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Original Article

Interactions of amyloid precursor protein intracellular domain (AICD) with copper and DNA fragment reveal conformational changes that trigger AD

D. Jagadeesh Kumar[2], M. Govindaraju[1,*], Priya Narayan[2], P. Ramasamy[1], H. G. Nagendra[2], K. S. Jagannatha Rao[3] and K. R. K. Easwaran[1]

[1]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India; [2]Department of Biotechnology, Sir M. Visvesvaraya Institute of Technology, Bangalore, India; [3]Department of Biotechnology, KL University, Vijayawada, AP, India.

ABSTRACT

Neurofibrillary tangles and Amyloid plaques are central to the progression of Alzheimer's disease [AD]. It has been well substantiated that the Amyloid precursor protein is cleaved enzymatically at its C terminal end yielding the APP intracellular domain [AICD]. It has been shown that AICD is an intrinsically unstructured molecule involved in AD pathology and appears to be a potential candidate in understanding the complexity of this disease. However, the relevance of AICD mechanism in neurodegeneration is poorly understood. Recent evidences reveal that AICD is localized in the nucleus, and upon binding to DNA, gene expression appears to get altered, and this could be regarded as the third hallmark of AD. Reports have highlighted that higher concentrations of copper induces a neurotoxic effect, which could enhance the AD pathogenesis. Hence, our work using circular dichroism and computational studies focuses on the interactions of AICD with copper and DNA which indicates that AICD-Cu complex interacts with the DNA and triggers conformational perturbations leading to AD.

KEYWORDS: Alzheimer's disease (AD), copper, AICD, circular dichroism, docking studies, DNA.

*Corresponding author: govindaraja@iisc.ac.in

INTRODUCTION

Alzheimer's disease (AD) is considered as a neurodegenerative disorder primarily characterized by progressive impairment of memory, decreased cognitive function, paranoia and decline in language function. According to the World Alzheimer's Report (2019) over 50 million people are affected by dementia. This according to the reports is said to reach 152 million by 2050. The current cost of dementia care is estimated at US $1trillion which may double by 2030. The pathogenesis of (AD) is extremely complex and involves the formation of neuropathological lesions, amyloid plaques (Aβ) and neurofibrillary tangles (NFTs) [1]. Recent evidences [2, 3] reveal that the amyloid precursor-protein (APP) is cleaved by gamma-secretase through proteolysis in a controlled manner at the C terminal end. Through this an additional 58 amino acid residue catabolite, the APP intracellular domain (AICD) is formed in the cytosol [4]. This is in-turn cleaved by epselon cleavage and caspase3 into JCASP (**VMLKKKQYTSIHHGVVEVDA**) and C31 (**AVTPEERHLSKMQQNGYENP TYKFFEQMQN**) fragments, and the C31 is believed to be involved in neuronal death [5, 6]. It is also well evidenced that the 656I-667V hydrophobic cluster, the 667-VTPEER- 672 which is the helix capping box at the N terminal end, and the 684-NPXY-687 which is the type I β-turn are known to make up the structure of AICD.

Further, it is also known to contain a nascent helix for residues 664-DAA-666, 675-SKMQQNGYE-683, and 688-KFFEQM-693. The conserved regions of AICD are also known to interact with intracellular adaptor proteins [7]. The consensus motif is seen to be the 682-YENPTY- 687 sequence which is involved in clathrin-mediated endocytosis and phosphotyrosine binding (PTB). It is also revealed that the AICD is produced in either the unphosphorylated or phosphorylated form. The residues (654 and 668) and a Ser (655) are the sites of phosphorylation [8]. The AICD is also known to interact with signaling proteins such as BACE1, p53, APP, LRP1 and GSK3β. This is known to lead to an enhanced Aβ generation, and cascading events of apoptosis, tau phosphorylation, Ca^{2+} signaling and cytoskeletal dynamics [9]. The AICD also is known to bind to adaptor proteins which involves in the regulation of its stability and cellular localizations [10, 11]. The binding of AICD to adaptor proteins helps in gene transcription regulation, and other cellular events like NF- κB pathway activation, calcium signaling, and processing of APP [11]. Human cell line studies have shown the interaction of AICD with FoxO proteins. These proteins translocate into the nucleus during oxidative stress with AICD. The AICD in this stage is known to act as a transcriptional co-factor. The AICD then acts as a transcriptional co-factor of *FoxO* thus inducing transcription of *Bim*, known to be a pro- apoptotic gene that activates the cell death machinery [10].

