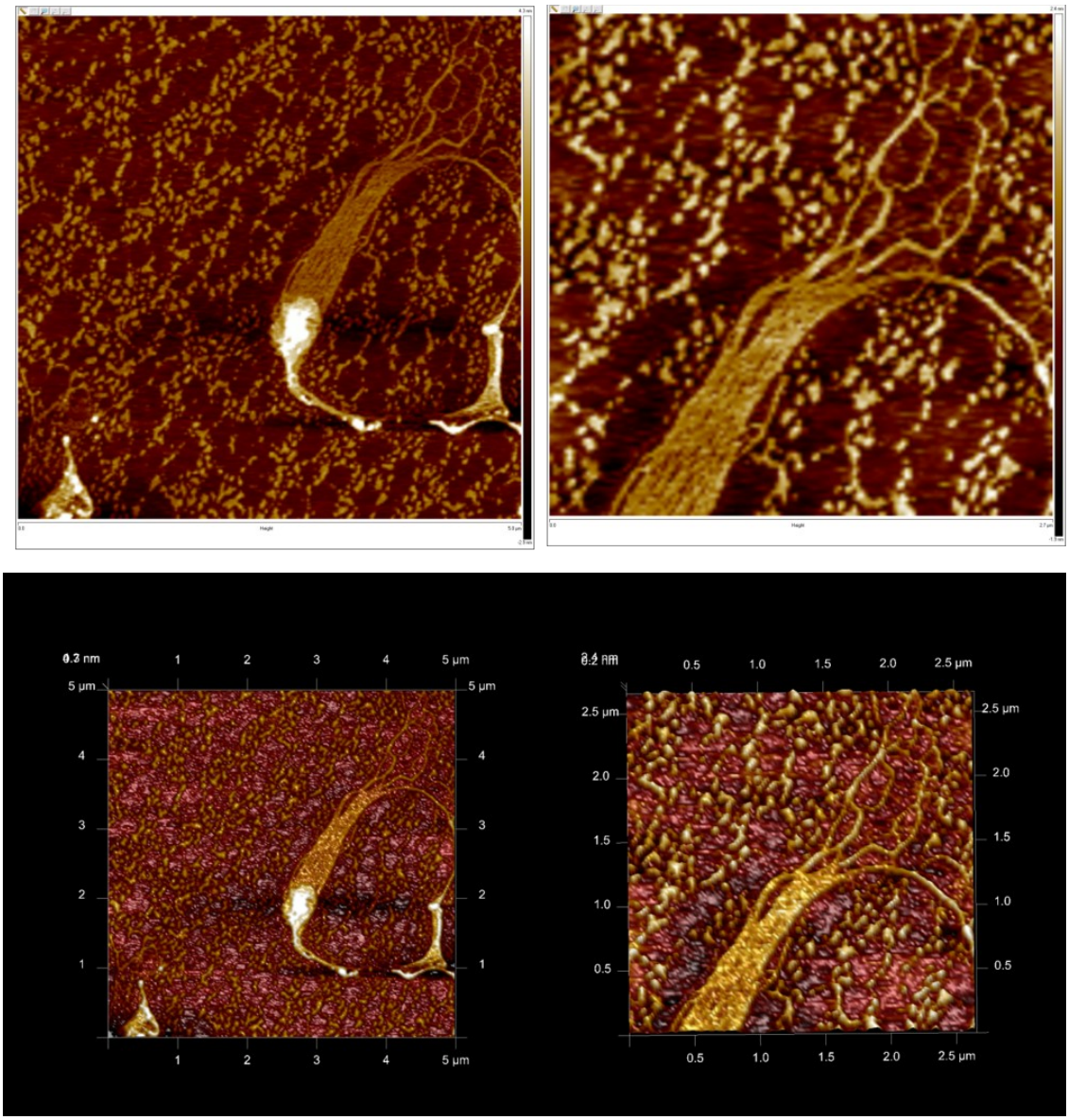

Figure 1: Self-assembly of Lys. HCl at 1 mM concentration reveal formation of globular structures which have tendency to aggregate and form fibrils.

Since the globular aggregates tend to assemble to fibril shape gradually, we also performed a time dependent AFM analysis to ascertain the morphological changes which might happen as amino acid is incubated for longer time in deionized water. Hence, the AFM microscopic images

were recorded after incubating Lys. HCl in deionized for 1 day (24 hours), 3 days and 10 days (Figure 2). It can be deciphered from the AFM image that Lys. HCl aggregates to fibrils during the course of 24 hours (1day) which gradually changes to tube like structures in 3 days. As the time of incubation is increased to 10 days the morphologies reveal thick irregular bundle like structures (Figure 2 g-i).

