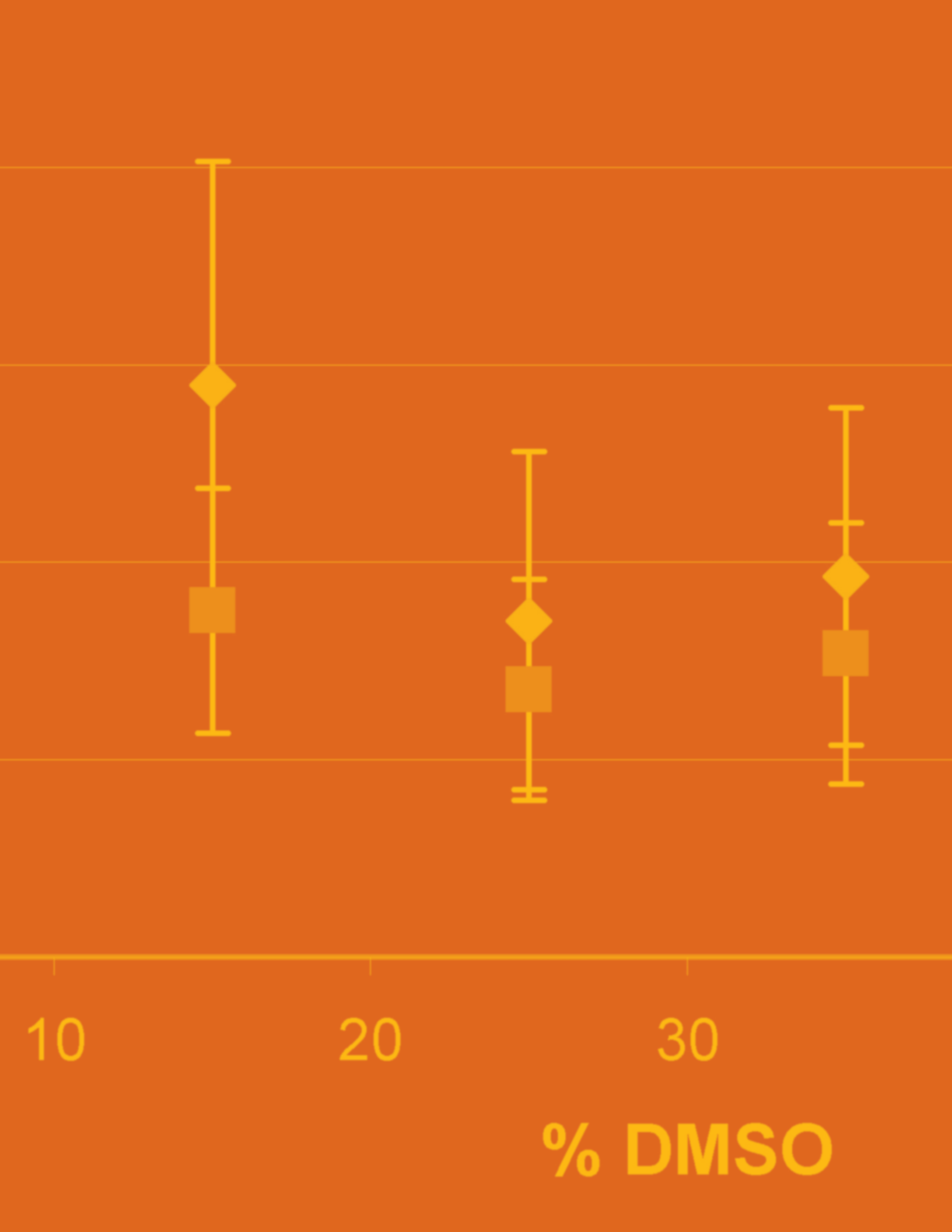
10
20
30
% DMSO

Solvent Effects on the Kinetics of Amyloid-β Oligomerization

Undergraduate Researcher
Allison Meisner
University of Connecticut

Faculty Mentor
William L. Klein
Department of Neurobiology and Physiology,
Northwestern University

Postdoctoral Mentor
Mary P. Lambert
Department of Neurobiology and Physiology,
Northwestern University

Graduate Student Mentor
W. Paige Hall
Department of Chemistry,
Northwestern University

Abstract

Amyloid-β oligomers, also known as amyloid-β derived diffusible ligands (ADDLs), have been implicated in numerous studies as being a possible cause for Alzheimer's disease (AD). The goal of this study was to investigate the kinetics of the oligomerization of the monomer amyloid-β (Aβ) into ADDLs in various ratios of dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS). The results indicate that by changing the intermolecular structure of water, DMSO affects protein interactions and possibly hydrogen bonding, both of which are needed in the formation of ADDLs. In addition to affecting water, DMSO appears to affect the ability of Aβ monomers to form hydrogen bonds, which are integral to Aβ oligomerization. This information will eventually be used to study the formation of ADDLs with a localized surface plasmon resonance (LSPR) nanosensor, with the goal of preventing or reversing the formation of ADDLs, thus leading to a cure or treatment for AD.

