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Undesirable impact on structure and stability of insulin on addition of (+)-catechin hydrate with sugar

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ABSTRACT

Insulin (*In*) based formulation has been used over decades for the cure of *In*-dependent diabetic patients, however, more attempts are still required to improve the remedial use of *In*. In this regard, the use of green tea has become widespread nowadays. However, it is unknown that (+)-catechin hydrate (CAT), a major component of green tea which enhances anti-diabetic activity of *In*, will or will not enhance the structure and stability of *In* if ingested with sugars. Interestingly, by using biophysical techniques, present study reveals the fact that the use of sugar during the intake of green tea extract may produce unwanted effects on *In* which may further lead to some disorders associated with *In* stability and also create obstacle in successful implications of *In* formulations.

1. Introduction

Insulin (*In*), one of the most important mammalian hormones, regulates blood glucose levels, cell growth and fat metabolism etc. [1–3] Human *In* self associates to form a hexamer in presence of zinc ions under physiological conditions which is the most stable form of *In* [3–5]. Undoubtedly, hexamer reversibly changes into monomer which interacts with *In* receptor [3,4]. However, monomer is highly prone to fibril formation that may lead to pathogenesis of misfolding-based diseases such as type 2 diabetes mellitus, Parkinson's disease and Alzheimer's disease [6,7].

According to Wild et al. [8], the number of people suffering from *In* dependent diabetes worldwide is rising at a frightening rate with a proposed 366 million people likely to be diabetic by the year 2030, whereas 191 million were estimated in 2000. Undeniably, *In*-based formulation has been used for more than 75 years [9], however, much endeavour is still required for stability of hexamer unit of *In*, which is among the most demanding tasks in development of protein formulations and has been the subject of extensive clinical, biochemical and biophysical studies.

Nowadays, green tea is becoming most commonly used beverage worldwide. The natural polyphenol such as catechin, a component of green tea is found to interact with various proteins, especially *In*, in order to decrease their aggregation [10–12]. Nonetheless, epigallocatechin gallate component of green tea also enhances *In* activity attributed to *In* hexamer stability responsible for decreased aggregation [13].

For past years, there is vast debate on the use of green tea with sugars. Green tea consumption has always been at the centre of discussions about human health, disease and longevity. Still, it is not apparent that catechin will enhance the structure and stability of *In* if ingested with sugars. Therefore, it is necessity of time to explore the stability of *In* in presence of catechin and sugars in order to make a successful path from administration site to target site and also to evade undesirable outcome in biopharmaceutical formulations as well as in human body.

In present study, we have depicted the influence of (+)-catechin hydrate (CAT) on structure and stability of *In* in presence of sugars at physiological pH by using various biophysical techniques. Besides, the diverse range of sugars is well known for maintaining various protein stability and activity by their efficacy against peptide or protein aggregation associated with neurodegenerative diseases, the structure and stability of insulin in the presence of trehalose and sucrose, which have received special interest in biochemical adaptation, rarely studied so far [14–19]. Here, we have used two different sugars (trehalose and sucrose) in order to check whether trehalose being an exceptional stabilizer [14–19], may lead to some different effects. To the best of our knowledge, there is not a single report on impact of green tea component along with sugar on the structure and stability of *In*. Therefore, implementation of this study may help to resolve these unexposed aspects in literature which cannot be averted from the necessity of today's research.

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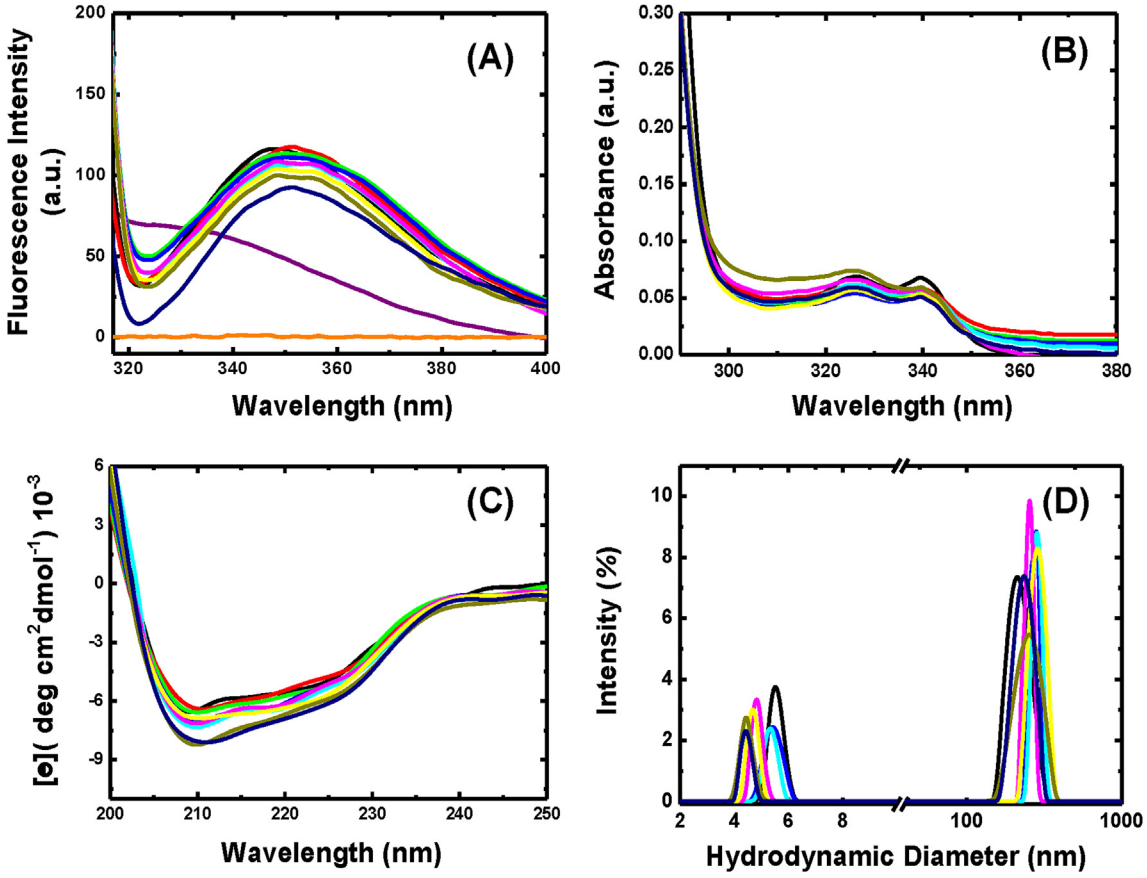

