Journal of the Indian Chemical Society 98 (2021) 100057

Contents lists available at ScienceDirect

Journal of the Indian Chemical Society

journal homepage: www.editorialmanager.com/JINCS/default.aspx

Revisiting magnetic field effects in homogeneous medium and bio-mimicking environments with emphasis on acridine derivatives☆

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ARTICLE INFO

Keywords:
Magnetic field
Photo-induced electron-transfer
Acridine derivatives
Micelles
Serum albumin
β-Lactoglobulin

ABSTRACT

A weak external magnetic field, very close to the hyperfine interactions of the system, can acts as a tool to monitor spin dynamics and assess distance between the components of the spin-correlated transient radical pair or radical ion pair (RIP). The present review focuses on the magnetic field effect (MFE) on the photo-induced electron-transfer (PET) reactions among acridine derivatives and classical as well as biological electron acceptor or donor moieties, which produce spin-correlated RIPs, in homogeneous solvents, heterogeneous micellar media and in biological nanocavities of proteins. Although a confined medium is preferred to observe prominent MFE, yet unanticipated MFE on PET between acridine derivatives [Acridone (AD) and Acridine Yellow (AY)] and classical electron donors is obtained even in homogeneous medium when it consists of impurities like water molecules. In a comparative study of interaction of another acridine derivative, Proflavin (PF^+) with two electron donors which are amines of aromatic nature, MFE on PET reveal that the bulk and the structure of the electron donor govern the mechanism as well as the spin dynamics of PET. While studying interaction of PF^+ with a different amine which is aliphatic in nature, MFE on PET implies that it is the nature of the solvent matrix which determines the spin dynamics of PET. The cause of discrepancy in the experimental and calculated values of $B_{1/2}$ for 9-amino acridine – methyl viologen system has been delineated. Apart from micellar medium, prominent MFE on PET is also observed while studying the interaction of PF^+, AY and AD with tryptophan residues present in the nanocavities of serum albumins since the inter-radical distance within primary geminate RIP is enough to make exchange interaction negligible.

1. Introduction

As the phenomenon of Photo-induced Electron-Transfer (PET) reactions is extensively prevalent in chemical and biological sciences, understanding as well as control of these reactions is a thriving area of research today. PET involves electron transfer from an electron rich donor to an electron deficit acceptor, while one of them remains in the photo-excited state. It may be either intermolecular, i.e. occurs between two separate molecules serving as electron donor and acceptor or intramolecular when both the donor and the acceptor are parts of the same molecule linked with a spacer. In general, PET is identified by quenching of fluorescence. However, as a consequence of PET, non-fluorescent radical pairs (RPs) or radical ions pairs (RIPs) (or in some cases combination of radical and radical ion) are formed as intermediates in the reaction pathway. These non-fluorescent transients (short-lived) species can be detected not by steady-state fluorescence, but by using transient absorption techniques, among which nanosecond-resolved laser flash photolysis (LFP) is quite popular and easy to handle. Thus, the occurrence of PET may be confirmed *via* detection of the presence of radical/radical ions in the system. As radicals/radical ions contain free electrons, so they are prone to be perturbed by an externally applied magnetic field (MF) [1–4]. A weak MF in the range of 0.01–0.08 T has an enormous capacity to modulate the reaction pathways containing radicals/radical ions by manipulating their spin dynamics. The magnetic field effect (MFE) is a subtle amalgamation of dynamics of diffusion and spin of the initially formed geminate radical pairs originating from PET. The geminate radical pairs or radical ions pairs within solvent cage are individually disintegrated through diffusion in the solvent and achieve an ideal distance where the interaction through exchange (J) becomes negligible and the MF present within the system generated through electron and nuclear

☆ This review article is dedicated to Professor Dulal Chandra Mukherjee.