Though there are some studies on the AICD sequence and structure [12], much more need to be deciphered with respect to the structural aspects and the binding properties that might provide clues towards drug design. Recent evidences have revealed that the AICD is responsible for developmental activities and calcium homeostasis [13, 14]. Experimental evidences also reveal the interaction of different proteins with the AICD, thus bringing to light the protein-protein interaction network [13]. Reports also reveal 'conformational switching' in AICD upon phosphorylation [15]. It is also known that AICD increases the GSK3β activity thereby triggering a cascade of events leading to mitochondrial death [16, 17]. The importance of DNA and its role in metal binding has been well elucidated [18, 19]. DNA and its binding to metals has been a cause for concern as it is known to affect the replication process, protein synthesis and altered gene expression [20]. Recent research has pointed out that metal toxicity is the key factor responsible for the onset and progression of neurological disorders [18]. Studies on DNA metal interactions have revealed that copper and zinc play an important role in altering the DNA function due to conformational changes [21]. The involvement of copper has been well studied and has been of particular interest as they play a crucial role in oxidative stress and DNA damage [22, 23]. Reports also reveal that Aβ peptides are also involved in conformational changes of DNA thereby contributing to AD pathogenesis [24]. The studies reported in this paper provide an understanding of the interactions of AICD and AICD-Cu complex with DNA, which offer clues towards the conformational perturbations AD pathogenesis.

MATERIALS AND METHODS

DNA sample

The synthetic DNA having sequence **GCAATCTAATCCCTA** was procured from Sigma Aldrich. The sample was dissolved in mili Q water and required dilutions were made from the stock solution using 5 mM Tris-HCl buffer (pH of 7.4).

AICD peptide

The AICD fragment with sequence TSIHHGVVEVDA and molecular weight of 1956 Da was synthesized, purified and used for further studies.

Copper chloride ($CuCl_2.2H_2O$)

The dihydrate form of copper chloride was procured from Merck Schuchard. 9.5 mg of $CuCl_2.2H_2O$ was dissolved in 1.1 ml of milliQ water to arrive at a stock concentration of 50 mM.

UV/VIS absorption studies

Absorption studies are a common technique to explore the interaction of metals and proteins with DNA. The investigations of DNA with and without copper chloride were carried out using Jasco V-530 spectrophotometer (Jasco, Japan) equipped with a Peltier temperature controller. DNA samples (1μg/μl) in the presence and absence of $CuCl_2$ were recorded at wavelengths between 220 nm and 320 nm with a 1-cm path-length quartz cuvette.

Fluorescence studies

Probing the structure and dynamics of proteins and nucleic acids was achieved by Fluorescent spectroscopy. Fluorescence emission experiments were carried out using equimolar concentrations of DNA and EtBr (1:1). The binding pattern of EtBr-DNA as well as the effect of varying concentrations of copper between 25 μM and 500 μM were analysed. Further, the DNA/EtBr solutions were excited at 530 nm, and emission spectra were recorded from 550 nm to 650 nm using Jasco J-600 spectrofluorimeter.

Circular dichroism studies

Circular dichroism (CD) is a useful technique to study the conformations of DNA and proteins. The conformational change of DNA with copper and AICD peptide was measured using Jasco J-715 spectropolarimeter in a 1 mm path length quartz cuvette. Recordings were done at 1 nm intervals. The spectra were recorded with an average of four scans was taken to improve the signal-noise ratio. Native DNA (1 μg/μl) and its complex with copper (100 μM to 500 μM) and AICD fragment (1 μg/μl) was recorded in the wavelength between 200 nm and 320 nm at pH 7.4.

In silico molecular docking studies

Computational molecular docking was carried out to understand the interactions between AICD, copper and DNA. The selected target DNA sequence (GCTCTAATCCCCG) was chosen as these are predominantly present in brain [25] and showed sequence similarity with the one taken for *in vitro* studies. Further, these sequences are also present in the promoter region and are known to bind to various proteins and metals like copper, zinc etc. [25]. The B-DNA sequence was retrieved from the protein data bank [26] with PDB ID: (2LKX). The modeled AICD fragment TSIHHGVVEVDA (12 residue, 729-740) was used for docking analysis with B- DNA. The metal ion ligand (Cu (II) with PubChem CID: 27099) was taken from Pubchem database [27]. The ligands and target DNA were minimized by using CHARMM force field with potential energy -978.23719 kcal/mol. Docking was carried out using CDOCKER protocol in Discovery Studio 3.5 (http://www.accelrys.com/) [28], which is a CHARMM-based molecular dynamics (MD) simulated-annealing algorithm [29]. While DNA is kept rigid, the ligands, AICD and copper are treated as flexible and *via* the minimization step the docked poses are refined. Thus, the optimized structure of the AICD-Cu complex was docked with DNA to investigate the conformational perturbations.

