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Probing inclusion complexes of cyclodextrins with amino acids by physicochemical approach

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L-Leucine (PubChem CID: 6106)
L-Isoleucine (PubChem CID: 6306)

ABSTRACT

Formations of host-guest inclusion complexes of two natural amino acids, *viz.*, L-Leucine and L-Isoleucine as guests with α and β-cyclodextrins have been investigated which include diverse applications in modern science such as controlled delivery in the field of pharmaceuticals, food processing etc. Surface tension and conductivity studies establish the formation of inclusion complexes with 1:1 stoichiometry. The interactions of cyclodextrins with amino acids have been supported by density, viscosity, refractive index, hydration and solvation number measurements indicating higher degree of inclusion in case of α-cyclodextrin. L-Leucine interacts more with the hydrophobic cavity of cyclodextrin than its isomer. With the help of stability constant by NMR titration, hydrophobic effect, H-bonds and structural effects the formations of inclusion complexes have been explained.

1. Introduction

Cyclodextrins (CD) are the members of cyclic oligosaccharide family containing six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units, which are bound by α-(1–4) linkages (Dinar, Sahra, Seridi, & Kadri, 2014; Szejtli, 1998). CDs have a torus-shaped ring structure with polar hydrophilic rims and relatively hydrophobic inner cavity (Fig. 1) (Yang et al., 2013). Due to this type of unique structure they can build up host–guest inclusion complexes (ICs) with various small molecules having hydrophobic moiety, e.g., vitamins, amino acids, ionic liquids etc (Mathapa & Paunov, 2013; Valle, 2004). The hydrophobic part of the guest molecule is accommodated into the hydrophobic cavity of CD whereas the polar part of the guest (if present) makes association with the polar rims resulting stabilisation of the IC (Szejtli, 1996). For this reasons CDs have vast applications in the field of pharmaceuticals, pesticides, foodstuffs, toilet articles, textile processing industry, supramolecular host-guest chemistry, molecular encapsulation etc (Connors, 1997; Gao et al., 2006). CDs form stable host-guest ICs with essential amino acids e.g., arginine, histidine, lysine, phenyl alanine, glutamic acid (Roy, Ekka, Saha, & Roy, 2014; Saha, Ray, Basak, & Roy, 2016), ionic liquids e.g., 1-butyl-4-methylpyridinium iodide (Datta, Barman, & Roy, 2015; Roy, Roy, Das, & Barman, 2016; Roy, Saha, Barman, & Ekka, 2016), RNA nucleosides (Roy, Roy et al., 2016; Roy, Saha et al., 2016) etc. as guest molecules.

In the present study we have attempt to ascertain the nature of formation of ICs of α and β-CD with two natural α-amino acids, *i.e.*, L-Leucine (L-Leu) and L-Isoleucine (L-Ile) in 0.001, 0.003, 0.005 mass fractions of α and β-cyclodextrins in aqueous media. Aim of this work is to explore the formation, carrying and controlled release of the two essential amino acids by forming IC with host CDs without chemical & biological modification of the guests.

L-Leu is used in the biosynthesis of proteins and is essential in humans, *i.e.*, our body cannot synthesize it and thus it must be incorporated from outside, which may be done using α and β-CD as carriers (Etzel, 2004; Rosenthal, Angel, & Farkas, 1974). It is a major component of the subunits in ferritin, astacin etc. proteins and L-Leu is an activator of mTOR; it is the only dietary amino acid that has the capacity to directly stimulate muscle protein synthesis (Cota et al., 2006; Nelson & Cox, 2000). On the other hand L-Ile is used

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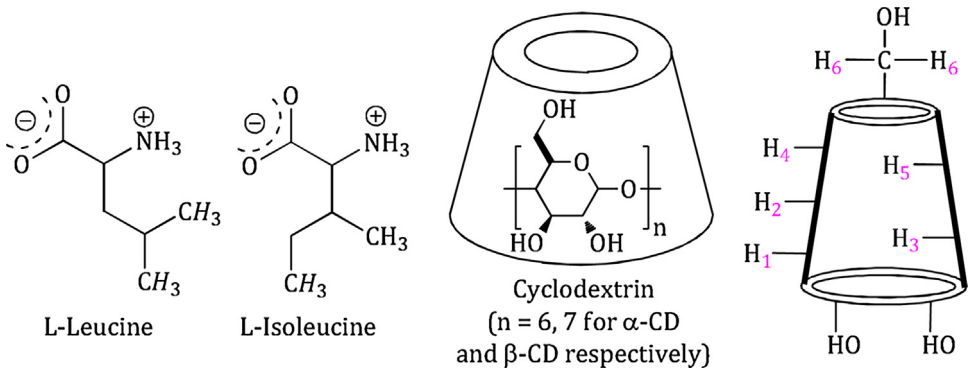

Fig 1. Molecular structure of L-Leu, L-Ile in aqueous solution and cyclodextrin molecule with interior and exterior protons.

in the biosynthesis of proteins and it is also essential in humans. L-Ile is synthesized from pyruvate employing leucine biosynthesis enzymes in other organisms such as bacteria (Kisumi, Komatsubara, & Chibata, 1977; Nelson & Cox, 2000).