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https://doi.org/10.1016/j.jics.2021.100057
Received 20 November 2020; Accepted 25 January 2021

spin interactions, i.e., hyperfine interactions (HFI) make spin flipping, leading to mingling of singlet (S) and triplet ($T_{\pm}$,T_0) states, thus promoting intersystem crossing (ISC). Then an externally applied MF similar to HFI or more can remove the degeneracy among triplet states through Zeeman splitting, therefore only S ↔ T_0 channel is functional and the population of RIPs with parent spin state formed through PET increases (as depicted in Fig. 1). Generally, singlet spin-correlated geminate RPs/RIPs generate exciplex, i.e., recombination product within the solvent cage while triplet spin-correlated RIPs favor to form free radicals/radical ions. Consequently, on application of MF an increase in exciplex luminescence or transient absorbance of free radicals/radical ions signifies that the parent geminate radical pairs are singlet or triplet born respectively. The importance of PET depends on its production of free radicals, rather than their recombination. Hence, MFE makes it possible to validate the generation of a radical/radical ion, certifies their original spin configuration and reveals the reaction mechanism. Moreover, MFE is able to signify the 'optimal separation' among the geminate radical ions, where spin flipping as well as formation of free ions or recombined products occur. The separation distance can be retained by changing temperature, viscosity or dielectric of the medium for intermolecular PET and by varying chain length that links donor and acceptor for intramolecular PET reactions. However, optimization of separation distance by confining the radical ions within heterogeneous organized assemblies has gained much attention because those systems mimic biological cell membrane [5–12]. Additionally, our group has reported that MFE is appreciably exhibited in biomolecular environment and it is quite competent to assess the separation distance between electron donor and acceptor within protein or DNA undergoing PET [13,14].

One of the prime mechanisms for ISC in spin correlated RPs is hyperfine coupling (HFC). The extent of ISC is suppressed by application of a weak MF in the range of 0.01–0.08 T. The corresponding MFE increases with increasing field strength and eventually reaches saturation when the Zeeman splitting exceeds HFC interaction. The field at which half the saturation is attained is termed as $B_{1/2}$ and this is an important parameter that depends in a characteristic way on the HFC constants of the magnetic nuclei [15–18].

Exploration of reactions of acridine derivatives with nucleic acids has fascinated the scientists for many years [19–23] as they are good intercalators because of the planar nature of the acridine moiety. In fact, acridine derivatives are also recognized as potential photosensitizers in photodynamic therapeutical treatment [24–26]. It is observed that comparatively less reports are existing which talk about the interaction of proteins with acridine derivatives and also explore their participation in PET reactions.

The present review highlights MFE on the precursors of PET in homogeneous medium, bio-mimicking systems and biological nanocavities of proteins involving a number of derivatives of acridine (as depicted in Fig. 2). To decipher the roles of the acridine derivatives in PET reactions and to characterize the intermediates originating from them, it is necessary to initially explore their interactions with classical electron acceptor or donor moieties. Such studies are carried out in bio-mimicking heterogeneous media to impose a restricted environment so that MFE on the precursors of PET is appreciable and also to provide differential occupational sites to the acceptors and donors. Further, PET involving these acridine derivatives trapped in protein cavities and associated MFE are also discussed in this review.

2. Experimental techniques

Ultra Violet - Visible absorption spectra were obtained using an absorption spectrophotometer (Unicam UV-500). Steady-state and time-resolved fluorescence studies were carried out using Spex Fluoromax-3 spectro-fluorimeter and Time-Correlated-Single-Photon Counting (TCSPC) kinetic spectrophotometer (Edinburgh) respectively. The transient absorption spectra were obtained by Nanosecond Flash Photolysis (Applied Photophysics) with Nd:YAG (Lab series, Model Lab 150, Spectra Physics) laser. Excitation of the sample was done with 355 nm (FWHM = 8 ns) laser and the transients were probed by a pulsed xenon lamp (250W) through absorption. MFE on absorption spectra was observed by keeping a pair of electromagnetic coils inside the sample chamber through which direct current is passed.