RESULTS

UV absorption studies

The UV spectrum of the DNA fragment with the addition of different concentrations of $CuCl_2$ is shown in Fig. 1A. The absorption maxima at 260 nm on addition of $CuCl_2$ increases with increasing concentration with simultaneous shift of the peak to 250 nm (blue shift). The bands observed at 200 and 225 nm also show changes in the presence of $CuCl_2$. Fig. 1B shows the UV spectra of the DNA fragment with increasing concentrations of the peptide, AICD. With increase in concentration of AICD, the absorbance maxima also show an increase. The UV spectra of AICD peptide alone and with various concentrations of $CuCl_2$ (Fig. 1C) show absorbance maximum peak at 275 nm with an increase in the absorbance and blue shift to 270 nm. The negative band observed around 250 nm arises from the AICD as is seen in Fig. 1C where it shows large increase in the negative ellipticity on addition of $CuCl_2$.

Fluorescence studies

The fluorescence spectra of DNA-EtBr complex with and without copper are shown in Fig. 2A. The fluorescence maximum at 595 nm shows a decrease in the emission intensity upon addition of $CuCl_2$. The fluorescence spectra of AICD peptide with and without DNA are shown in Fig. 2B. AICD shows an intensity maximum at 342 nm which decreases in intensity upon addition of DNA.

Circular dichroism studies

The Circular dichroism (CD) spectra of GCAATCTAATCCCTA sequence DNA show positive band at 269 nm, a negative peak at 247 nm and a positive peak around 220 nm. On addition of $CuCl_2$ (Fig. 3A) a decrease in the positive peak at 269 nm with an evident red shift to 273 nm (4 nm), a decrease in the negative peak with a red shift to 249.2 nm and a decrease in intensity of the positive peak at 220 nm [30] is observed. Upon