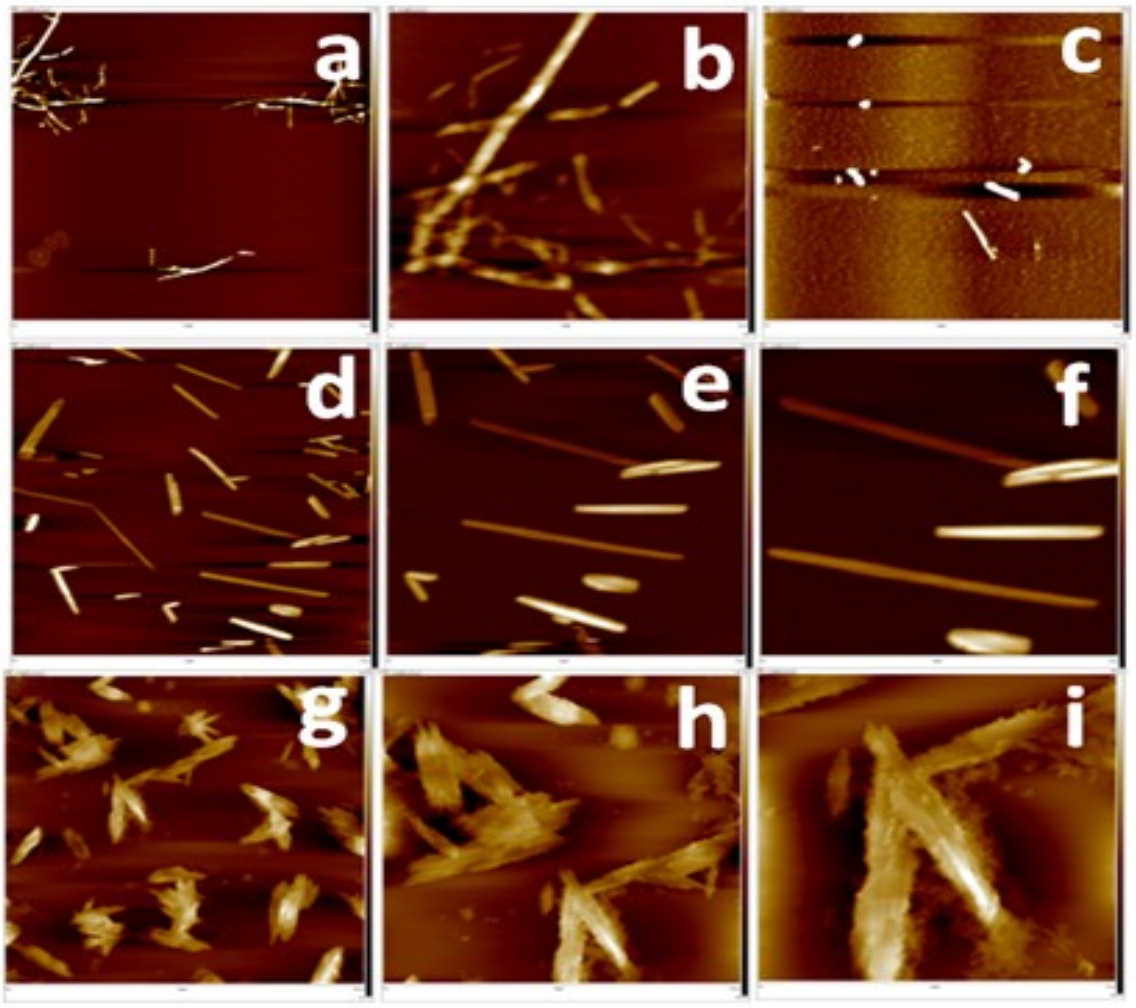

Figure 2: AFM images of self-assembly of Lys.HCl in deionized water after different time intervals of incubation and under different magnifications. (a-c) AFM image of Lys.HCl under different magnification after 24 hours (1 day) of incubation; (d-f) AFM image of Lys.HCl under different magnification after 3 days of incubation; (g-i) AFM image of Lys.HCl under different magnification after 10 days of incubation.

Further, to decipher the amyloid nature of these aggregates Congo red and Thioflavin T assays (ThT) were done in solution as well as ThT stained structures were observed under fluorescent microscope (Figure S3, ESI). It is reported that ThT reveal enhanced fluorescence on binding with amyloidogenic fibres while congo red binding with amyloid cause hyperchromicity through UV.