3. MFE on PET in homogeneous solvent when donor and acceptor are unlinked

As already discussed in the Introduction, in general MFE experiments are carried out in micellar media or highly viscous solvents because these constrained environments diminish the chances of escape and help to retain the spin-correlation between the partners of geminate radical ions pairs. Very few examples are cited in literature where MFE on PET is obtained in homogeneous medium [16,27–29]. Interactions of Acridone (AD) and Acridine Yellow (AY) with classical electron donors like *N, N′*-dimethylaniline (DMA), 4,4′-bis(dimethylamino)diphenylmethane (DMDPM) and *N,N′*- diethylaniline (DEA) are studied in alcoholic medium (Ultra Violet-spectroscopic grade ethanol which contains 0.1% water) using LFP technique with an associated weak MF [30,31]. LFP study shows that PET reaction occurs between the two acridine derivatives (acceptor moieties in PET) and the organic amines (donor moieties) in ethanol medium. It is interesting to note that contrary to the conventional observation of MFE in restricted environment, appreciable MFE is detected on the PET reactions in the homogeneous alcoholic medium. This anomalous observation has been rationalized in the light of the existence of water molecules in alcohol as contaminants which may help to form non-covalently linked acceptor-donor system by means of hydrogen bonds (Fig. 3). In fact, this non-covalent interaction among the radicals imparts pseudo-confinement to the RPs/RIPs formed through PET and preserves the optimal separation between the radical ions so that spin correlation is maintained in spite of minimizing *J*. A critical remark in this context is that suitable functional groups of acridine derivatives can form hydrogen bond with appropriate electron donors in homogeneous solvents, which may give rise to appreciable MFE on PET.

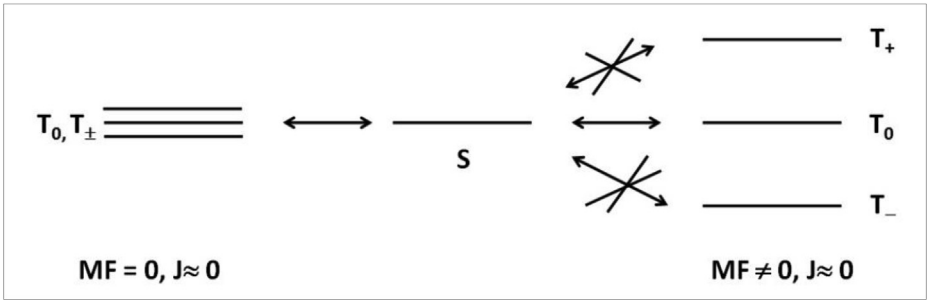
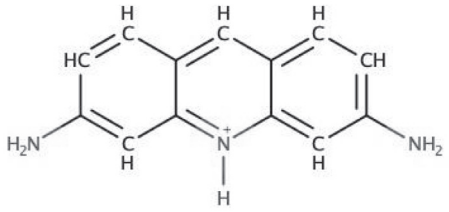
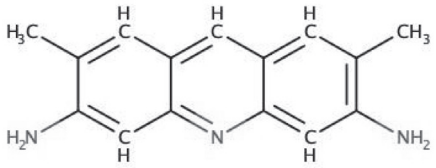


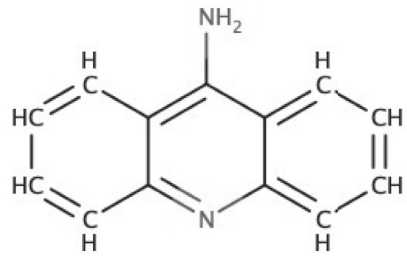
Fig. 1. The effect of an externally applied MF on the energy states of Singlet (S) and Triplet (T). Only S↔T_0 transition remains functional due to Zeeman splitting.