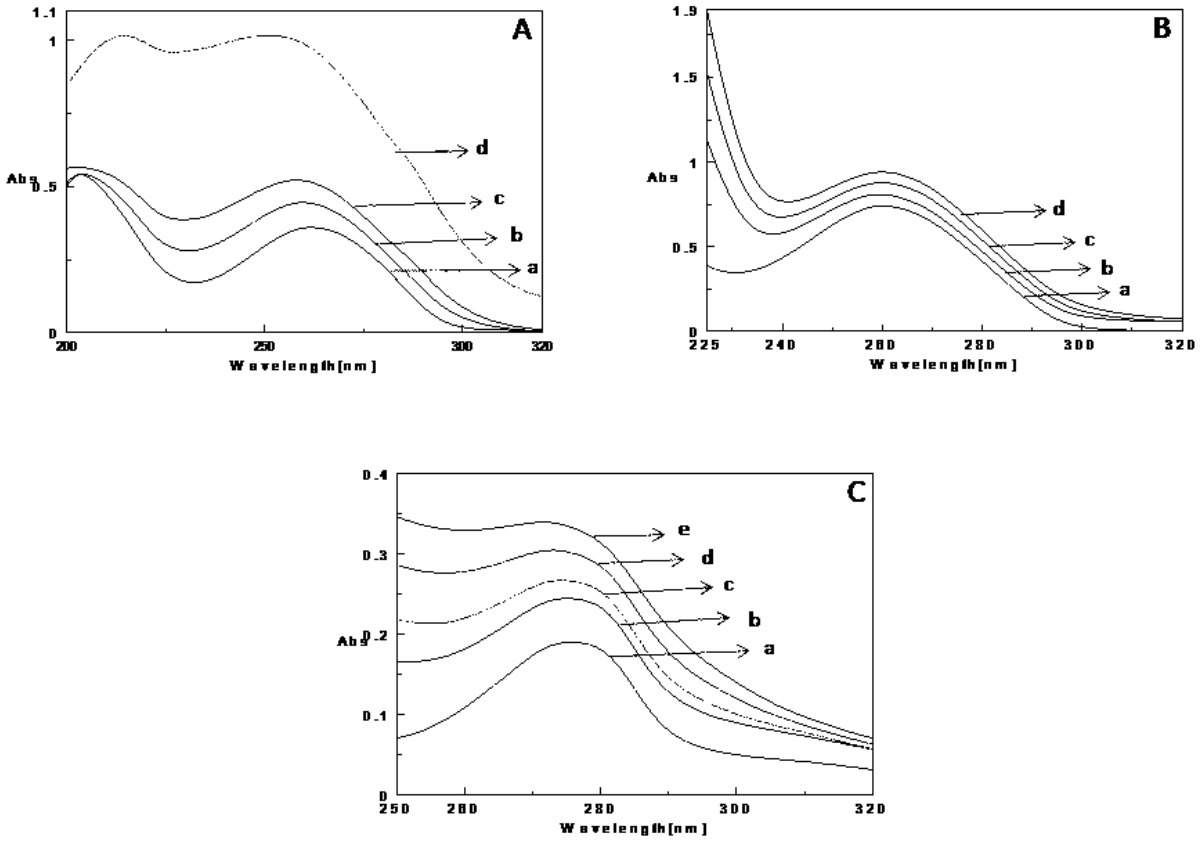

Fig. 1. UV/Vis spectra of DNA in the presence of $CuCl_2$ and AICD Peptide (TSIHHGVVEVDAA).
1A. a) DNA alone, b) DNA with 100µM $CuCl_2$, c) DNA with 250µM $CuCl_2$, d) DNA with 500µM $CuCl_2$.
1B. a) GCA sequence alone, b) DNA with 10µM AICD c) DNA with 20µM AICD, d) DNA with 30µM AICD.
1C. a) AICD alone. b) AICD with 50 µM $CuCl_2$, c) AICD with 100 µM $CuCl_2$, d) AICD with 250 µM $CuCl_2$ e. AICD with 500 µM $CuCl_2$.

addition of AICD (Fig. 3B), the magnitude of the positive peak reduces with a maximum change at negative peak of 246.6 nm to 244.5 nm (2 nm) and abrupt changes in the peak at 207 nm and a blue shift at 199.5 nm (6 nm). Fig. 3C shows the CD spectra of DNA in the presence of both AICD and $CuCl_2$. The AICD shows a negative peak maximum at 198 nm and changes in the spectra are seen with the reduced peak intensity even with addition of 100 µM of $CuCl_2$ (Fig. 3D).

Molecular docking studies

The results of molecular docking analysis of DNA with AICD fragment and copper are presented in Fig. 4 and Table 1. The best docked conformations of AICD with DNA, and the possible binding of AICD at multiple sites on the DNA are shown in Fig. 5.

DISCUSSION

The increase in absorbance and blue shift of the absorption band at 260 nm of the DNA fragment is due to strong binding of copper and AICD. Hyperchromism and blue shift occur due to the damage in DNA double helix providing evidence that copper binds with the base pairs of DNA [31]. The reduction in the fluorescence intensity of the band at 595 nm of the DNA on addition of $CuCl_2$ may be due to the quenching action of Cu with DNA-EtBr complex and it gives the information about the DNA binding affinity of copper and indicates the intercalation mode of binding between the copper and the DNA base pairs [32, 33].

The CD spectra of GCAATCTAATCCCTA sequence DNA is in the typical B conformation [34].

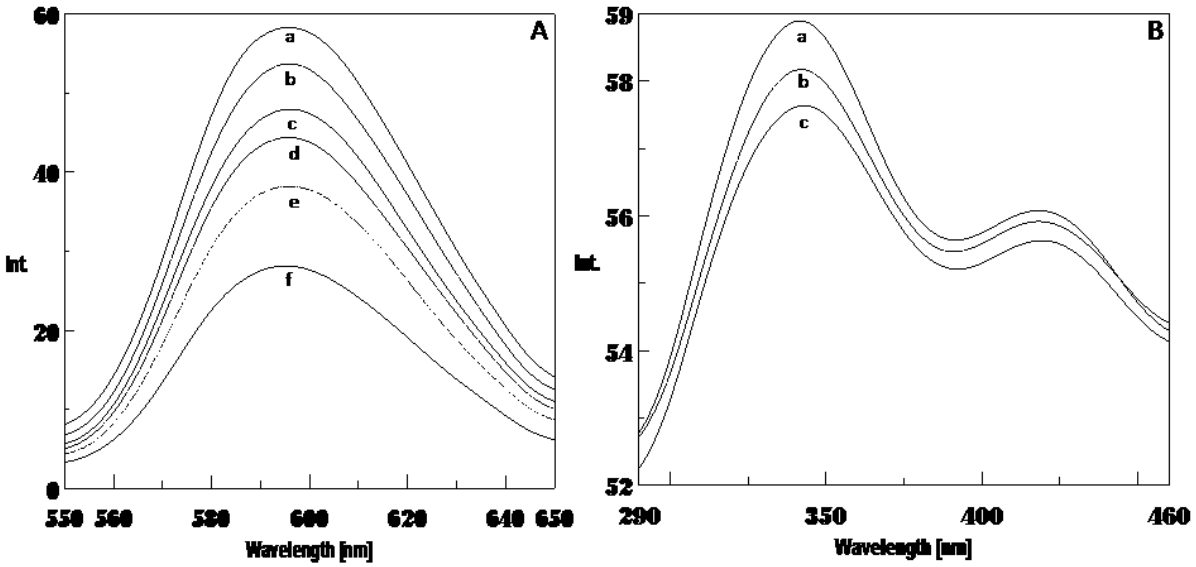

Fig. 2. Fluorescence spectra of DNA in the presence of $CuCl_2$ and AICD Peptide (TSIHHGVVEVDAA).
2A. a) GCA sequence alone, b) DNA with 25µM $CuCl_2$, c) DNA with 50µM $CuCl_2$, d) DNA with 100µM $CuCl_2$, e) DNA with 250µM $CuCl_2$, f) DNA with 500µM $CuCl_2$.
2B. a) AICD alone, b) AICD with 50µM GCA, c) AICD with 60µM GCA.

