5-Fluorotryptophan as dual probe for ground-state heterogeneity and excited-state dynamics in apoflavodoxin

Visser, N. V.; Westphal, A. H.; Nабuurs, S. M.; van Hoek, A.; Mierlo, C. P. M. van; Visser, A. J. W. G.; Broos, Jaap; van Amerongen, H.

Published in:
FEBS Letters

DOI:
10.1016/j.febslet.2009.07.022

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 02-04-2017
5-Fluorotryptophan as dual probe for ground-state heterogeneity and excited-state dynamics in apoflavodoxin

N.V. Visser a, A.H. Westphal b, S.M. Nabuurs b, A. van Hoek a, C.P.M. van Mierlo b, A.J.W.G. Visser c, J. Broos d, H. van Amerongen a*

aLaboratory of Biophysics, Microspectroscopy Centre, Wageningen University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands
bLaboratory of Biochemistry, Microspectroscopy Centre, Wageningen University, P.O. Box 8128, 6700 ET Wageningen, The Netherlands
cDepartment of Physics, University of Strathclyde, Scottish Universities Physics Alliance, Photophysics Group, Glasgow G4 0NG, United Kingdom
dDepartment of Biophysical Chemistry and Groningen Biomolecular Science and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

A R T I C L E I N F O

Article history:
Received 16 June 2009
Revised 13 July 2009
Accepted 13 July 2009
Available online 18 July 2009

Edited by Richard Cogdell

Keywords:
Fluorescence anisotropy
19F NMR
Picosecond fluorescence
Protein folding
Protein stability

A B S T R A C T

The apoflavodoxin protein from Azotobacter vinelandii harboring three tryptophan (Trp) residues, was biosynthetically labeled with 5-fluorotryptophan (5-FTrp). 5-FTrp has the advantage that chemical differences in its microenvironment can be sensitively visualized via 19F NMR. Moreover, it shows simpler fluorescence decay kinetics. The occurrence of FRET was earlier observed via the fluorescence anisotropy decay of WT apoflavodoxin and the anisotropy decay parameters are in excellent agreement with distances between and relative orientations of all Trp residues. The anisotropy decay in 5-FTrp apoflavodoxin demonstrates that the distances and orientations are identical for this protein. This work demonstrates the added value of replacing Trp by 5-FTrp to study structural features of proteins via 19F NMR and fluorescence spectroscopy.

1. Introduction

The recent discovery of more homogenous fluorescence decay of single 5-fluorotryptophan-containing proteins as compared to single tryptophan-containing ones has considerably simplified interpretation of excited-state kinetics of tryptophan [1]. Experiment and theory show that photo-induced electron transfer to a nearby amide group in the peptide bond is responsible for lifetime heterogeneity. Photo-induced electron transfer is largely suppressed by fluoro-substitution at the 5th position of the indole of tryptophan [2]. This decrease in electron transfer rate is due to a higher ionization potential of the fluoro-indole residue compared to the one of the indole of tryptophan. Consequently, compared to tryptophan, the fluorescence lifetime of 5-fluorotryptophan is longer and the corresponding fluorescence decay kinetics is much more homogenous.

Since the fluorine nucleus has a magnetic moment, 19F NMR spectroscopy can be used to obtain information about the chemical environment of 5-fluorotryptophan (5-FTrp) in proteins. By using both NMR and time-resolved fluorescence techniques one should in principle be able to obtain detailed information on ground-
rapidly migrates to Trp128, which acts as energy sink and is as a result the main emitting fluorophore of apoflavodoxin.

In this paper we compare the spectroscopic properties of native apoflavodoxin with those of apoflavodoxin, in which fluorinated tryptophan residues are incorporated.

2. Materials and methods

2.1. Replacement of tryptophan residues by fluorinated analogs in apoflavodoxin

Replacement of the tryptophans of apoflavodoxin by 5-FTrp analogs is done using an Escherichia coli tryptophan auxotroph system, as previously described [10]. The cells produce flavodoxin in which 5-FTrp analogs instead of tryptophan are incorporated. Flavodoxin is isolated and purified and subsequently apoflavodoxin is obtained through removal of the non-covalently bound FMN. The gene for Azotobacter vinelandii flavodoxin II was excised from the Lac-promoter containing pUC18 vector, using restriction enzymes KpnI and SphI, and ligated into the KpnI/SphI digested pMa plasmid [11]. This vector contains the heat-inducible lambda Pr promoter, allowing strict expression control of flavodoxin. Mass spectrometry showed that the 5-FTrp incorporation efficiency was at least 99%.

2.1.1. 19F NMR measurements

The 19F NMR spectrum has been recorded at 376.5 MHz on a Bruker DPX 400 MHz NMR machine, which is equipped with a 10 mm 19F probe. For chemical shift referencing, trifluoroacetic acid and 4-fluoro-phenylalanine are added to the flavodoxin sample. The NMR sample contains 100 mM potassium pyrophosphate and 10% D2O, pH 6.0.