Hence, Congo red (CR) binding assays were performed with the aggregates formed by Lys.HCl. To get a comparative analysis of the results CR was also co-incubated with other single amino acids like Phenylalanine (Phe),[3] Tyrosine (Tyr),[8] Cysteine (Cys),[11] Methionine (Met)[11] and Tryptophane (Trp)[25] since their self-assembly to amyloid like structures is already reported. The CR binding assay revealed the increase in absorbance and similar red shift with Lys.HCl aggregates as other single amino acid assemblies confirming its amyloid like characteristics. (Figure 3a). The fluorescence spectra of ThT with Lys.HCl aggregates too revealed enhancement in fluorescence indicating its amyloid nature (Figure 3b).

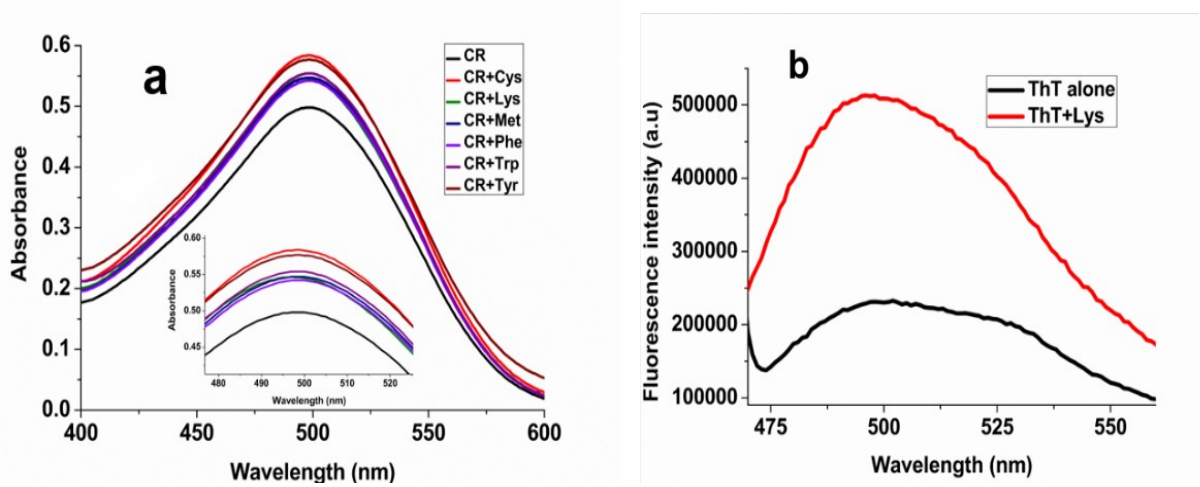

Figure 3: Congo red and ThT binding assay with Lys aggregates.

The toxic nature of amyloid assemblies is very well reported in literature, and the cell vaibility assay of neural cell lines with amyloidogenic sequences causes decrease in the cell viablity. The tetrazolium dye assay MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is a reliable in vitro assay which is routinely used for the quantitative measurement of Aβ toxicity in cultures of different neuroblastoma cells.[26] The MTT assay analysis reveals that structures formed by Lys were cytotoxic in a dose dependent manner (Figure 5). The cytotoxicity anaysis was done at four different concentrations of amino acids: 1 mM, 3 mM, 6 mM and 10 mM to assess the dose dependent cytotoxicity. To assure formation of fibrils amino acids were heated with cell medium (excluding FBS) at 90 ^{o}C for 30 minutes and gradualy cooled and incubated overnight

before treating it with SH-SY5Y cell lines. Alanine was taken as negative control since it does not aggregate and already reported for no cytotoxic effect even at very high concentrations (4 mg/mL, 20 mM)[3] while phenylalanine was taken as positive control since its toxicity is well reported.[4]