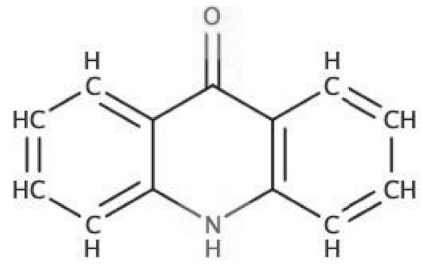
Proflavin (PF^+)



Acridine Yellow (AY)



9-Aminoacridine (9AA)



Acridone (AD)

Fig. 2. Structures of various acridine derivatives discussed in this review.

4. Study of MFE on PET in bio-mimicking micellar systems

Interaction of uni-positive acceptor moiety, Proflavin (PF^+) with classical electron donors DMA and DMDPM in homogeneous and heterogeneous solvent matrices shows that the bulk and structure of the donor determine the mechanism as well as the spin dynamics of PET [32]. LFP study proves that PET takes place in PF^+-DMA and PF^+-DMDPM systems in both homogeneous and heterogeneous solvents. On applying a weak external MF to PF^+-DMDPM system in SDS medium there is enhancement in optical density in the region assigned to $PF^{\bullet}$ and $DMDPM^{\bullet+}$ implying that the precursors of PET are triplet born. Similarly, while a weak external MF is introduced to PF^+-DMA system, there is increase in absorbance of $PF^{\bullet}$ while absorbance of $DMA^{\bullet+}$ decreases (as shown in Fig. 4). This observation is fascinating! We have proposed that $PF^{\bullet}$ is generated by two mechanisms: one is through PET from DMA to PF^+ and the other is by hydrogen abstraction from the tail region of SDS. Decrease in optical density in the wavelength region of $DMA^{\bullet+}$ suggests that the initial state of spin of the precursors of PET in PF^+-DMA system is singlet and the enhancement in optical density in the region corresponding to $PF^{\bullet}$ may be attributed to hydrogen abstraction, which possibly occurs in the triplet state. This proposition is substantiated by augmentation in optical density on introduction of MF in the region characteristic to $PFH^{\bullet+}$, an intermediate formed in the hydrogen abstraction pathway. Thus, the initial state of spin of the precursors of

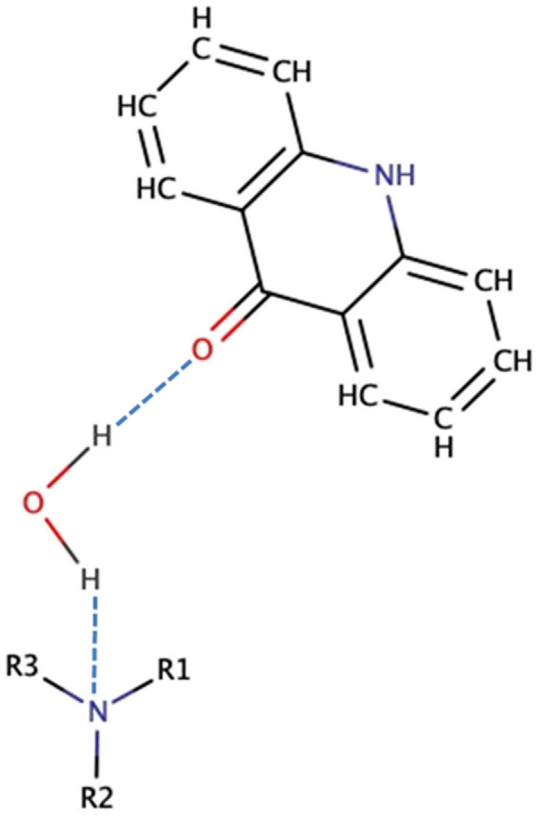


Fig. 3. Non-covalently linked acceptor-donor in Acridone – organic amine systems; additional intervening water molecules may be present.

