12-1995

Synthesis and Photophysics of the Optical Probe N1-Methyl-7-azatryptophan

R. L. Rich
Iowa State University

Alexandre V. Smirnov
Iowa State University

A. W. Schwabacher
Iowa State University

Jacob W. Petrich
Iowa State University, jwp@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/chem_pubs
Part of the Chemistry Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/chem_pubs/683. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.
Synthesis and Photophysics of the Optical Probe
N1-Methyl-7-azatryptophan

Abstract
The development of a new intrinsic optical probe of protein structure and dynamics, N1-methyl-7-azatryptophan (1M7AT), is reported. The utility of this nonnatural amino acid derivative lies in its single-exponential, long-lived fluorescence decay (21.7 ± 0.4 ns) and in its high fluorescence quantum yield (0.53 ± 0.07). Its absorption and emission maxima are red-shifted 10 and 65 nm, respectively, from those of tryptophan. These characteristics permit its unambiguous detection with unprecedented discrimination against emission from multiply occurring native tryptophan residues. In a mixture of these two amino acids, no tryptophan signal is detected until the tryptophan: N1-methyl-7-azatryptophan ratio exceeds 75:1. Consequently, N1-methyl-7-azatryptophans ideal for studying the interactions of small peptides containing it with large proteins.

Keywords
1 methyl 7 azatryptophan, amino acid derivative, tryptophan, tryptophan derivative, drug synthesis, fluorescence, molecular dynamics, photochemistry

Disciplines
Chemistry

Comments
Synthesis and Photophysics of the Optical Probe \(N_1\)-Methyl-7-azatryptophan

R. L. Rich, A. V. Smirnov, A. W. Schwabacher, and J. W. Petrich*

Contribution from the Department of Chemistry, Iowa State University, Ames, Iowa 50011

Received June 16, 1995*

Abstract: The development of a new intrinsic optical probe of protein structure and dynamics, \(N_1\)-methyl-7-azatryptophan (1M7AT), is reported. The utility of this nonnatural amino acid derivative lies in its single-exponential, long-lived fluorescence decay (21.7 ± 0.4 ns) and in its high fluorescence quantum yield (0.53 ± 0.07). Its absorption and emission maxima are red-shifted 10 and 65 nm, respectively, from those of tryptophan. These characteristics permit its unambiguous detection with unprecedented discrimination against emission from multiply occurring native tryptophan residues. In a mixture of these two amino acids, no tryptophan signal is detected until the tryptophan: \(N_1\)-methyl-7-azatryptophan ratio exceeds 75:1. Consequently, \(N_1\)-methyl-7-azatryptophan is ideal for studying the interactions of small peptides containing it with large proteins.

Introduction

The difficulties in using tryptophan as an optical probe of protein structure and dynamics are well known. Tryptophan has an intrinsic nonexponential fluorescence decay, and it occurs multiply in most proteins of consequence. We have devoted considerable effort to the development and characterization of the nonnatural amino acid 7-azatryptophan as an alternative optical probe.\(^{8,19}\) and other groups have subsequently begun to exploit its properties.\(^{20,21}\) 7-Azatryptophan has a single-exponential fluorescence decay (780 ps in water, pH 7 and 20 °C), and the locations of its absorption and emission maxima permit it to be detected unambiguously in the presence of up to 10 tryptophan residues.\(^{8,11,16}\) Furthermore, it is amenable to peptide synthesis and can be incorporated into bacterial protein.\(^{8,11}\) These qualities are extremely useful, especially when short-time dynamics are of interest and when there are a limited number of tryptophans present. We have, however, demonstrated in a recent study of biotinylated 7-azatryptophan in complex with avidin that the relatively low fluorescence quantum yield of 7-azatryptophan (0.03 in water, pH 7) can diminish its utility when long-time dynamics are of interest.\(^{19}\)

In order to address problems where long-time dynamics are of interest and many tryptophan residues are present, it is necessary that the optical probe have both a long-lived excited state and a high fluorescence quantum yield. We have already suggested that \(N_1\)-methyl-7-azatryptophan (Figure 1) conforms to these requirements\(^{19}\) on the basis of our understanding of the photophysics of the 7-azaindole chromophore.\(^{9-19}\) The most significant nonradiative properties of 7-azaindole (in particular, those that distinguish it from indole) are determined by the \(N_1\) proton and its interactions with the solvent. In alcohols\(^{9,15,17,22,23}\) (and to a small degree in water)\(^{13,24-26}\) this proton participates in an excited-state double-proton transfer. Internal conversion promoted by the interaction of this proton with the solvent has also been suggested.\(^{27}\) The importance of the \(N_1\) proton in the nonradiative process of 7-azaindole is demonstrated most vividly by the methylation of \(N_1\): in water, the fluorescence lifetime and quantum yield increase from 910 ps and 0.03 to 21.0 ns and 0.53, respectively.

\[\text{Figure 1. Structures of (a) tryptophan, (b) 7-azatryptophan, and (c) } N_1\text{-methyl-7-azatryptophan.}\]

---

*To whom correspondence should be addressed.

