Defining the Core Structure of the α-Lactalbumin Molten Globule State

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Molten globules are partially folded states of proteins which are generally believed to mimic structures formed during the folding process. In order to determine the minimal requirements for the formation of a molten globule state, we have prepared a set of peptide models of the molten globule state of human α-lactalbumin (αLA). A peptide consisting of residues 1-38 crosslinked, via the native 28-111 disulfide bond, to a peptide corresponding to residues 95-120 forms a partially folded state at pH 2.8 which has all of the characteristics of the molten globule state of αLA as judged by near and far UV CD, fluorescence, ANS binding and urea denaturation experiments. The structure of the peptide construct is the same at pH 7.0. Deletion of residues 95-100 from the construct has little effect. Thus, less than half the sequence is required to form a molten globule. Further truncation corresponding to the selective deletion of the A (residues 1-19) or D (residues 101-110) helices or the C-terminal 310 helix (residues 112-120) leads to a significant loss of structure. The loss of structure which results from the deletion of any of these three regions is much greater than that which would be expected based upon the non-cooperative loss of local helical structure. Deletion of residues corresponding to the region of the D helix or C-terminal 3_10 helix region results in a peptide construct which is largely unfolded and contains no more helical structure than is expected from the sum of the helicity of the two reduced peptides. These experiments have defined the minimum core structure of the αLA molten globule state.

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Introduction

Elucidation of the structure and the interactions which stabilize molten globule states is a key issue in protein folding (Barrick & Baldwin, 1993; Christensen & Pain, 1991; Creighton, 1997; Eliezer et al., 1998; Kuwajima, 1996; Ptitsyn, 1995; Raschke & Marqusee, 1997). Unfortunately, their dynamic nature, conformational heterogeneity and sometimes limited solubility make direct structural studies extremely difficult. Protein dissection studies using peptide models have proven to be an effective technique for characterizing the intermediate states of both BPTI and cytochrome c (Oas & Kim, 1988; Staley & Kim, 1990; Wu et al., 1993). We have adopted a protein dissection approach which allows, for the first time, the elucidation of the minimum core structure of the molten globule state of αLA.

The molten globule state of αLA has become a paradigm for evaluating the properties of stable partially folded proteins (Kuwajima, 1996; Kuwajima et al., 1989; Ptitsyn, 1995). αLA forms a molten globule state under a variety of conditions, including at low pH and at neutral pH at low salt concentrations in the absence of Ca²⁺ (Creighton & Ewbank, 1994; Dolgikh et al., 1981; Kronman et al., 1965; Kuwajima et al., 1976). The properties of the low pH molten globule form of αLA, known as the A-state, have been the most thoroughly characterized (Alexandrescu et al., 1993; Baum et al., 1989;
The A-state of the intact protein is characterized by the absence of a near UV circular dichroism (CD) signal and a Trp fluorescence emission maximum between the native and fully unfolded emission maxima (Dolgikh et al., 1981; Sommers & Kronman, 1980). In constrast, the A-state contains a significant amount of secondary structure as judged by far UV CD (Dolgikh et al., 1981; Kuwajima et al., 1976). The A-state also binds the fluorescent hydrophobic dye ANS and is relatively compact (Dolgikh et al., 1985; Kronman et al., 1967; Kuwajima et al., 1975; Semisotnov et al., 1991).

\( \sigma \)LA is a 123 residue protein comprised of two subdomains, the \( \alpha \)- and \( \beta \)-domains. The \( \alpha \)-domain is comprised of four \( \alpha \)-helices denoted A through D and one small region of \( 3_{10} \) helix near the C terminus (Figure 1). The \( \beta \)-domain, consisting of the remainder of the protein, contains three short \( \beta \)-strands and some loops and coils. There are two hydrophobic cores within \( \sigma \)LA. One consists of residues from the A helix, the B helix and the C-terminal \( 3_{10} \) helix and is contained within the \( \alpha \)-domain. The other, known as the hydrophobic box, is made up of residues from the C and D helices of the \( \alpha \)-domain and residues from the \( \beta \)-domain. There is good evidence that the \( \alpha \)-domain is structured in the molten globule state and that the \( \beta \)-domain is less ordered (Baum et al., 1989; Peng et al., 1995; Wu et al., 1995). Kim and co-workers have shown that an 82 residue construct consisting of the entire \( \alpha \)-domain is able to fold into a molten globule-like state (Peng & Kim, 1994). It is still unclear what constitutes the minimum structured core of the \( \sigma \)LA molten globule and what are the crucial interactions that are responsible for stabilizing this structured core. Here we describe studies of a set of peptide models derived from the \( \alpha \)-domain which address these important questions.