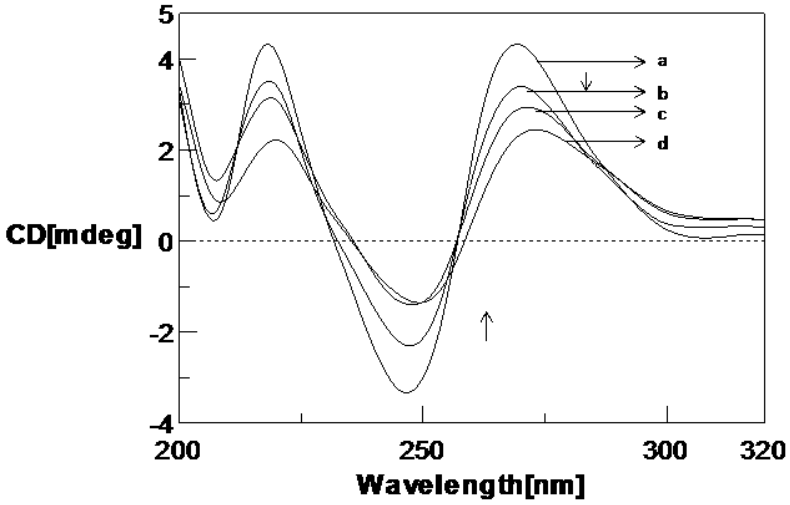

Fig. 3A. CD spectra of DNA in the presence of copper ions. a) GCA sequence alone, b) DNA with 100µM $CuCl_2$, c) DNA with 250µM $CuCl_2$, d) DNA with 500µM $CuCl_2$.

The former band is due to base stacking (indicated by UV maxima at 260 nm) and the latter due to the helicity observed in the DNA [35, 36]. The observed changes in the CD band peak with blue shift are due to the binding of AICD with base pairs of DNA indicating that AICD induced DNA damage and secondary conformational change.

Changes in the CD spectra of DNA on addition of varying amounts of $CuCl_2$, shown in Fig. 3A, due to the helicity decrease and peak shift is supportive of the binding of copper ions to the base pairs of DNA which is an indicative of conformational changes. The observed changes in DNA may be attributed towards the interaction of Cu through

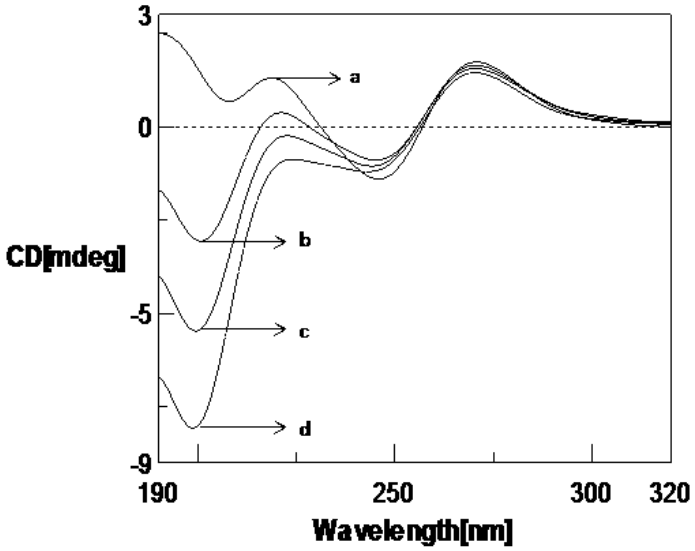

Fig. 3B. CD spectra of DNA in the presence of AICD peptide (TSIHHGVVEVDA). a) GCA sequence alone, b) DNA with 10µM AICD, c) DNA with 20 µM AICD, d) DNA with 30 µM AICD.