Introduction and Background

By the most recent count, 4.5 million Americans have been diagnosed with Alzheimer's disease, more than double the number who had the disease in 1980. This number is expected to grow in the coming years, and experts predict that by 2050, between 11.3 million and 16 million Americans will have AD. Very few families have not been affected in some way by the debilitating neurological disorder. Currently, the outlook for patients is bleak, with the average death occurring eight years after the onset of symptoms. However, if a treatment that delays the onset of AD by as little as five years were discovered, the number of people dying from the disease could decrease by as much as 50% in 50 years.[1]

Recently, progress has been made toward this end. Increases in the concentration of a 42-amino acid peptide, amyloid-β (Aβ), have been correlated with the presence of AD.[2] Aβ self-assembles into both the insoluble fibrils that form plaques and the soluble oligomers ADDLs.[3–5] Plaques, which can be seen only in an autopsy, are one of two markers in the brain for AD (the other being tangles of hyperphosphorylated tau).[6,7] However, since ADDLs and fibrils both originate from the same monomer, Aβ, ADDLs may be also be a potent AD marker in living patients. It has been shown that there is a relationship between ADDL formation and memory failure, which is a hallmark of AD.[3–5] Additionally, two separate studies have concluded that ADDLs may cause the early stages of AD.[8,9] Furthermore, a recent study has demonstrated that ADDL levels are substantially higher in the postmortem brain tissue of AD patients than in tissue of other patients.[10] Perhaps even more significantly, it has been shown that ADDL levels are higher in the antemortem cerebrospinal fluid (CSF) of AD patients than in fluid of healthy people.[11]

Since ADDLs are now a well-established biomarker for AD that may be detected early enough to be clinically relevant, the task turns to using this knowledge to treat or prevent the disease. To this end, one of the methods for detecting ADDLs, the LSPR nanosensor,[12] has made advances that may lead to early detection of AD in living patients. This nanosensor uses silver nanoparticles labeled with ADDL antibodies to detect very low concentrations of ADDLs.[12] The idea is to use the sensing capabilities of this assay to study the formation of ADDLs. The final goal is to use this knowledge of ADDL formation kinetics to prevent or reverse formation of the oligomers and eventually develop methods to prevent or reverse AD.

This study is designed to investigate the oligomerization of Aβ in different solvents. Once this is accomplished, the Van Duyne lab will use its LSPR nanosensor to monitor the oligomerization of the Aβ monomers. To achieve this, the proper balance of DMSO and phosphate-buffered saline (PBS) must be found. DMSO is used because it has been shown to have the ability to slow ADDL formation.[13] However, 1-ethyl-3-[3-dimethyl-laminopropyl]carboiimide hydrochloride (EDC), which is used to covalently link the monomers to the sensor surface, requires an aqueous environment, so PBS is also used.[12] The goal of this experiment is to examine the rate of oligomerization in different amounts of DMSO. Once this data is gathered, future experiments with the LSPR nanosensor will be carried out and

can be used to study the formation of ADDLs, which could then be used as a treatment for AD.

Approach

The dot blot, established by the Klein lab, is a frequently used method of determining ADDL concentration. A solution (in this case, 1.5 µL) is blotted on very thin film. After the blotted samples have dried, the blot is blocked with 20 mL 5% milk in Tris-Buffered Saline Tween-20. Primary antibodies are added, followed by a rinse and then the addition of secondary antibodies. After a further rinsing, film is developed and analyzed. The use of antibodies as well as the film development is detailed below.[14]

As mentioned previously, a mixture of DMSO and PBS was used as the solvent for the ADDLs. Additionally, because nitrocellulose is soluble in DMSO, nylon film was used instead of a nitrocellulose membrane. Due to the fact that ADDL levels among patients can vary by several orders of magnitude,[15] the nanosensor is required to be able to detect a range of ADDL concentrations. For this reason, solutions of synthetic ADDLs of concentrations in the 10 nM to 10 µM range were used in this study. Also, since the goal of this study was to determine the appropriate solvent ratio for slowing ADDL oligomerization, the concentration of ADDLs needed to be monitored over time. To accomplish this, the ADDLs were spotted at varying intervals over the course of 60 min. After the ADDLs were spotted on the nylon film, they were first treated with NU-1 monoclonal antibodies, which have been shown to bind only to ADDLs,[16] and then with monoclonal antimouse HRP-linked IgG antibodies, which bind to NU-1.