\(^{1}\) Abstract published in Advance ACS Abstracts, November 15, 1995.


\(^{22}\) Avouris, P.; Yang, L. L.; El-Bayoumi, M. A. Photochem. Photobiol. 1976, 24, 211.


0002-7863/95/1517-11850$09.00/0 © 1995 American Chemical Society
Synthesis of Peptides. Synthesis of peptides containing 7-azatryptophan (7AT) or N1-methyl-7-azatryptophan (1MTAT) was performed as described elsewhere, and the purity of the peptides was verified by HPLC. The peptide sequences listed in Table 1 employ the standard one-letter abbreviations of the natural amino acid residues and our chosen abbreviations for the optical probes.

**Spectroscopic Measurements.** Fluorescence lifetimes were obtained by means of time-correlated single-photon counting. Owing to the long fluorescence lifetime (Table 1) of N1-methyl-7-azatryptophan, a 50-ns full-scale time window was required to characterize it properly. The fluorescence decays of mixtures of one N1-methyl-7-azatryptophan to varying amounts of tryptophan were measured in order to determine what level of tryptophyl fluorescence produces an appreciable background signal. These measurements were performed on a 3-ns time scale in order to be more sensitive to onset of the tryptophyl fluorescence lifetime, especially its subnanosecond component (Table 2).

The fluorescence decays of the mixtures were adequately fit to one or a sum of two exponentially decaying components: \( F(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \), where \( A_1 + A_2 = 1.00 \). Two exponentials are required that the entire 7-azaindole population in water is capable of tautomerization.

<table>
<thead>
<tr>
<th>Table 1. Summary of Photophysical Data</th>
<th>( \lambda_{em}^{max} ) (nm)</th>
<th>( \lambda_{em}^{max} ) (nm)</th>
<th>( \epsilon^0 ) (M(^{-1}) cm(^{-1}))</th>
<th>( \phi_f )</th>
<th>( \tau_1 ) (ns)</th>
<th>( \tau_2 ) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tryptophan</td>
<td>2805</td>
<td>35113</td>
<td>540013</td>
<td>0.1803</td>
<td>0.22 ± 0.01</td>
<td>0.620 ± 0.050</td>
</tr>
<tr>
<td>7-azatryptophan (7AT)</td>
<td>28813</td>
<td>397113</td>
<td>620011</td>
<td>0.03 ± 0.01</td>
<td>1.00</td>
<td>0.780 ± 0.01013</td>
</tr>
<tr>
<td>N-ac(7AT)NHNH$_2$</td>
<td>28931</td>
<td>397113</td>
<td>62001</td>
<td>0.02 ± 0.01</td>
<td>1.00</td>
<td>0.830 ± 0.01013</td>
</tr>
<tr>
<td>N-ac-KACP(7AT)NCD-NH$_2$</td>
<td>28931</td>
<td>396</td>
<td>62001</td>
<td>0.03 ± 0.01</td>
<td>0.84 ± 0.04</td>
<td>0.850 ± 0.01013</td>
</tr>
<tr>
<td>N-Boc-N1-methyl-7-azatryptophan</td>
<td>28941</td>
<td>830011</td>
<td>830011</td>
<td>0.47 ± 0.02</td>
<td>1.00</td>
<td>1.61 ± 1.10</td>
</tr>
<tr>
<td>N1-methyl-7-azatryptophan (1MTAT)</td>
<td>28940</td>
<td>830013</td>
<td>830013</td>
<td>0.53 ± 0.07</td>
<td>1.00</td>
<td>27 ± 0.4</td>
</tr>
<tr>
<td>SIH(1MTAT)EKL</td>
<td>28940</td>
<td>83001</td>
<td>83001</td>
<td>0.52 ± 0.06</td>
<td>1.00</td>
<td>16 ± 0.3</td>
</tr>
</tbody>
</table>

* Zwitterionic forms of all amino acids, measured at 20 °C. Naturally occurring amino acids are abbreviated using the standard nomenclature.

* Fluorescence lifetimes fit to a double exponential.

* Charge transfer to the side chain is generally considered to be the major nonradiative process in tryptophan, and different charge transfer rates owing to a distribution of ground-state conformers is attributed to the origin of the nonexponential fluorescence decay in tryptophan. Although 7-azaindole is capable of photolization, we have argued that charge transfer to the side chain is not operative in 7-azatryptophan because it is not thermodynamically favorable. This argument is confirmed by the absence of nonexponential decay in the 7-azatryptophan tripeptide (Table 1) and its presence in all tryptophan-containing tripeptides.

(24) Chapman, C. F.; Maroncelli, M. J. Phys. Chem. 1992, 96, 8430. Chapman and Maroncelli, however, present an alternative explanation requiring that the entire 7-azaindole population in water is capable of tautomerization.


temperature (-21°C). Fluoromax fluorimeter. All measurements were conducted at room temperature (20°C).