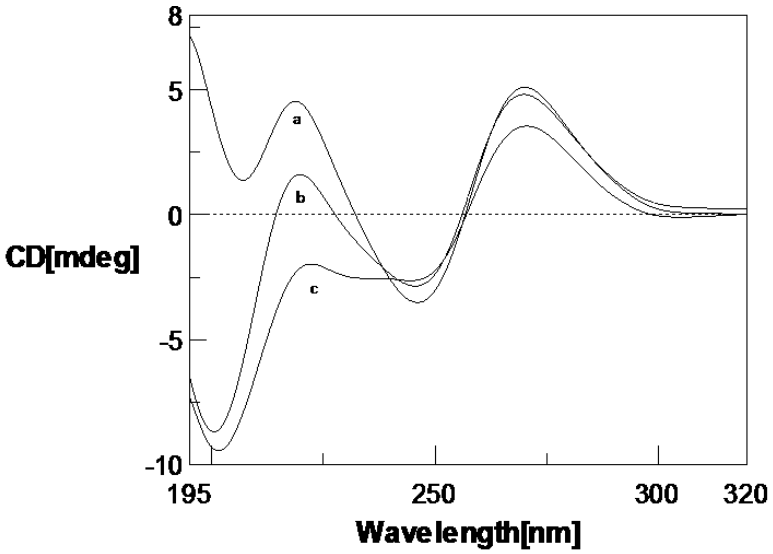

Fig. 3C. CD spectra of DNA in the presence of AICD peptide (TSIHHGVVEVDA) and $CuCl_2$. a) GCA sequence alone, b) DNA with 20 µM AICD, c) DNA with 20 µM AICD and 100 µM $CuCl_2$.

electrostatic interactions resulting in the electronic perturbations and disruption of secondary structure [37, 38]. Similarly, it is revealed that AICD-Cu complex induces more DNA damage as shown in Fig. 3C. Our results show that AICD peptide and Cu individually are able to induce conformational changes in DNA. The increase in absorbance and blue shift is due to the strong interaction of copper with AICD molecule with key amino acid residues like His, Asp, Ala and Tyr. These binding residues were found to be similar to the binding residues of the Aβ peptide [39]. Similar results were also observed in the computational docking studies. Hyperchromism and blue shift occur due to the damage in DNA double helix providing evidence that copper binds with the base pairs of DNA [35].

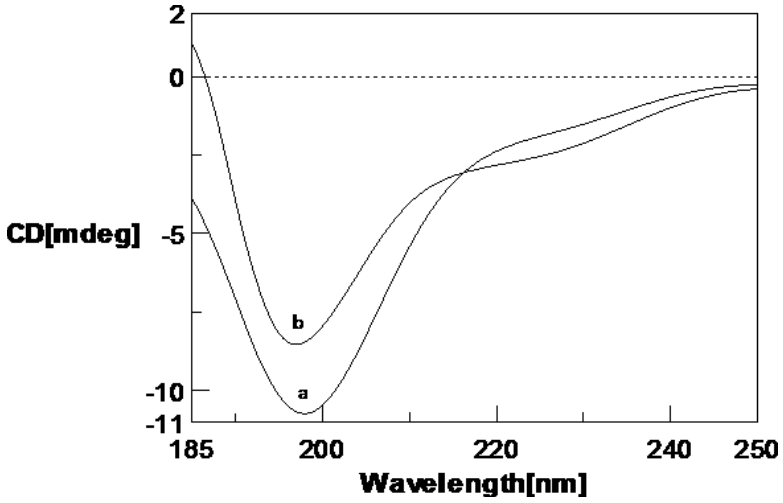

Fig. 3D. CD spectra of AICD peptide (TSIHHGVVEVDA) and $CuCl_2$. a) AICD peptide (20 µM), b) AICD with 100 µM $CuCl_2$.

The spectral variations in DNA are due to specific binding which may be due to the incoming Cu and AICD peptide, thus pushing apart the strands of DNA that may widen the major grove causing perturbation of B-DNA.

Change in the peaks at 220 nm and 207 nm of DNA is indicative of secondary structure change which is attributed to changes with respect to the winding angle. It is also well elucidated that in addition to the changes in the winding angle, changes in the tilt of the base pairs also occur. Further, the distance of the base pairs from the helix axis and geometric axis also changes. This tilt in the base pairs is also known to change sugar puckering from anti to syn stacking pattern. This in turn may cause unwinding of DNA [40]. Variations among the DNA secondary structures is dependent on the extent of twisting which may cause a decrease in the right handedness leading to altered B-DNA conformation. This is in line with previous studies which reveal that Aβ, tau and metals are capable of interacting with nuclear proteins and DNA causing DNA damage and conformational change that may cause changes in gene expression [41]. Taken together, our studies emphasize on the interaction of AICD and Cu with DNA causing changes in its conformation. Further, changes in the conformation of the AICD peptide have also been observed. The results show that an AICD and copper ion strongly bind to the major groove between the base pairs of the helical form of nucleic acids and alter the DNA conformation.