Fig. 1. Analysis of interaction of *In* and CAT by using Tpy-$CuCl_2$: (A) fluorescence spectra, (B) UV-vis absorbance spectra, (C) far-UV CD spectra and (D) DLS of *In* in buffer (black) and presence of varying concentration of CAT such as 3.4 μM (red), 6.8 μM (green), 17.2 μM (blue), 34.4 μM (cyan), 68.8 μM (pink), 103 μM (yellow), 138 μM (dark yellow) and 172 μM (navy). CAT in buffer and Tpy-$CuCl_2$ in buffer are shown as purple and orange spectra in Fig. 1A, respectively. For DLS study, some of the lower concentrations of CAT are not shown due to their insignificant effects on the d_H of *In* and also for more clarity. CAT stock solution is 0.5mg/5 mL. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2. Materials and methods

2.1. Materials

Insulin (11061-68-0) lot No. 91077C—lot no. 12C470-G human recombinant, (+)-catechin hydrate; CAT (≥98%), sucrose, trehalose, terpyridine (Tpy), deuterium oxide (D_2O) and 1-Anilinonaphthalene-8-sulfonate (ANS) were purchased from Sigma Aldrich, USA. Copper(II) chloride dihydrate ($CuCl_2{\cdot}2H_2O$), anhydrous sodium phosphate monobasic and sodium phosphate dibasic dihydrate were purchased from Sisco Research Lab (SRL), India. All chemicals were of high purity and analytical grade.

2.2. Sample preparation

The protein samples were prepared in 10 mM sodium phosphate buffer at ~ pH 7.3 with 0.2 mg/mL insulin concentration for all measurements. 5 μL of ANS solution (1mg/1 mL) was used for each sample during ANS fluorescence study. For gravimetric measurements of all samples, AND (Japan) balance having a precision of ± 0.00001 g was used. The buffer used for all samples in the present study was prepared in distilled deionized water with resistivity of 18.3 Ωcm. All mixtures were filtered with 0.22 μm disposal filter (Millipore, Millex-GS) through syringe before using for the experiments and were incubated for 1 h at 25 °C in order to obtain complete equilibrium before performing experiments. The stability of insulin was studied in the absence and presence of 0.01, 0.025, 0.05, 0.1, 0.5, 1.0, 1.5 and 2.0 M of trehalose and sucrose for all experiments. The stock solution of CAT was prepared in buffer at 0.5 mg/5 mL (344 μM). The CAT concentrations used in the range from 3.4 to 172 μM which were chosen by considering the bioavailability of the CAT in human body [20]. The external probe, $TpyCuCl_2$ used for the fluorescence and UV-vis study was prepared by the following the method mentioned elsewhere [21] and 5 μL of 50 μM solution of this probe in buffer was used for each sample.

2.3. Steady state fluorescence measurements of insulin

Steady state fluorescence emission spectra measurements were performed at 25 °C in Cary Eclipse fluorescence spectrofluorimeter (Varian optical spectroscopy instruments, Mulgrave, Victoria, Australia). The instrument was equipped with an intense xenon flash lamp and a Peltier-type temperature controller with a precision of ± 0.05 °C. The excitation wavelength at 280 nm was used for intrinsic fluorescence. All spectra were recorded at a concentration 0.2 mg/mL of insulin in the various concentrations of CAT, sugars and also in the mixtures of CAT and sugar, using a slit width of the excitation and emission at 5 nm and 10 nm, respectively, between 285 and 400 nm. For synthesized external probe, excitation wavelength of 298 nm was used and emission was observed from 300 to 400 nm for all samples consisting of CAT as well as insulin. All spectra were averaged of three scans. For ANS binding fluorescence study, excitation wavelength at 380 nm was used for all samples and emission spectra from 400 to 650 nm were observed. Other parameters were kept the same. The fluorescence spectra of CAT were subtracted for each sample consisting of both CAT and insulin. The thermal fluorescence study was not performed in the present study because of the decreasing stability of CAT as a function of temperature.

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