**Results and Discussion**

The absorption and emission spectra of N1-methyl-7-azatryptophan and tryptophan are shown in Figure 2. Table 1 summarizes the steady-state and the time-resolved data. Because of the shift in the absorption and emission spectra of N1-methyl-7-azatryptophan with respect to tryptophan, and because of the very large fluorescence quantum yield of N1-methyl-7-azatryptophan (0.53 ± 0.07), it is expected that its fluorescence decay can be uniquely detected in the presence of many background tryptophan residues. A comparison of the fluorescence decays of N1-methyl-7-azatryptophan, tryptophan, and 7-azatryptophan is given in Figure 3. To illustrate the usefulness of N1-methyl-7-azatryptophan as an optical probe in an environment containing multiple tryptophans, we measured the fluorescence decay lifetime of the mixture of these two amino acids. The fluorescence decay profile of the mixture exactly overlaid that of N1-methyl-7-azatryptophan up to a 75-fold excess of tryptophan, where the average fluorescence lifetime begins to become perceptibly shorter. The tryptophyl contribution to the fluorescence decay becomes much more apparent as the probe:tryptophan ratio approaches 1:200 (Table 2, Figure 4). Few, if any, naturally occurring biological systems contain this many tryptophans; clearly, emission from our optical probe would be unambiguously observed when incorporated into proteins. Finally, the single-exponential fluorescence decay of N1-methyl-7-azatryptophan permits a simplified interpretation of time-resolved data. Any change in its fluorescence decay can be directly attributed to its environment.

**Sensitivity to the Environment of the Fluorescence Properties of the Chromophores 7-Azaindole and N1-Methyl-7-Azaindole.** Unlike the photophysics of tryptophan where blue shifts or red shifts of the fluorescence spectrum can only be crudely interpreted in terms of, respectively, nonpolar or polar environments and where nonexponential decay can only be rationalized by a sweeping invocation of conformational heterogeneity, the delicate nature of the photophysics of 7-azaindole provides the possibility of gaining much more detailed and specific information on the environment of the chromophore. This is because the photophysics of 7-azaindole can only be satisfactorily explained by understanding in microscopic detail its interactions with its solvation environment: a continuum picture of the environment is not sufficient.

It has been suggested that if the 7-azaindole chromophore is located in a hydrophobic pocket, it will exhibit a long...
However, sufficiently different from that of tryptophan to provide a significant contrast in mixtures. In our work with 7-azatryptophan, we have improved the optical selectivity of the probe by methylating N1-methyltryptophanamide (NATA) because it is known to have an anomalous single-exponential lifetime of 3-ns duration. This lifetime component is expected to stand out in starkest contrast against the 21-ns lifetime of 7-azatryptophan. In the measurements displayed in this figure and reported in Table 2, tryptophan itself affords the more rigorous test of one of the primary nonradiative processes in 7-azaindole is nonradiative excited-state proton transfer, as observed in dimers,31 in alcohols,9,15,17,22,23 and to a much lesser extent in water.13,24-26 

Table 1 provides a comparison of the behavior of 7-azaindole and N1-methyl-7-azaindole as fluorescent probes in systems of varying complexity. While 7-azatryptophan and the tripeptide NAc-Pro-7-AzaTrp-Asn-NH2 exhibit single-exponential fluorescence decay, the octapeptide NAc-Lys-Ala-Cys-Pro-7-AzaTrp-Asn-Cys-Asp-NH2 provides a nonexponential fluorescence decay. Clearly, the nonexponential fluorescence decay in the octapeptide must be induced directly by the amino acid side chain residues or indirectly by their ability to reorganize water about the chromophore.25 On the other hand, both N1-methyl-7-azatryptophan and the octapeptide Ser-Ile-Ile-Asn-(1M7AT)-Glu-Lys-Leu display single-exponential fluorescence decay because of the absence of the N1 proton. Although methylation of N1 in 7-azaindole eliminates excited-state proton transfer as a nonradiative process, it should not be assumed that the fluorescence properties of N1-methyl-7-azaindole or of 1M7AT are insensitive to the environment. For example, the fluorescence lifetime of N1-methyl-7-azaindole increases by a factor of ~6 in going from cyclohexane to D2O as a solvent.26

Conclusions

Previously we have shown that 7-azatryptophan can be uniquely detected in an environment of up to a 10-fold tryptophan excess. We have improved the optical selectivity of the probe by methylating N1. N1-Methyl-7-azatryptophan (1M7AT) has numerous advantages over other intrinsic fluorescent probes currently in use. It has red-shifted absorption and emission spectra with respect to those of tryptophan and a very high fluorescence quantum yield. This latter feature allows for a shorter data collection time and analysis of smaller or more dilute sample volumes. This probe is characterized by a single long-lived monoeponential fluorescence decay that is also clearly distinguishable from that of tryptophan. In addition, 1M7AT is amenable to incorporation into peptide sequences. These combined factors allow for unambiguous detection of the probe signal in biological systems where site-specific analyses are expected to be difficult, if not impossible, owing to an overwhelming tryptophan content. The immediate and most powerful use of 1M7AT will be to incorporate it into small peptides of known biological interest and to study the interactions of these tagged peptides with larger proteins.

JA951963E