As evident from the docking studies (Fig. 4 and 5 & Table 1), the amino acid residues of AICD, namely Ser730, Ile731, and His733 interact with Thy87, Gua89 of DNA C-chain and Cyt 70 of B chain nitrogenous bases of B-DNA and form 5 hydrogen bonds across the major groove. The molecular docking results shown in the figures indicate that the major groove interactions are the relevant binding modes for the AICD fragment across the TAATCC. The sequence logo of DNA is also shown in Fig. 5D. It is observed that Ser730 bonds with Thy87 (bond length 2.807˚A), Ile731 with Thy87 (bond length 3.043˚A and 2.936˚A), and His733 with Gua89 of C chain (bond length 3.043˚A). It is also seen that the His733 interacts with CYT70 of the B Chain (bond length 2.581˚A). (Table 1). Hence formation of hydrophobic and hydrogen bond indicates interaction between AICD and DNA. AICD interacts in the major groove of the DNA having a Cdocker score of −45.2375 kcal/mol. Interestingly, our results reveal the interaction of AICD at different binding sites on the DNA molecule, indicating a higher binding probability/affinity to DNA. Similarly copper also interacts with nucleotides Thymine 86 and 87 of C chain and Adenine72 and 73 of B chain. Thymine and Adenine form strong covalent metal-adenine-thymine complexes. Thus in the current study, the *in silico* docking studies

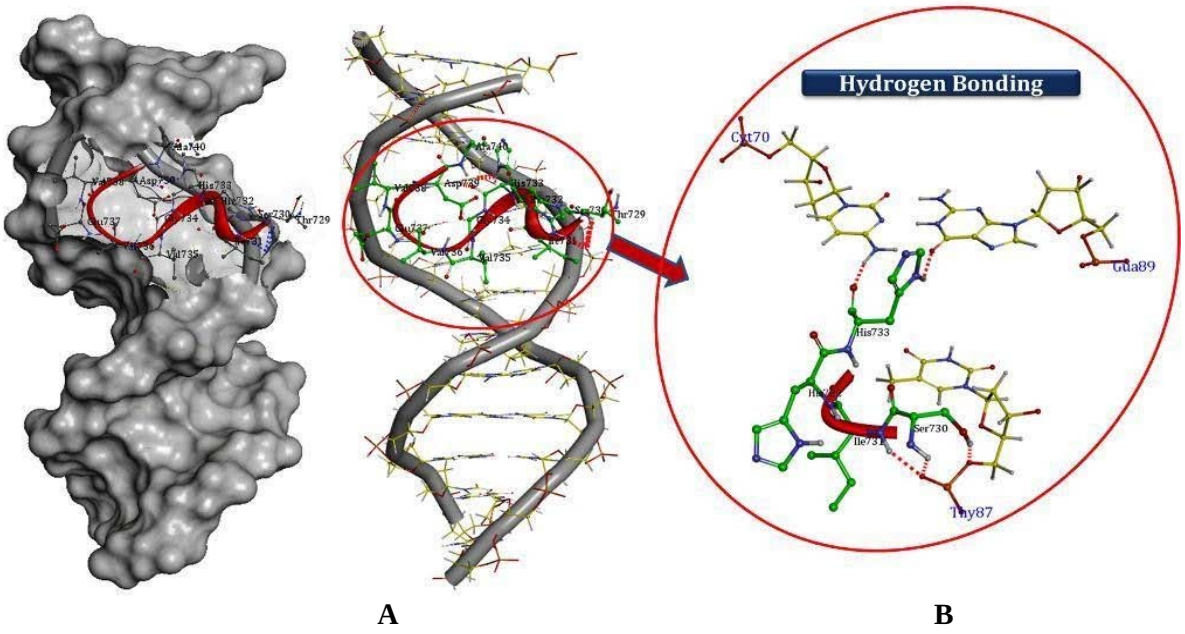

Fig. 4. A) Surface view of molecular-docked AICD- DNA complex.
B) Molecular-docked model of AICD showing major groove binding with DNA duplex of sequence with a human TAATCC DNA binding site [PDB code 2LKX: B-CHAIN – GCTCTAATCCCCG and C–CHAIN–CGGGGATTAGAGC. Top predicted binding poses are considered. The green color amino acid residues denote the DNA binding residues of AICD and nucleotides are shown in Yellow (CYT70 B chain and Thy87 GUA 89 of C chain). The amino acid atoms of Ser730, Ile731, and His733 with intermolecular H-bonds are shown in Red lines.