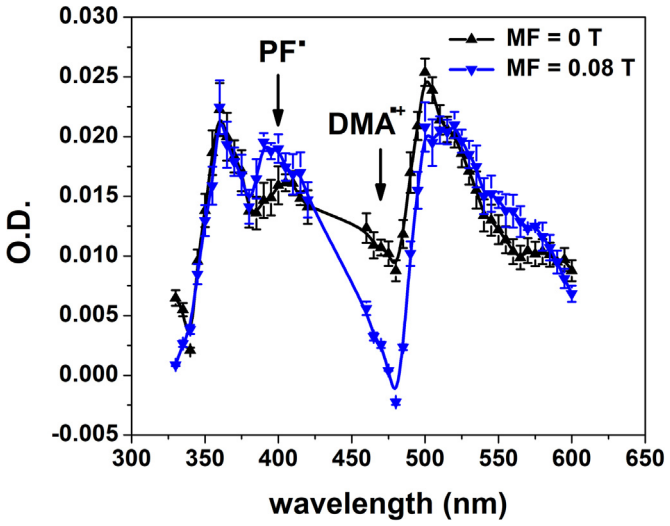


Fig. 4. Absorption (transient) spectra obtained from laser flash photolysis (λ_{ex} = 355 nm) of PF^+ (0.1 mM) and DMA (3 mM) (i) without (▲) and (ii) with (▼) MF in SDS medium at a delay of 0.6 μs.

PET for PF^+-DMA system is singlet whereas that for PF^+-DMDPM system is triplet. Now, DMA and DMDPM primarily differ in their bulk and DMDPM may be treated as a dimer of DMA, where a $–CH_2–$ bridge joins two DMA units. Thus, DMDPM effectively induces ISC of $^1(PF^+)^*$ and PET predominantly takes place in triplet state, whereas DMA cannot induce ISC of $^1(PF^+)^*$ as efficiently as DMDPM and hence PET preferably takes place in the singlet state. Moreover, one of the determining factors for exhibiting appreciable MFE is the geminate characteristic of the solvent separated ion pair (SSIP) within an external solvent cage. A geminate cage containing a radical cation and a radical anion is electrostatically stable and is capable of exhibiting MFE. But in case of PF^+-DMA system, the geminate cage contains a radical and a radical cation, ($PF^\bullet$ $DMA^{\bullet+}$) and hence, is not electrostatically stable enough to show MFE. On the contrary, for PF^+-DMDPM system, the bulk of $DMDPM^{\bullet+}$ helps to overcome the electrostatic instability factor by maintaining inter-radical separation distance and consequently ($PF^\bullet$ $DMDPM^{\bullet+}$) shows appreciable MFE. Thus, from this study it is evident that the structure of the electron donor dictates the reaction mechanism and dynamics of spin of PET reaction.

To explore the impact of microenvironment on the mechanistic pathway and spin dynamics of PET, we have resorted to investigation of interaction between PF^+ and an amine of aliphatic nature, viz., triethylamine (TEA) in homogeneous solvent and also heterogeneous micelles using absorption/fluorescence spectroscopic techniques and LFP coupled with an external MF [33]. Steady-state absorption study suggests that a complex is formed in the ground state between the aliphatic TEA and the uni-positive PF^+ and LFP study confirms that PF^+—TEA complex has charge transfer characteristics. Moreover, the occurrence of PET in both the solvent matrices is validated by LFP studies. Both positively charged (CTAB) as well as negatively charged (SDS) micelles have been used as heterogeneous media in this study. Now, MFE on PET reaction in the ionic micelles suggests that the precursors of PET in PF^+-TEA system is singlet born in case of SDS medium while it is triplet born for CTAB medium. To explain this intriguing observation we have to verify the sites which are preferentially occupied by the donor and acceptor within the micelles as the parting distance among the radical or radical ions within SSIP is a vital factor controlling MFE. TEA prefers to stay in the hydrophilic environment in the region of the polar head groups of the micelles. In SDS medium, PF^+ will be pulled towards the polar head groups (as they are negatively charged) via electrostatic attraction and thus the donor and acceptor will come in extremely close vicinity of each other while in case of CTAB medium the head groups will exert a repulsive force on PF^+ and consequently PF^+ will prefer to stay away from the micellar head into the solution, thus maintaining the optimal distance between acceptor and donor necessary to sustain the geminate property of the SSIP cage. Thus, in SDS micelle the partners in SSIP come very close to each other and fail to achieve the favorable distance of separation, and eventually introduction of MF will decrease the optical density of $PF^\bullet$ and $TEA^{\bullet+}$ indicating the occurrence of PET in the singlet spin state. On the contrary, in CTAB micelles, the optimal separation between the electron donor and the acceptor is maintained and appreciable MFE is shown by $PF^\bullet$ and $TEA^{\bullet+}$ indicating that PET takes place primarily in the triplet state. Thus, the spin dynamics and mechanism of PET is dependent on the nature of the medium.