Peptides corresponding to the sequences of the isolated A, B and C helices have been shown to be largely unstructured in isolation in aqueous solution (Demarest et al., 1998; Kuhlman et al., 1997; Shimizu et al., 1996), while peptides containing the D and C-terminal \( 3_{10} \) helices have shown some propensity to adopt non-native residual structure (Demarest et al., 1998, 1999; Smith et al., 1995). These experiments have demonstrated that formation of the molten globule is not due to usually stable elements of secondary structure. Clearly longer range interactions are required. Our peptide models allow us to define these interactions, and to determine the minimum sequence necessary to form the \( \sigma \)LA molten globule state.

Not all proteins form stable molten globule states or fold through molten globule intermediates and there has been considerable speculation about which features of proteins dictate the ability to form a molten globule. The peptide constructs described here are considerably smaller than many proteins which fold by a two state process. Thus, comparison of our construct with other proteins of similar size should help elucidate the sequence requirements for molten globule formation. In particular, it is of interest to determine if the ability to form a stable molten globule state is due to the total number and/or size of the hydrophobic residues or if more subtle effects are responsible.

**Results**

**A peptide model of the molten globule state**

We have prepared a 64 residue peptide which corresponds to the primary sequence of the A, B and D \( \alpha \)-helices and the C-terminal \( 3_{10} \) helix of human \( \sigma \)LA. This model system, denoted AB-D\(_{95-120}\), is comprised of residues 1-38 (AB) crosslinked by the native 28-111 disulfide to residues 95-120 (D\(_{95-120}\)). The construct corresponds to the \( \alpha \)-domain of \( \sigma \)LA with most of the C-helix deleted. Residues 95 to 100 include several positions derived from the C-terminal portion of the C helix. These residues were included since I95 forms part of the hydrophobic box in the native state which also includes residues from the B and D helices. Equilibrium sedimentation experiments with AB-D\(_{95-120}\) were performed at two concentrations, 4 \( \mu \)M and 50 \( \mu \)M, at pH 2.8, 20 °C. The data from the dilute sample were well fit by a single species with an apparent molecular weight which is within the experimental uncertainty of the known monomer molecular weight. The more concentrated sample showed evidence of self-association. CD spectra of AB-D\(_{95-120}\) are also independent of concentration below 10 \( \mu \)M peptide concentrations. Consequently, all subsequent experiments were performed at 4 \( \mu \)M peptide.

AB-D\(_{95-120}\) forms a highly helical molten globule state. The helicity, as judged by CD, of the crosslinked peptide is much greater than the sum of the helicity of the reduced peptides (Figure 2(a)). The residual CD signal from the reduced peptides is primarily due to structure present in the D\(_{95-120}\) fragment (Demarest et al., 1998). Formation of the

![Figure 1. Ribbon diagram of the crystal structure of human \( \sigma \)LA created using MOLSCRIPT (Acharya et al., 1991; Kraulis, 1991). The \( \alpha \)-domain is shown in black and the \( \beta \)-domain is displayed in grey. The 28-111 disulfide bond is illustrated. The N terminus is indicated and the A, B, C, D and \( 3_{10} \) helices are labeled.](image-url)
heterodimer results in a threefold increase in \( \theta_{222} \) from \(-5200 \) to \(-14,500 \ \text{deg cm}^2 \text{dmol}^{-1} \). This value of \( \theta_{222} \) could result from 29 of the 64 residues occupying a fully helical conformation. The helical content of our peptide accounts for all the helicity observed in the \( \alpha \)-domain model of Kim and co-workers, strongly suggesting that the extra 18 residues in their construct are unstructured (Peng & Kim, 1994). One of the classical spectroscopic signatures of the molten globule state of \( \alpha \)-LA is the absence of a near UV CD signal. The near UV CD spectrum of AB-D\(_{95-120}\) at pH 7.0 is also virtually identical to the spectrum of AB-D\(_{95-120}\) at pH 2.8. These experiments demonstrate that AB-D\(_{95-120}\) also forms a molten globule-like state at pH 7.0.