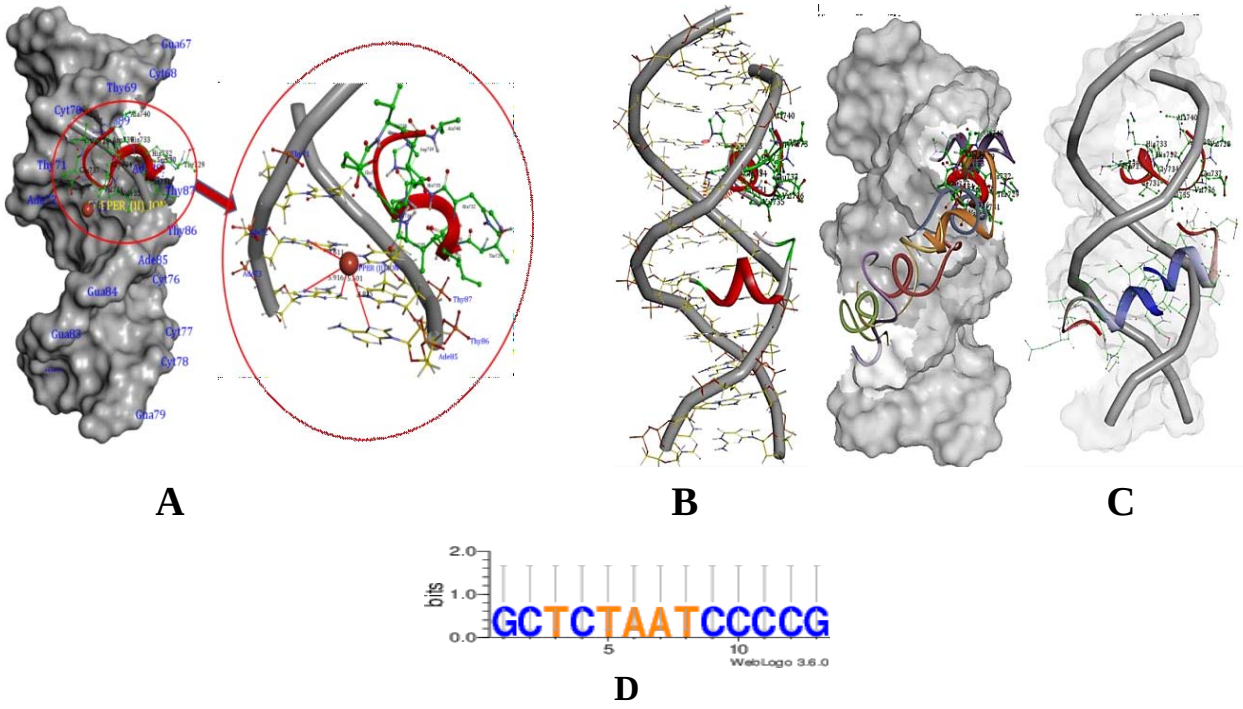

Fig. 5. A) Copper docked with AICD and DNA; **B)** Best docked conformations of AICD with DNA; **C)** Binding of AICD at multiple sites on DNA**; D)** Sequence logo signature of DNA.

Table 1. Interaction of Copper and AICD amino acids with B DNA Nucleotides with hydrogen bond distances.

SL NO	AICD	DNA B-chain Residues	DNA C-chain Residues	Hydrogen Bond and Distance in A˚
1	SER 730	-	THY87	N-H-O-2.807
2	ILE731	-	THY87	O-H-O-3.043 N-H-O -2.936
3	HIS733	-	GUA89	N-H-O -2.893
4	HIS733	CYT 70	-	N-H-O -2.581
5	Copper(II)	ADE72 ADE73	THY87 86 THY87 87	-

shows inter- actions of AICD and copper in the major groove of DNA via formation of hydrogen bond and hydrophobic bond with docking score of −45.2375 kcal/mol. The molecular docking results also show interactions of AICD and copper in the major groove of DNA *via* hydrogen bond and hydrophobic bond formation with docking score of −45.2375 kcal/mol. Thus the *in silico* exercises revealed possible binding mode of AICD and copper with DNA which is in line with the spectroscopic results indicating strong interactions of these ligands across B-DNA leading to the conformational change and instability, which are potential triggering events in the pathogenicity of AD.

CONCLUSION

In conclusion, our UV spectroscopy studies showed increased intensity of the bands on addition of AICD peptide and $CuCl_2$. This is attributed to the binding of AICD and $Cucl_2$ to the DNA and possible denaturation and opening up of the DNA. Fluorescence studies indicated that the conformation of the nucleotide sequence was affected due to copper binding and showed that the interaction of copper and AICD with DNA is very strong. The CD data of GCAATCTAATCCCTA sequence DNA was found to be in the B conformation. Our results reveal that on addition of copper, changes occurred in the positive as well as the negative peaks, indicating that copper binds to DNA as is corroborated via *in-silico* docking studies as well. Our data further reveal changes in conformation of DNA on addition of AICD. Any change in DNA conformation is related to its damage. The CD study shows a decrease of band at 270 nm which is due to damage of DNA. Taken together, this may indicate DNA damage is induced by AICD. Our *in-silico* studies provide valuable information on the DNA conformational changes of secondary structures upon binding to some specific residues of AICD and Cu and their probable role in an early event pathogenesis of AD. Hence our results may provide clues towards the binding modes of AICD and Cu with DNA and their possible roles in the pathogenesis of AD. Our study is one of the first preliminary biophysical studies of the amyloid precursor protein intracellular domain with Cu and DNA. The detailed studies on the interaction of DNA with AICD and Cu at the molecular level using NMR are in progress.

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CONFLICT OF INTEREST STATEMENT

The authors declare that this paper has no conflict of interests.

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