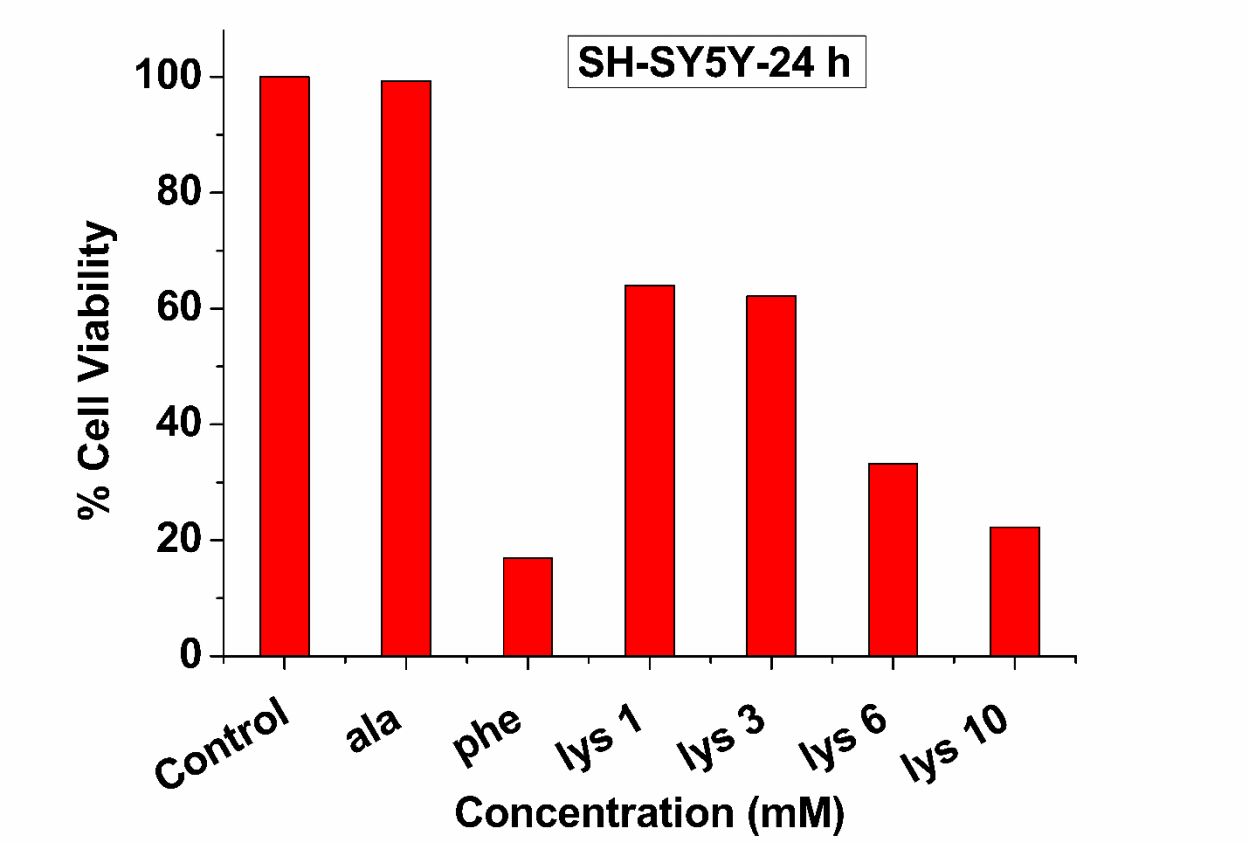

Figure 3: Cell viability assays of L-Lysine.HCl on SH-SY5Y after 24 hours.

The cytotoxicity analysis by MTT assay suggest Lys aggregates decreased cell viability. The cell viability was reduced to 60% at 1 mM and further reduced to nearly 25% at 10 mM concentration of L-Lys.HCl. The results observed in MTT assay may have important implications for associating the cytotoxicity of Lys aggregated to rare genetic in-born errors of

metabolisms Hyperlysinemia. Notably the symptoms associated with Hyperlysinemia are severe neurological problems like seizures, intellectual disability, memory loss and neuronal dystrophy. Hence the neurotoxicity caused by Lys aggregates observed in MTT assay may be the cause of these neuro-pathological problems.

Conclusions

In conclusion, we have reported self-assembled structures formed by Lys.HCl and its amyloid like aggregation. From the AFM studies it can be surmised that the Lys.HCl first assembles to globular oligomers which gradually changes to fibers. The morpholgies of the aggregated formed were studied under varying concentration and the time periods by AFM. The aggregates could also bind amyloid dyes like ThT and Congo red in solution confirming their amyloid characteristics. Further, the MTT assay sugget that the Lys.HCl aggregates were cytotoxic and reduced the cell viablity of SH-SY5Y neural cell lines. Hence, the association of these aggregates to hyperlysinemis a disease caused by genetic inborn error of metabolism which causes accumulation of lysine may be a possiblity and needs to be studied in greater detail in future.

Associated Content

The supporting information of this manuscript is available and contains additional figures and methods.

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Author Contributions

NG proposed the concept and drafted article. NG, BK and VSK worked on data characterization and analysis by microscopy and biophysical assay, SW and DB performed, interpreted and drafted the cytotoxicity data. RS and KBJ worked on data characterization and analysis by microscopy. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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