Truncation mutants define the critical core required to form the molten globule

We have prepared four additional peptides which correspond to deletion variants of AB-D\(_{95-120}\). Removal of residues 95 to 100, resulting in a construct denoted as AB-D\(_{101-120}\), has little effect on the structure as judged by CD. The value of \( \theta_{222} \) decreases from \(-14,500 \) to \(-13,600 \ \text{deg cm}^2 \text{dmol}^{-1} \) (Figure 4(a)). The Trp fluorescence emission maximum and the ability of the construct to bind ANS are also unaffected by removal of residues 95 to
100. Residues 95 to 100 were originally included since they form part of one of the hydrophobic cores in the native state and pack against the B helix. The truncation experiment indicates that they are not critical for formation of the molten globule. Further truncation of this 58 residue construct results in a dramatic loss of structure. A peptide corresponding to residues 1-38 crosslinked to residues 101-111, designated AB-D 101-111, lacks the C-terminal 3_10 helix and is only slightly structured \( \theta_{222} = 4000 \text{ deg cm}^2 \text{ dmol}^{-1} \). A third peptide consisting of residues 1-38 crosslinked to residues 111-120, denoted AB-D 111-120, is missing the D helix and is also largely unstructured \( \theta_{222} = -4200 \text{ deg cm}^2 \text{ dmol}^{-1} \). The \( \theta_{222} \) values of both AB-D 101-111 and AB-D 111-120 are no greater than the sum of the ellipticity of the corresponding reduced peptides (Figure 4(b)). This clearly demonstrates that the regions corresponding to the D helix and the C-terminal 3_10 helix are required to stabilize the structure observed in AB-D 95-120 and AB-D 101-120. Removal of residues 1 through 19, which includes the A helix, also results in a significant decrease in structure. A peptide consisting of residues 20-36, denoted the B peptide, crosslinked to D95-120 has a value of \( \theta_{222} \) equal to \(-7700 \text{ deg cm}^2 \text{ dmol}^{-1} \). This is only 50% larger than the value expected from the sum of the reduced peptides, \(-5000 \text{ deg cm}^2 \text{ dmol}^{-1} \). A peptide corresponding to the isolated A helix is slightly structured (less than 10% helical), but the helical content of this peptide cannot account for the large difference in helicity between the B-D95-120 pair and AB-D95-120 (Figure 4(a)). Thus the sequence encompassing the A helix must provide some stabilizing interactions that promote further structure formation in the molten globule state. Overall, the truncation experiments demonstrate that a 58 residue fragment, less than half of the protein, is sufficient to form a molten globule state.

Table 1. Comparison of CD, fluorescence and ANS binding properties of AB-D 95-120, AB-D 101-120 and the native, molten globule and denatured states of human αLA

<table>
<thead>
<tr>
<th>Protein</th>
<th>( [\theta]_{222} ) (deg cm(^2) dmol(^{-1}))</th>
<th>Trp fluorescence ( \lambda_{\text{max}} ) (nm)</th>
<th>ANS fluorescence ( \lambda_{\text{max}} ) (nm)</th>
<th>( c_M ) (M urea)</th>
<th>Apparent ( m ) value (cal M(^{-1}) mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native αLA (pH 7.0, 10 mM Ca(^{2+}))</td>
<td>-9300</td>
<td>332</td>
<td>( \sim 522 )</td>
<td>7.0 ± 0.5</td>
<td>1260 ± 70</td>
</tr>
<tr>
<td>Molten globule αLA (pH 2.8, 1 mM EDTA)</td>
<td>-12,000</td>
<td>342</td>
<td>470</td>
<td>5.8 ± 0.6</td>
<td>590 ± 40</td>
</tr>
<tr>
<td>Denatured αLA (pH 2.8, 10 M urea)</td>
<td>-3500</td>
<td>356</td>
<td>( \sim 525 )</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AB-D 95-120 (pH 2.8)</td>
<td>-14,500</td>
<td>345</td>
<td>480</td>
<td>3.5 ± 0.6</td>
<td>570 ± 60</td>
</tr>
<tr>
<td>AB-D 101-120 (pH 2.8)</td>
<td>-13,600</td>
<td>346</td>
<td>480</td>
<td>2.7 ± 0.6</td>
<td>570 ± 60</td>
</tr>
</tbody>
</table>

Also included are the \( c_M \) and apparent \( m \) values obtained by fitting the urea-induced unfolding curves.