Dimethyl viologen (MV^{2+}) is a popular electron acceptor which yields a stable radical cation ($MV^{\bullet+}$) when it takes part in PET [34]. Additionally, there are some reports on the ability of MV^{2+}towards acceptance of H atom [35]. Contrary to our previous reports where the other acridine derivatives act as electron acceptor, it is found that 9-aminoacridine hydrochloride hydrate (9AA-HCl) behaves as the electron donor while interacting with MV^{2+} [17]. However, we have already reported the electron accepting capacity of 9AA-HCl from amines like DMA and TEA [36]. Actually, the value of reduction potential of 9AA-HCl, MV^{2+}, DMA and TEA determines whether the acridine derivative would behave as an acceptor or donor moiety while participating in PET. Redox potential value of 9AA-HCl is −0.781 V and that of methyl viologen is −0.37 V, which justifies the function of the acridine derivative as a donor and that of MV^{2+} as an acceptor during PET. LFP technique helps to detect the nature of the species involved in each of the photoinduced interactions and ascertain that although PET as well as excited state proton transfer (ESPT) occur in homogeneous alcoholic solvent, but only PET is established in CTAB micellar medium. MFE proves that the electron transfer occurs primarily in the triplet spin state in CTAB micelles. It is inferred from the disagreement in calculated and experimental values of $B_{1/2}$ (Fig. 5) that electron hopping mechanism prevails in the system

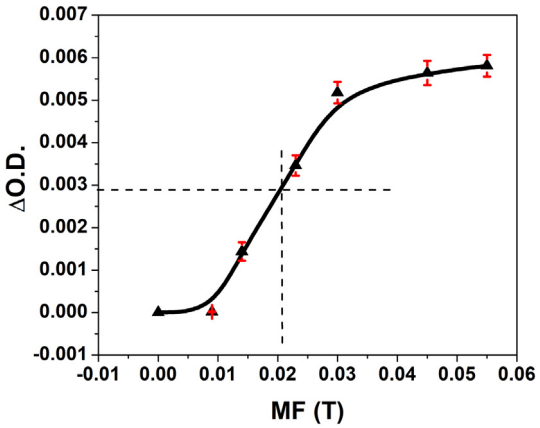


Fig. 5. Variation of O.D. with external MF for 9AA-HCl (50 μM) and MV^{2+}(6 mM) at 405 nm in 5% CTAB solution at a time delay of 0.75 μs after pulsing of laser, utilized for determination of the $B_{1/2}$ value experimentally.

due to large effective concentration of the acceptor moieties.

5. Study of MFE on PET in protein nanocavities

Study of the behaviour of probe molecules trapped in nanocavities of proteins, like those in serum albumins [viz., Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA)] as well as in β-Lactoglobulin (βLG) is intriguing since the behaviours of these molecules are quite different when they are confined compared to the situation when they are in solution as free moieties. Our group has reported the photochemistry of many such probe molecules (in addition to acridine derivatives) trapped in protein pockets [37–40].