2.2. Fluorescence measurements

Steady-state fluorescence spectra were obtained with a Horiba Jobin Yvon Fluorolog 3.2.2 spectrofluorometer. All spectra were corrected for wavelength-dependent instrumental response characteristics. Time-resolved polarized fluorescence measurements and associated analysis of total fluorescence intensity decay and fluorescence anisotropy decay are described in detail in [9]. 5-FTrp shows a low intrinsic anisotropy ($r_0 = 0.13$) when excited at 300 nm [2] and therefore an excitation wavelength of 308 nm ($r_0 = 0.26$) was chosen.

3. Results and discussion

To verify whether uniform 19F-labeling of the tryptophans of flavodoxin is achieved, a 19F NMR spectrum of flavodoxin has been acquired (Fig. 1). The 19F NMR spectrum clearly shows that indeed all three tryptophans of flavodoxin have been replaced by 5-FTrp in 5-FTrp flavodoxin, as three distinct 19F NMR resonances of equal amplitude are observed. These three distinct NMR resonances also show that the chemical environments of the three 5-FTrp residues differ in flavodoxin.

The fluorescence excitation and emission spectra of 5-FTrp apoflavodoxin are red-shifted as compared to the ones of native apoflavodoxin (Fig. 2). A similar observation is made for the absorption and fluorescence spectra of N-acetyl-l-tryptophanamide and N-acetyl-dl-5-fluorotryptophanamide in different solvents, particularly dioxane [2]. The more red-shifted excitation (absorption) spectrum of 5-FTrp compared to the one of tryptophan allows excitation of 5-FTrp at wavelengths between 305 and 310 nm, where tryptophan hardly absorbs light.

Time-resolved fluorescence properties of 5-FTrp apoflavodoxin are determined in this study using excitation at 308 nm and detection at 363 nm. Total fluorescence decay of native 5-FTrp apoflavodoxin turns out to be mono-exponential, with a fluorescence lifetime of 4.14 ns (Fig. 3A). In contrast, in case of non-fluorinated native apoflavodoxin the measured fluorescence decay is slightly heterogeneous and consists of a short lifetime component of 1.34 ns (7% amplitude) and a predominant long lifetime component of 4.18 ns (93%), leading to an average fluorescence lifetime of 4.0 ns (see Supporting information). Time-resolved fluorescence anisotropy of native 5-FTrp apoflavodoxin shows rapid decay on the picosecond time scale followed by a much slower decay process on the nanosecond time scale (as well as depolarization due to the rotational correlation time of the protein of 10.4 ns), similar to as detected for native apoflavodoxin [9] (Fig. 3B). The 60-ns correlation time observed is due to resonance energy transfer from 5-FTrp167 to 5-FTrp128 and matches, within the 30-ns error, the 50-ns correlation time detected for native apoflavodoxin [9]. The 3.3-ns transfer correlation time observed is due to resonance energy
we experimentally observe that energy transfer from 5-FTrp74 to 5-FTrp167 is 2.09 times faster than energy transfer from Trp74 to Trp167 (i.e., 0.303 ns$^{-1}$ versus 0.145 ns$^{-1}$).

The data presented here show that replacement of tryptophan residues in a protein by their 5-fluoroTrp analogs preserves the three-dimensional structure of the protein involved. This conclusion is drawn because native apoflavodoxin contains three tryptophans that have specific relative positions and orientations in the hydrophobic core of the molecule. These positions and orientations must remain unaltered upon incorporation of 5-fluoroTrp analogs to explain the time-resolved fluorescence data obtained. Far-UV CD spectra of flavodoxin and 5-FTrp flavodoxin are comparable (see Supporting information) and thus secondary structure content of both proteins is similar. The 5-FTrp containing protein is isolated and purified in its holol form, which is yellow due to non-covalent binding of FMN. Tight binding of FMN to 5-FTrp apoflavodoxin reveals additional proof for preservation of the three-dimensional structure of apoflavodoxin upon replacement of tryptophan residues by their 5-FTrp analogs. Firm binding of FMN occurs primarily through a very specific combination and geometry of hydrogen bonds and aromatic interactions of FMN with apoflavodoxin, implying that the three-dimensional structures of apoflavodoxin and 5-FTrp apoflavodoxin must be similar. The incorporation of 5-FTrp analogs has the advantage that now both NMR and fluorescence spectroscopy can be used to extract information about the submolecular properties of a protein molecule. In case of apoflavodoxin, although the chemical environment of the three tryptophan residues in apoflavodoxin is shown to be different, the corresponding fluorescence decay of the protein turns out to be mono-exponential. This observation supports that indeed excitation energy is transferred in a radiationless manner to the excited state of 5-FTrp128, which subsequently emits detectable fluorescence.

In case of single-tryptophan containing protein molecules, the combination of NMR and fluorescence spectroscopy enables one to directly correlate the signature of the fluorescence lifetime to the chemical environment of the particular tryptophan residue involved. The use of this dual, non-perturbing probe also holds great promise for studies of protein stability and protein folding as recently reviewed for 19F NMR applications [12].

**Acknowledgments**

The Netherlands Organization for Scientific Research supported this work. We thank Hortense Mazon (Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands) for mass spectroscopy measurements. The receipt of a Scottish Universities Physics Alliance distinguished fellowship (to A.J.W.G.V.) is gratefully acknowledged.

**Appendix A. Supplementary data**


**References**