Figure 4. Effects of truncation on the CD spectra of AB-D 95-120. (a, i) Ribbon diagram of human αLA with the residues from AB-D 95-120 shaded black. The residues which were not included in AB-D 95-120 are lightly shaded. The A, B, D and 3_10 helices are labeled as is the 28 to 111 disulfide. The position of residues 20, 28, 95, 101, 111 and 120 are indicated. (a, ii) CD spectra of AB-D 95-120 (filled triangles), B-D 95-120 (open circles) and AB-D 101-120 (open diamonds). (b, i) Ribbon diagram of human αLA with the residues of AB-D 101-120 shaded. (b, ii) CD spectra of AB-D 101-120 (open diamonds), AB-D 101-111 (open/inverted triangles) and AB-D 111-120 (open triangles).
Many experiments have demonstrated that unfolding of the molten globule state of zLA is non-cooperative (Griko et al., 1994; Haynie & Freire, 1993; Schulman & Kim, 1996; Schulman et al., 1997; Shimizu et al., 1993). The molten globule of zLA does display a sigmoidal loss in CD signal upon chemical denaturation, even though the transition is non-cooperative (Uchiyama et al., 1995). Kuwajima and co-workers have used the midpoint of the urea transition, \( c_M \), as a rough measure of the apparent stability of the molten globule states formed by variants of zLA (Uchiyama et al., 1995). The \( c_M \) of all the model peptides is below that of the molten globule of zLA (Figure 5, Table 1). The apparent stability of the largest peptide model AB-D95-120 approaches the apparent stability of the molten globule state of zLA most closely. Deletion of residues 95-100 affects the apparent stability only marginally. However, deletion of the A, D or C-terminal 3 \( \alpha \) regions decreases the apparent stability so drastically that no pretransition is observed. These results provide excellent additional evidence that the A, D, and C-terminal 3 \( \alpha \) regions are all important for forming the molten globule. Deletion of residues 95 to 100 strongly suggest that the integrity of the hydrophobic box is not important for formation of the molten globule. These observations are entirely consistent with recent mutagenesis studies, which have shown that mutation of I95 has no significant effect on the molten globule state (Song et al., 1998; Wu & Kim, 1998).

At first glance, the truncation experiments appear to be in contrast to the studies of Kim and Schulman (Schulman & Kim, 1996). In fact, our studies are complementary to their experiments, and there is no inherent contradiction. In their experiments, proline mutants were made in each of the helices of the \( \alpha \)-domain. Substitution with a proline decreased the overall helicity of the molten globule by the amount expected for the removal of the individual helix (except for proline insertion into the C helix, which had no significant effect on the helicity). In that sense, the structure can be viewed to be formed non-cooperatively. However, disruption of each helix does not necessarily eliminate all of the potential stabilizing interactions involving that region of the protein. In particular, non-specific interactions are still likely. Complete removal of each helical region, as in our truncation experiments, is a more drastic probe and eliminates any possibility that the region could interact with the remainder of the protein. The truncation experiments show that a striking loss of structure results from the deletion of segments corresponding to individual regions of secondary structure. The loss of helical structure is considerably greater than would be expected if removal of the fragment did not perturb the structure of the remainder of the protein. In this sense, mutually stabilizing interactions between the helical elements are important for the stability of the molten globule. Taken in combination, the work reported here together with the earlier studies of Kim and co-workers helps to provide a unified view of the interactions which stabilize the molten globule state (Schulman &

**Figure 5.** Urea unfolding curves of AB-D91-111 (inverted triangles), B-D95-120 (squares), AB-D101-120 (triangles), AB-D95-120 (diamonds) and human zLA (circles) at pH 2.8, 20 °C.