During the study of interaction of AY and PF^+ with HSA it has been observed from the LFP study that PET occurs from the sole Tryptophan (Trp) residue of the serum albumin to the acridine derivatives and MFE ascertains that the parental spin state of the precursors of PET is triplet [41]. Even in absence of protein environment, PET could be observed between PF^+ and bare Trp with a significant MFE, where the optimal parting between the donor and acceptor is maintained by using AOT as reverse micellar solvent matrix [18]. In the presence of protein, even though the study is carried out in homogeneous medium of phosphate buffer, nevertheless considerable MFE can be noticed because of pseudo-confinement of RIPs imparted by the intricate structure of the protein that assists to maintain the optimal features necessary for MFE. The distance between the geminate RIPs governs MFE and it is significantly observed when the separation distance lies between 10 and 20 Å. In fact, the stereoview of the docked conformations illustrates that distance between Trp and AY is 1.33 nm while that between Trp and PF^+ is 1.29 nm, which is ideal for the observation of MFE, otherwise it could not be ensued. Thus, the protein pocket in HSA provides the proper confinement which is ideal for MFE.

Further, interaction of AD is studied with both the serum albumins [42,43]. AD is chosen to explore its interaction with proteins as it contains a keto group. It has previously been reported that molecules containing keto functional groups (eg. methyl ethyl ketone, acetone etc), can cause denaturation of proteins [44]. In case of AD, the keto group can probably impart ample modification in the secondary and tertiary structures of the model proteins as proven by the circular dichroism spectroscopic studies. Even though BSA shows sequence homology with HSA by about 76%, the key distinction between them is that HSA contains only one tryptophan residue (Trp 214) while BSA has two (Trp 212 and Trp 134). Now, Trp 214 present in HSA and Trp 212 present in BSA are both positioned inside hydrophobic domain IIA of the respective proteins and they are housed in a comparable environment which is markedly distinct from the environment of Trp 134 of BSA in hydrophilic domain IB. In both AD-HSA and AD-BSA systems PET is detected using LFP. However, in AD-HSA system appreciable MFE is detected on the precursors of PET (as depicted in Fig. 6) while no such effect is found in AD – BSA system. This is attributed to the differential modes of approach of AD towards Trp residues within proteins. In HSA, AD exclusively approaches towards Trp 214, the only Trp residue of the protein. AD is stuck in the cleft of IIA domain within HSA and nicely holds to the optimal parting distance from Trp 214, necessary condition for MFE to be observed. However, in BSA, AD primarily approaches towards Trp 212 located in domain II, after that it tries to penetrate within the rigid structure of BSA to access Trp 134 inside domain I, which is supported by the Time Resolved Area Normalised Emission Spectra. This dynamic behaviour of AD within BSA is unfavourable for retaining the optimized parting distance within initially formed RIPs of AD and Trp 212, which are geminate in nature, required condition to make *J* negligible and MFE significant. Thus, no MFE is observed in AD–BSA system.

This work is further extended with AD and βLG, a small whey protein containing a couple of Trp residues viz. Trp 19 and Trp 61 within dissimilar environments [45], attempting to confirm whether AD is able to approach both the Trp residues in βLG similar to its approach within BSA or specifically enter within the microenvironment of βLG approaching only one of the Trp residues. Various spectroscopic observations suggest the interaction of AD with only Trp 19 residue of the βLG protein. Contrary to the previous reports of PET involved in interaction of AD with the serum albumins, hydrogen abstraction supported by hydrogen bonding occurs during the interaction of the whey protein with AD, which is ascertained by LFP. The hint of occurrence PET between AD and serum albumins are supported by reduction of fluorescence lifetime of the proteins in presence of AD, while in presence of βLG, fluorescence lifetime of the protein is increased by addition of AD, which gives a sign of the participation of rotamers of Trp 19. However, no MFE is observed on the radical pairs which are detected by LFP in AD-βLG system. This is probably due to the dynamic nature of Trp 19 which undergoes rotation to yield its rotamers and thus the stability of geminate pairs is not high enough to show appreciable MFE.

