SHORT COMMUNICATION

Enhanced antibacterial, antioxidant and anticancer activity of caffeic acid by simple acid-base complexation with spermine/spermidine

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\textbf{ABSTRACT}

Caffeic acid (CA) is a naturally occurring plant-derived polyphenol possessing diverse biological properties. However, the poor water-solubility of CA restricts its widespread applications. On the other hand, biogenic amines such as spermine and spermidine are natural constituents in eukaryotes. In this work, we present water-soluble complexes of CA with spermine and spermidine by exploiting the acid-base interaction. Four different compositions have been prepared by varying the CA to amine ratios, whose chemical structures have been probed in detail using Fourier-transform infrared spectroscopy (FT-IR) and nuclear magnetic resonance (NMR) studies that have revealed the acid-base interaction between the constituent precursors. The obtained acid-base complexes at their native pH values have shown enhanced antibacterial and antioxidant activities than pristine CA. Further, the CA-polyamine complexes have shown high anticancer performances in the concentration range that is compatible with the normal cell lines.

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1. Introduction

Caffeic acid (CA) is a natural product found in several fruits and plant sources (Khan et al. 2021). This phytochemical has gained significant attention in recent years due to its diverse applications like antimicrobial, anti-glycemic, antioxidant, anti-inflammatory and anticancer (Li et al. 2019; Fidelis et al. 2020; Aćimović et al. 2021). It is also regarded as an effective adjuvant with several antibiotics and vaccines (El-Missiry et al. 2021; Pawar et al. 2021; Solairaja et al. 2021). A recent review article summarises the chemical and pharmacological aspects of CA in hepatocarcinoma (Espíndola et al. 2019). Despite the vast beneficial characteristics, the poor water-solubility of CA is a constrain for several practical applications, due to which a variety of synthetic derivatives like esters, glycosides etc., have been developed (Collins et al. 2019; Li et al. 2019). Among these, the saccharide-modified derivatives have shown promise in enhancing the water solubility of CA; nevertheless, such derivatives signify its covalently modified version (Mbagwu et al. 2020).

On a different note, biogenic amines such as spermine and spermidine are natural constituents in eukaryotes. These biogenic amines are known for their high antioxidant activity, which has an essential in vivo function of quenching singlet molecular oxygen to protect the replicating DNA from oxidative damage (Khan et al. 1992). While spermine is known for its neuroprotective action against anoxia at millimolar level concentration, spermidine is known for its role in inducing autophagy to maintain neuronal and cellular homeostasis (Ferchmin et al. 2000; Ghosh et al. 2020). These endogenous polyamines also play an important role in proper neuronal development and brain function (Xu et al. 2020). Moreover, several amines and polycationic species are reported to possess enhanced self-promoted uptake by the microbes to provide antimicrobial characteristics (Myers and Clark 2021).

Recently, our group reported a new strategy of facile acid-base complexation of CA with dopamine to enhance the water-solubility to the tune of ~35 times (Mude et al. 2022). In the current study, we report new acid-base complexes of CA with spermine/spermidine to further enhance its water solubility. Due to the higher number of amine groups per molecular in spermine/spermidine as compared to dopamine, we aimed to load more CA per molecule and to study the effect on the subsequent biological characteristics. The obtained complexes have been characterised using Fourier-Transform Infrared (FT-IR), $^1$H, $^{13}$C, and two-dimensional $^1$H-$^1$H nuclear Overhauser effect spectroscopy (2D $^1$H-$^1$H NOESY) nuclear magnetic resonance (NMR) studies to unearth the acid-base interaction between the constituent precursors. The products have been studied for their antibacterial, antioxidant, cytotoxicity and anticancer activities.

2. Results and discussion

The plausible chemical structures of the complexes of CA with spermine and spermidine are presented in Figure S1. A total of four different compositions, namely, 1:1 CA:Spermine, 1:2 CA:Spermine, 1:1 CA:Spermidine and 1:2 CA:Spermidine, were prepared. Figure 2 presents the FT-IR spectroscopic results of the CA:polyamine complexes. Pristine CA exhibited the characteristic carbonyl stretching vibrations at 1646 cm$^{-1}$ and aromatic vibrations at 1615 cm$^{-1}$, 1527 cm$^{-1}$, and 1450 cm$^{-1}$. On the
other hand, the polyamines exhibited –NH₂ twisting and wagging vibrations at 1318 cm⁻¹ and 819 cm⁻¹, respectively (Bertoluzza et al. 1983). After complexation between CA and the polyamines, these twisting and wagging vibrations of amine groups were disappeared and new peaks at 1389 cm⁻¹ and 1268 cm⁻¹. Furthermore, a new broad peak at 1036 cm⁻¹ was appeared that can be attributed to the C-O stretching of the ammonium ester (Trivedi et al. 2015). These observations indicated the effective complexation between CA and the polyamines.