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**Conclusions**

Near and far UV CD, fluorescence, ANS binding and urea denaturation experiments clearly demonstrate that AB-D95-120 forms a molten globule-like state with spectroscopic properties very similar to the A-state of zLA. Our truncation experiments show that the A, B, D and C-terminal 3 \( \alpha \) helix regions are all important for forming the molten globule. Deletion of residues 95 to 100 has only a very minimal effect upon the structure and stability of the peptide construct. Residues from this region of the protein chain, in particular I95, contribute to the hydrophobic box in the native state. The very minor effects observed upon deleting residues 95 to 100 strongly suggest that the integrity of the hydrophobic box is not important for formation of the molten globule. These observations are entirely consistent with recent mutagenesis studies, which have shown that mutation of I95 has no significant effect on the molten globule state (Song et al., 1998; Wu & Kim, 1998).
Kim, 1996). The proline mutant experiments show that interactions between fully formed helices are not required, while our truncation experiments demonstrate that interactions involving these regions of the protein must be present to form the molten globule state.

Our studies have also demonstrated an important role for residues contained within the region corresponding to the D helix (residues 101-110). Mutations in the A helix and the C-terminal 3\_10 helix have previously been shown to perturb the stability of the molten globule as have mutations near Cys28 (Song et al., 1998; Uchiyama et al., 1994; Wu & Kim, 1998). These results have been interpreted to indicate that the A and B helices as well as the C-terminal 3\_10 helix form a stable core structure. Our experiments with the truncation constructs are consistent with an important role for these regions, but in addition indicate that the D helix region makes a crucial contribution to the stability of the zLA molten globule state.

What is the effect of the remainder of the protein chain on the core region containing the A, B, D and C-terminal 3\_10 helices? The A-state of human zLA has a \( \theta_{222} \) value of \(-12,000\) deg cm\(^2\) dmol\(^{-1}\). This corresponds to roughly 34% helicity or nominally 42 residues in a helical conformation. The helicity of AB-D\(_{95-120}\) or AB-D\(_{101-120}\) does not entirely account for all of this structure. AB-D\(_{95-120}\) and AB-D\(_{101-120}\) are also less resistant to urea denaturation than the molten globule of zLA. These observations suggest that either residual structure in the C helix and/or in the \( \beta \)-subdomain exists in the molten globule of zLA or that the presence of the rest of the protein stabilizes additional structure in AB-D\(_{95-120}\). Alternatively, the extra disulfide bonds absent in our model peptides may stabilize additional structure (Ikeguchi et al., 1992).

An important question in protein folding concerns the key factors that contribute to the formation of a stable equilibrium molten globule state. Comparison of our peptide models with natural proteins that are of approximately the same size and fold in a two-state fashion is particularly interesting in this regard. Nearly all such proteins lacking detectable kinetic or equilibrium stable intermediates have a limited size and most, but not all, lack disulfides (Fersht, 1997; Jackson, 1998; Kuhlman et al., 1998). AB-D\(_{95-120}\) and AB-D\(_{101-120}\) are 64 and 58 residues, respectively. Both are well below the maximum size of proteins that have been shown to fold via a two-state mechanism, thus, size cannot be the sole determining factor. The total number and the percentage of hydrophobic residues found in both AB-D\(_{95-120}\) and AB-D\(_{101-120}\) are not significantly different from what is found in proteins which fold by a two-state process. In this regard it is interesting to note that early \textit{de novo} designed polypeptides often exhibited molten globule-like features and typically are only slightly larger than AB-D\(_{95-120}\) (Betz et al., 1993; Kamtekar et al., 1993). For example, the \( \alpha-4 \) family of designed four helix bundles are approximately 70 residues in length and exhibit at least some of the properties associated with molten globule states (Betz et al., 1993). The family of four helix bundle proteins studied by Hecht and coworkers are comparable in size to \( \alpha-4 \) and many of these proteins appear to be molten globule-like (Kamtekar et al., 1993). These comparisons indicate that more subtle primary sequence effects, likely including the distribution of hydrophobic and hydrophilic residues, may determine the ability to form a molten globule state (Davidson & Sauer, 1994; Raleigh et al., 1995).