6. Conclusion

MFE depends upon the system possessing states which differ in their magnetic properties (S and T states). If application of MF can modulate the reaction channels for these states, i.e. either close or boost selected reaction channels, then MFE on the product distribution is possible. Although singlet - triplet ISC operates via a number of mechanisms, in the present review, we have discussed only HFC mechanism in details as it is operative in presence of weak MF and is pertinent to our work. In this review, we have revisited our earlier works related to MFE on PET involving acridine derivatives in homogeneous medium, bio mimicking micellar systems and also in protein nanocavities. It is found that MFE shows the 'signature' of the parental state of spin of the precursors of PET and also acts as an evaluator of distance among the geminate radical ions, which are the primary products of PET.

Unusual observation of MFE in homogeneous medium is rationalized in the light of hydrogen bond formation, which brings the donor and the acceptor molecules nearer to each other in the excited state. MFE in heterogeneous micellar media show that the structure of donor moiety and nature of medium govern the mechanism and spin dynamics of PET. If the photophysical interactions of PF^+ with a couple of amine electron donors, which are aromatic in nature, is compared, then from MFE on the precursors of PET it could be inferred that the overall mechanism and dynamics (both diffusion and spin) are controlled to a great extent by the bulk and structure of donor. Further, the key phenomena that have been observed in the interaction of PF^+ with triethylamine are PET and complex formation in the ground state. MFE on PET implies that it is the solvent matrix which dictates the spin dynamics of PET owing to the differential occupational sites of PF^+ provided by two ionic micelles of opposite charges. Finally, a comparison of absence and presence of MFE has been made in various protein environments, viz. HSA, BSA and βLG.

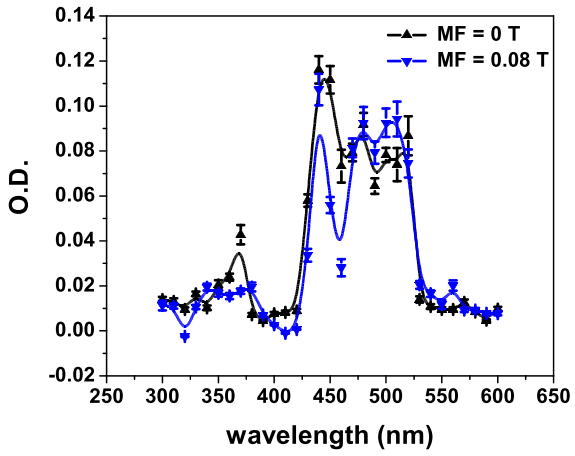


Fig. 6. Absorption (transient) spectra obtained by laser flash photolysis (λ_{ex} = 355 nm) of 0.2 mM AD + 30 μM HSA in phosphate buffer (pH 7.4) solution (i) without (▲) and (ii) with (▼) MF at a delay of 0.1 μs.

Prominent MFE on PET is observed while studying the interaction of PF^{+} and AY with Trp present in the nanocavities of serum albumins (HSA and BSA) since the inter-radical distance within primary geminate RIP is ~1.2–1.3 nm, which is enough to reduce *J* almost to zero. However, although the effect of MF is appreciably exhibited by AD trapped in the biological nanocavity of HSA, but it is completely absent when AD is present in the protein environment of BSA and β-Lactoglobulin. This has been rationalized by differential modes of approach of AD towards the tryptophan residues within these three proteins. Amalgamation of all these reports discussed in the review points towards the fact that LFP in combination with a weak external MF may be used as a 'spectroscopic ruler' to assess intermediate space within the partners of the geminate radical ion pairs.

Acknowledgement

This work was financially supported by the Chemical and Biophysical Approaches for Understanding Natural Processes (CBAUNP) and Biomolecular Assembly, Recognition and Dynamics (BARD) projects, SINP of Department of Atomic Energy (DAE), Government of India.

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