Further, Figures 3 and S4 show the ¹H NMR spectra of all four compounds—in deuterated dimethylsulfoxide (DMSO) as the solvent—in comparison to the parent precursors. Tables S1 and S2 summarise the chemical shift values corresponding to the ¹H NMR spectra of the complexes. In addition to confirming the characteristic signals of the constituent precursors, the ¹H NMR spectra also confirmed their integral values being in line with the feed stoichiometry. The ¹H NMR signals of the polyamines were more resolved after complexation with CA. Besides, the acidic protons of CA and the protons in the amine moieties got merged and appeared as a broad signal, which additionally confirmed the complexation between the precursors (Mude et al. 2022). Now, the question arises that which of the amine moieties—terminal or the middle ones—take part in the reaction. The ¹H NMR spectra reveals that the protons present in the carbons adjacent to the terminal amines experience a greater change in the chemical shift upon complexation than those present in the carbons next to the middle amines. This observation has indicated the participation of the terminal amines in the acid-base reaction.

The ¹³C NMR spectra of the obtained compounds are presented in Figures 5 and S6, and the corresponding chemical shift values in Tables S3 and S4, respectively. In this case too, the characteristic carbon signals of both the precursors were clearly observed. In all the complexes, the greater chemical shift of the carbonyl carbon of CA and the carbons nearer to the terminal amine moieties corroborated the observation from the ¹H NMR spectra on the participation of the terminal amines in the acid-base complexation reaction.

To derive additional insights on the spatial interactions between the protons in the 1:1 and 2:1 complexes of CA:Spermine and CA:Spermidine, 2D ¹H-¹H NOESY NMR spectra were recorded and the results are shown in Figure 7. While the off-diagonal cross-peaks shown in red colour represent the correlation spectroscopy (COSY) interactions, those shown in blue colour represent the nuclear Overhauser effect (NOE) interactions. In all the spectra, the long-range interaction between the acidic protons and amine protons giving rise to the broad cross peak was clearly observed. The NOE interactions between the protons present in the carbon adjacent to the terminal amine moieties and the broad peak (corresponding to the merged signal of acid and amine protons) were also observed (marked in the spectra). These prominent interactions additionally confirmed the closer proximity of CA to the terminal amine moieties and thus corroborated the observations from the ¹H and ¹³C NMR spectra.

The antimicrobial activity of the complexes was studied against E. coli and S. aureus using the Alamar blue assay (Figure 8). Since the as-prepared complexes were alkaline (pH ~8.2 to 9.4), apart from the native pH, we also studied the biological properties of the same at pH 7.5 so as to assess the stability and activity of the same at this
physiologically relevant pH that is employed in many applications. It is noteworthy that spermine displayed solubility issues at pH 7.5 and was hence excluded from the studies. It was observed that the CA:Spermine complexes exhibited higher antibacterial activity than the CA:Spermidine complexes. At the native pH values, the 1:1 and 2:1 CA:Spermine complexes exhibited minimum inhibitory concentration (MIC) values in the range of ~7.5–10 mM, whereas those of CA:Spermidine complexes were in the range of 10–15 mM. In all the cases, the antibacterial activity of the complexes decreased at pH 7.5, which indicated the necessity of maintaining the native pH to derive the maximum activity.

The antioxidant characteristics of the complexes have been studied following the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay. At the native pH values, the pristine spermine and spermidine exhibited >90% activity from sub-mM concentration of 0.625 mM, whereas pristine spermidine at pH 7.5 exhibited <10% activity even at 50 mM concentration (Figure S9(a, b)). The CA-spermidine complexes, in general, exhibited higher antioxidant activity than CA-spermine complexes. The 1:1 and 2:1 CA-spermidine complexes, at their native pH, were found to exhibit similar activity to pristine spermidine.

The biocompatibility of the CA:polyamine complexes has been ascertained using cytotoxicity assay against SNL 76/7 mouse fibroblast and HEK-293 human embryonic kidney cells (Figure S9(c–f)). The results, in general, revealed high biocompatibility of the complexes in their native pH till 12.5 mM concentrations. Many compositions exhibited much higher cell growth than the control samples up to a concentration of 5 mM, which could be due to the high neuroprotective effect of the CA:polyamine complexes at this concentration range.

The complexes have been further explored for their anticancer property against HeLa cells. The pristine spermine and spermidine exhibited significant anticancer activities to the tune of 70–80% in the concentration range of 0.5–12.5 mM (Figure S9(g, h)). On the other hand, the CA-polyamine complexes exhibited >95% anticancer activity in the concentration range of 0.5–2.5 mM both at native and 7.5 pH. These results showed the high anticancer properties of the complexes even at sub-mM concentrations.

4. Conclusions

Acid-base complexation of CA with spermine and spermidine resulting in water-soluble derivatives of CA was reported in this study, which revealed an enhancement in water solubility to the tune of >40–45 times. The detailed FT-IR and NMR studies revealed the binding of CA with the terminal amine moieties of the polyamines. The complexes exhibited enhanced antioxidant activity than the pristine CA. Furthermore, at their native pH values, the complexes exhibited substantial antibacterial activity against E. coli and S. aureus. The CA:polyamine complexes exhibited significant anticancer property against HeLa cells even at sub-mM concentrations both at native and 7.5 pH. Also, the complexes exhibited high biocompatibility against SNL 76/7 and HEK-293 cells up to ~10 mM concentration.
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Disclosure statement

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