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Conversion of 2′-substituted chalcones in the presence of BSA as evidenced by ^{1}H NMR studies

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ABSTRACT

The emergence of albumin as a biocatalyst has created continuous interest of researchers for its application not only in the field of asymmetric oxidations and reductions but also in chemical reactions such as additions, condensations and eliminations. In the present work we report the cyclization reactions in presence of an albumin protein, Bovine Serum Albumin (BSA). The work is focused on cyclization of 2′-hydroxychalcone and 2′-aminochalcone to flavanones and azaflavanone, respectively. The results are supported by ^{1}H NMR studies.

1. Introduction

Serum albumin, the most abundant protein in blood plasma binds reversibly with the variety of endogenous and exogenous substances, involved in their transportation, has emerged as biocatalyst in 1980. The binding properties of molecules, including drugs, to serum albumin are one of the most important factors in determining their pharmacokinetics [1] and have become an interesting research field in clinical medicines including life sciences and chemistry [2,3]. The interaction of compounds with this globular protein can affect their half life in-vivo. Because of easy availability of BSA and its 80% homology with Human Serum Albumin (HSA) [4,5], this albumin protein is largely used for interaction study with various substances that have the potential to be used as drugs. Bovine and human serum albumin has been found to be versatile catalysts [6] with a broad reactivity that includes reduction, oxidation, condensations and cycloadditions e.g., hydroformylation [7], hydrogenation [8], sulfide oxidation [9,10], Diels–Alder cycloaddition [11], aldol, Knoevenagel condensation [12] etc. BSA-reagent complexes are known to mimic metalloenzymes [13]. BSA immobilized on epoxy-functionalized polymer has been used as catalyst in Knoevenagel condensation [14]. PEGylated-BSA has been explored for use as drug carrier [15].

Chalcones are also common substructures in numerous natural products belonging to flavanoid family [16,17]. The compounds with the backbone of chalcone have been reported to exhibit diverse pharmacological effects including antimalarial, anticancer, antiprotozoal, anti-inflammatory, antibacterial, antifilarial, antifungal, antimicrobial, anti HIV, antiviral, anticonvulsant, antioxidant activities [18].

Chalcones are biosynthetic precursors of flavanoids including flavanones which also exhibit various interesting pharmacological activities [19,20], continue to attract attention due to their ample range of biological activities [21]. A close examination of biological activities exhibited by chalcones and flavanones suggest that both show similar biological activities [22,23].

Several methods have been reported for the synthesis of 2′-hydroxychalcones and their conversion to flavanones using bases [24], L-proline [25] and microwave conditions [26]. But, till date, we could not find any previous report which describes the flavanone synthesis by the use of a protein molecule. In plants chalcones are cyclized to flavanones with the help of enzyme chalcone isomerase [EC 5.5.1.6] [27]. Previously, we have reported interaction studies of various chalcones with BSA [28–40]. In these reports we found that differently substituted chalcones interact with BSA irrespective of position of substituent and proposed that different nucleophilic groups in the backbone of BSA interact with α-β unsaturated carbonyl group resulting in protein–ligand interaction. In continuation of our work when we studied the interaction of 4-methoxy-2′-hydroxychalcones with BSA we found that the precursors were

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converted to flavanones. In the present work, for the first time we report the conversion of 4-methoxy-2′-hydroxy chalcone and its amino derivative to respective flavanones using BSA.

2. Result and discussion

The synthesized 4-methoxy-2′-hydroxychalcone showed characteristic IR absorption peaks of C—H, C=C stretching vibrations at respective positions, and a characteristic (C=O) stretching vibration at 1639 cm^{-1}. The ^1H NMR spectrum of **(1)** in DMSO-d^6 shows signals at δ 12.94, 7.83–7.93, 7.48–7.67 and 3.83 ppm assignable to 2′—OH, CH=CH and —OCH_3 protons respectively. The synthesized 2′-hydroxychalcone was incubated with BSA at pH 5.5 in 0.1 M acetate buffer at room temperature in 10:1 molar ratio, which resulted in cyclization of 2′-hydroxychalcone into flavanone. The synthesis of flavanone was also confirmed by spectral data. The IR peak corresponding to **(1)** at 1639 cm^{-1} attributed to (C=O) group disappeared and a new peak at 1688 cm^{-1} arrived. The results are expected because in the latter compound, there is a lesser conjugation resulting in increase in force constant which is clearly visible in (C=O) stretching vibration. Cyclization of 2′-hydroxychalcone into flavanones was also confirmed by ^1H NMR spectrum where the peaks due to CH=CH protons of 2′-hydroxychalcone disappeared and characteristic ABX pattern is observed. In ^1H NMR spectrum of flavanone **(3),** a characteristic ABX pattern is observed: H_a, H_b and H_x appear as double doublets at δ 2.4 (dd, 1H, J = 3.2 and 16.8 Hz), 3.6 (dd, 1H, J = 13.2 and 16.8 Hz), 5.1 (dd, 1H, J = 13.2 and 3.2 Hz), 7.48–7.67 (m, 8H, Ar–H), 2.49 (s, 3H, OCH_3). Interestingly quantitative yield of flavanone (~50%) sufficient enough to carry out spectral studies like IR and ^1H NMR was obtained. The results were compared to control and are not based on qualitative HPLC but quantitatively the product was identified by Bruker 300 MHz NMR spectrophotometer. The spectral characteristics and physical data of the BSA assisted conversion of 2′-hydroxychalcones matched exactly with the flavanones synthesized by chemical means [41]. Fig. 1 shows the ^1H NMR of the products obtained after conducting different reactions. Fig. 1a is the ^1H NMR of compound **(1)**. In the subsequent ^1H NMR spectra (Fig. 1b and c) of reaction products conducted in presence of BSA clearly show two distinct peaks corresponding to —OCH_3, one for chalcone and another for flavanone in approximately 1:1 ratio. These ^1H NMR spectra also show the peaks for —OH of chalcone and three protons of flavanone showing characteristic ABX pattern. Fig. 1d represents the ^1H NMR spectra of the flavanone obtained after the treatment of 2′-hydroxychalcone with BSA. An important aspect of the present study is that the conversion takes place in presence of BSA alone. However, in previous studies where BSA is involved in the catalysis of various chemical reactions the protein is either in complex formation with metal complex [42] or with the reagents [43]. For example, hydroformylation and hydrogenation reactions has been carried out in presence of $Rh(CO)_2(acac)$/(HSA) and $[Ir(COD)Cl]_2$/HSA. In these reports the role of albumin is to introduce stereo specificity and is an extension of previously existing procedures because it is well known that Rh-complexes have been used in hydroformylation and hydrogenation reactions [44]. Similarly, sulfoxidation reactions also involve the albumin and an oxidant [45].