Here, the nature of the ICs and their interactions have been studied by surface tension, conductivity, density, viscosity and refractive index measurements by calculating the contributions towards the limiting apparent molar volume and viscosity-B coefficient of different groups of the two amino acids. NMR titrations have also been done by ^{1}H NMR spectroscopy to confirm the inclusion phenomenon and the binding constants have been calculated from the titration by using Benesi–Hildebrand method (Caso et al., 2015).

2. Experimental

2.1. Source and purity of samples

The above mentioned two amino acids and CDs of puriss grade were purchased from Sigma-Aldrich, Germany and used as it was. The mass fraction purity of L-Leu, L-Ile, α-CD and β-CD were ≥0.98, 0.98, 0.98 and 0.98 respectively.

2.2. Apparatus and procedure

Solubilities of the two CDs and that of the above two α-amino acids in aqueous CDs have been verified in triply distilled, deionized and degassed water. It was detected that these were quite soluble in aqueous CDs. All the stock solutions of L-Leu and L-Ile were prepared by mass (Mettler Toledo AG-285 with uncertainty 0.0001 g) and the working solutions were got by mass dilution at 298.15 K. Changes of molarity to molality were done using the densities of the solutions (Shoemaker & Garland, 1967). Sufficient precautions were made to decrease the evaporation during mixing.

pH values were measured by Mettler Toledo Seven Multi pH meter having uncertainty ±0.001. It was studied in a water bath with thermostat maintaining the temperature at 298.15 K, having uncertainty in temperature ±0.01 K.

Surface tensions of the solutions were determined by platinum ring detachment technique using a Tensiometer (K9, KRŰSS; Germany) at 298.15 K. Accuracy of the study was ±0.1 mN m^{-1}. Temperature of the system was maintained by circulating thermostated water through a double-wall glass vessel holding the solution.

Conductivities of the solutions were studied by Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μSm^{-1}. The study was carried out in a thermostated water bath at 298.15 K with uncertainty ±0.01 K. HPLC grade water was used with specific conductance 6.0 μS m^{-1}. The conductivity cell was calibrated using 0.01 M aqueous KCl solution.

The densities (ρ) of the solutions were studied by vibrating *U*-tube Anton Paar digital density meter (DMA 4500 M) having precision ±0.00005 g cm^{-3} and uncertainty in temperature was ±0.01 K. The density meter was calibrated by standard method (Shoemaker & Garland, 1967).

Viscosities (η) were determined by Brookfield DV-III Ultra Programmable Rheometer with spindle size 42. The detail has already been depicted before (Shoemaker & Garland, 1967).

Refractive indexes of the solutions were studied with a Digital Refractometer from Mettler Toledo having uncertainty ±0.0002 units. The detail has already been described before (Shoemaker & Garland, 1967).

^{1}H NMR spectra were recorded in D_2O at 300 MHz using Bruker ADVANCE 300 MHz instrument at 298 K. Signals are quoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O: δ 4.79 ppm). Data are reported as chemical shift. In each titration initially 0.5 mL 1.0 mM amino acid solution was taken and then 10 μL 10 mM CD solution was added into it at five several times.

3. Result and discussion

3.1. pH measurement proves the ionic states of the amino acids

Existence of zwitterionic forms of amino acids in aqueous solution can be understood with the help of pH measurement (Saha et al., 2016). The values of pH for L-Leu in aqueous α and β-CD ranges from 5.89 to 5.14 and 5.75–5.13 respectively at 298.15 K, while for L-Ile it ranges from 6.14 to 5.39 and 5.85–5.38 for α and β-CD respectively at the same temperature (Tables S1, S2). The pH value decreases with the increasing concentration of the respective amino acids and also with the increase of concentration of α and β-CD for both the two amino acids. These values clearly show the variation in their zwitterionic forms, *i.e.*, the amine and carboxylic acid groups exist in ionic forms —NH_3^+ and —COO^- respectively.

3.2. Surface tension study illustrates the inclusion and the stoichiometric ratio of the inclusion complexes

Surface tension (γ) measurement can be used to obtain valuable information about the formation of inclusion complex in CDs (Roy et al., 2014; Roy, Roy, & Roy, 2015). It is known that γ for aqueous solutions of pure α and β–CD do not show any remarkable change with increasing concentrations (Roy, Roy et al., 2016; Roy, Saha et al., 2016). The pH data of both these amino acids show the existence of NH_3^+ and COO^- in their zwitterionic forms. Thus the side groups being nonpolar, both the amino acids show surfactant