**Materials and Methods**

**Peptide synthesis**

Synthesis and purification protocols are described elsewhere (Demarest et al., 1998). Cys residues at positions 6 and 120 were changed to Ala. Oxidation of the reduced peptides was carried out in a 0.2 M Tris(hydroxymethyl)aminomethane buffer at pH 8.5 for 24 hours before quenching with 0.1% HCl on a semi-preparative Vydac C18 reverse phase HPLC column. The time courses of the oxidations were followed using HPLC with a reverse phase C18 analytical column. Initially, two peaks corresponding to the two reduced peptides which were being crosslinked were observed. As time progressed, three peaks corresponding to the desired heterodimer and to the two homodimers grew and the intensity of the peaks due to the reduced peptides diminished. No changes were observed after 24 hours. The identities of the purified peptides were confirmed using MALDI mass spectrometry. The purity of the peptide constructs was judged by analytical HPLC and was greater than 95% in all cases. Human zLA was purchased from Sigma. The sequences of the peptide models were taken from the human zLA sequence listed in the crystallographic study by Acharya, Phillips and coworkers (Acharya et al., 1989).

**Sedimentation equilibrium**

Solutions of AB-D\(_{95-120}\) were dialyzed against a buffer containing 2 mM phosphate, 2 mM borate, 2 mM citrate, and 10 mM NaCl at both pH 2.8 and pH 7.0. All truncation mutants were dialyzed against the same buffer, but only at pH 2.8. Experiments were performed at 20°C with a Beckman XL-A analytical ultracentrifuge, using rotor speeds of 30,000, 40,000, and 50,000 rpm. Analyses were performed at two separate peptide concentrations, 4 \( \mu \)M and 50 \( \mu \)M. Experiments were carried out using 12 mm pathlength, six-channel, charcoal-filled, Epon cells with quartz windows. Data were collected using continuous radial scanning at 219 nm for the 4 \( \mu \)M samples and 288 nm for the 50 \( \mu \)M samples. Partial specific volumes were calculated from the weighted average of the partial specific volumes of the individual amino acids (Cohn & Edsall, 1943). The data were globally fit with both a single species model with the molecular weight treated as a fitting parameter and to monomer-monomer equilibria with the molecular weight of the monomer held fixed. The HID program from the Analytical Ultracentrifugation Facility at the University of Connecticut was used for the fitting analysis.
CD

CD experiments were performed at 20 °C and at peptide/protein concentrations less than or equal to 4 μM on an Aviv Model 62A circular dichroism spectrometer. Unless stated otherwise in the text, all of the experiments were performed at pH 2.8. The same buffer which was used for the sedimentation equilibrium experiments was used for all of the spectroscopic studies. Wavelength scans were performed with a minimum of three repeats and a minimum averaging time of three seconds at each wavelength. Near UV wavelength scans were performed with a minimum of five repeats and a minimum averaging time of five seconds at each wavelength. A cuvette with a 10 cm pathlength and a 20 ml sample volume was used for the near UV wavelength scans. Peptide concentrations were determined from absorbance measurements using the method of Pace et al. (1995). Spectra are displayed as mean residue ellipticity versus wavelength. The fraction helix was calculated using the method of Rohl & Baldwin (1997):

\[
f_{\text{helix}} = \frac{(\theta_{222} - \theta_c)}{(\theta_{198} - \theta_c)}; \quad \theta_c = 2220 - (53T);
\]

\[\theta_c = \left(-44,000 + 250T\right)\left(1 - \frac{3}{N}\right).\]

θc and θ198 are the molar ellipticities for pure coil and pure helix, respectively, T is temperature in °C, and N is the number of amino acids in the peptide. The fraction helix was also calculated using the software package ANTHEPOT (Deleage & Geourjon, 1993) and very similar results were obtained. Urea denaturation curves were fit using standard methods (Pace, 1986; Uchiyama et al., 1995).

Fluorescence measurements

Fluorescence measurements were performed at pH 2.8, 20 °C using an ISSA Fluorolog spectrometer. An excitation wavelength of 279 nm was used for the Trp fluorescence experiments and an excitation wavelength of 370 nm was used for the 1-anilinonaphthalene-8-sulfonate (ANS) measurements. The concentration of the ANS stock solution was determined using a molar absorption coefficient of 7.8 × 10^4 M⁻¹ cm⁻¹ at 372 nm in methanol (provided by Molecular Probes).

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References


Dolgikh, D. A., Abaturov, L. V., Bolotina, I. A., Brazhnikov, E. V., Bychkova, V. E., Bushuev, V. N., Gilmanshin, R. I., Levedev, Y. O., Semisotnov, G. V.,


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