After ascertaining the role of BSA in conversion of 2′-hydroxychalcone, the work was further extended to explore the amino acid residue involved in the reaction. Based on the assumption that —SH being the best nucleophilic group among all present as side chain residues in protein experiments were designed to evaluate its role in the aforesaid conversion. Considering that thiol being the most nucleophilic amino acid present in the side chain of the protein backbone, experiments were conducted in presence of different concentrations of $HgCl_2$. The results presented in Fig. 2a and b are the ^1H NMR spectra of the products obtained in 0.004 mM and 0.04 mM concentration of $HgCl_2$, respectively. It can be observed that the peak for —OCH_3 protons in Fig. 2a corresponding to the amount of flavanone decreases in comparison to Fig. 1b and c which is further diminished when the $HgCl_2$ concentration was increased to 0.04 mM (Fig. 2b). $HgCl_2$ acts as poison to cysteine group of proteins [46]. The decrease in concentration of flavanone with increasing concentration of $HgCl_2$ indicates the involvement of cysteine group in this cyclization. On the basis of above experimental fact we propose that —SH group of cysteine of BSA is probably involved in reaction catalysis.

Once the reaction was established with 2′-hydroxychalcones, similar experiment was conducted with 2′-aminochalcone, **(2),** which was also converted to azaflavanone, **(4)**, when treated with BSA under similar conditions. The ^1H NMR of 1-(-2-aminophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one and 2,3-dihydro-2-(4-methoxyphenyl) quinolin-4(1H)-one have been presented in Fig. 3a and b respectively.

Based upon the above findings the mechanism of reaction has been proposed (Scheme 1). First of all, under acidic condition at pH 5.5 where the reaction was carried out, Michael addition of —SH group of cysteine takes place on carbonyl carbon which may be assisted by specific acid (H^+) or by any general acid group [47] of the side chain of BSA **(A)** and results in the formation of a tetrahedral intermediate **(B)**. The ring closure takes place when the ortho placed group (—NH_2 or —OH) substitutes the —S-BSA and then the 1, 3-enol is tautomerized to form the product **(D),** flavanone.

The mechanism is supported by the fact that addition of $HgCl_2$ inhibits the cyclization and with increase in concentration of $HgCl_2$ the amount of flavanone formed is reduced and 0.04 M of $HgCl_2$ is sufficient enough to stop this conversion.

Another important and novel finding in the present study is that a sulfhydryl group has been found to be involved in BSA catalyzed reactions previously, have reported the involvement of amino group in aldol condensation [12]. Sharma et al. [12] proposed a mechanism which involved the stabilization of intermediate by ionic liquid [bmim]Br used as a solvent in the study. The catalysis by BSA is generally employed under neutral to basic conditions (pH 7–11), which also indicate the involvement of —NH_2 group in catalysis [48]. The involvement of cysteine under acidic media and amino group in neutral or basic media in the catalysis by a single bio molecule highlights signifies the importance of serum albumin in different chemical reactions which need to be explored. Involvement of amino group in the cyclization of 2′-substituted chalcones to corresponding flavanones can be completely over ruled as the reaction is taking place under mild acidic conditions and at this pH (5.5) the —NH_2 group will be existing in its conjugate acid form and therefore will not be in a position to substitute the BSA-S- group (Scheme 1, structure **(B)**). Our attempts to convert 2′-substituted chalcones to respective flavanones under alkaline conditions were not successful, therefore the above mechanism has been proposed.

Chalcones and flavanones both have been of great interest to biologists and chemists because of the diverse biological activities and easy synthetic routes. These compounds are precursors for other biologically significant molecules. The results in the present study suggest that there is a possibility that flavanones may be synthesized from the chalcones during transportation through blood because of its major constituent protein, serum albumin, which is responsible for maintaining osmotic pressure and transportation of a number of compounds. A close similarity reported for bovine serum albumin and human serum albumin, indicate that similar results will be obtained in both cases.

Based on our findings we propose that the cyclization reaction can also occur during transportation of biologically active molecules. The present study not only indicates the role of