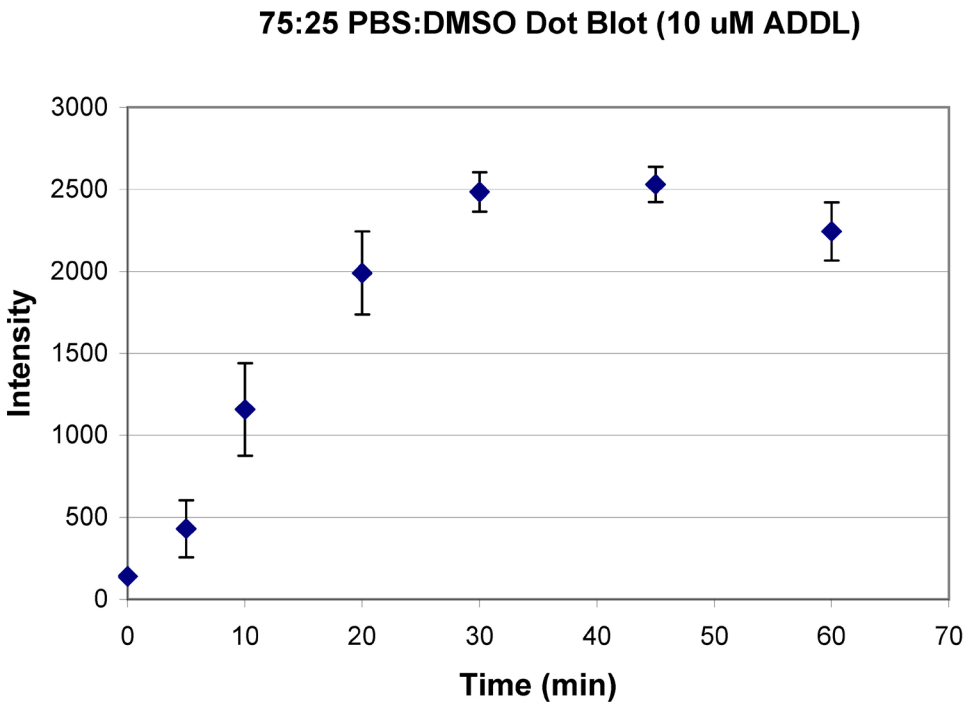

Figure 1. An example of the optical intensity data collected for the 10 µM ADDL dot blot with 25% DMSO over 60 min. A higher intensity indicates the presence of more oligomers.

The final step was to develop the nylon film using the Pierce SuperSignal chemiluminescence kit and Kodak processing software. The software was used to quantify the ADDL concentration, which it does by determining the optical intensity of the dot. Thus, a more luminescent dot will register a higher intensity, indicating a higher concentration of ADDLs. As a result, the detected ADDL concentration is not reported in terms of molarity but rather in terms of the arbitrary units of intensity given by the software program. Moreover, since NU-1 will bind only to oligomers and not monomers, a more luminescent dot signifies the presence of more ADDLs and less monomeric Aβ.

Results

Figure 1 shows the ADDL concentration of the 10 µM solution at 30 min and 60 min for various amounts of DMSO. The graph clearly indicates that a higher amount of DMSO will result in a reduced ADDL concentration (i.e., less oligomers and more monomers), with the exception of the point at 25% DMSO. Also, the graph shows that the ADDL concentration decreases as time increases, except when the solvent is 50% DMSO. Additionally, as the amount of DMSO increases, the difference between the concentration at 30 min and at 60 min decreases.

The above findings are similar in the graph of the 1 µM solution (Figure 2). There are only three points in Figure 3 because the 1 µM solution was not tested in 50% DMSO. The size of the error in Figure 3 for 25% DMSO seems to indicate that this point is not valid. Despite this, the 1 µM solution follows

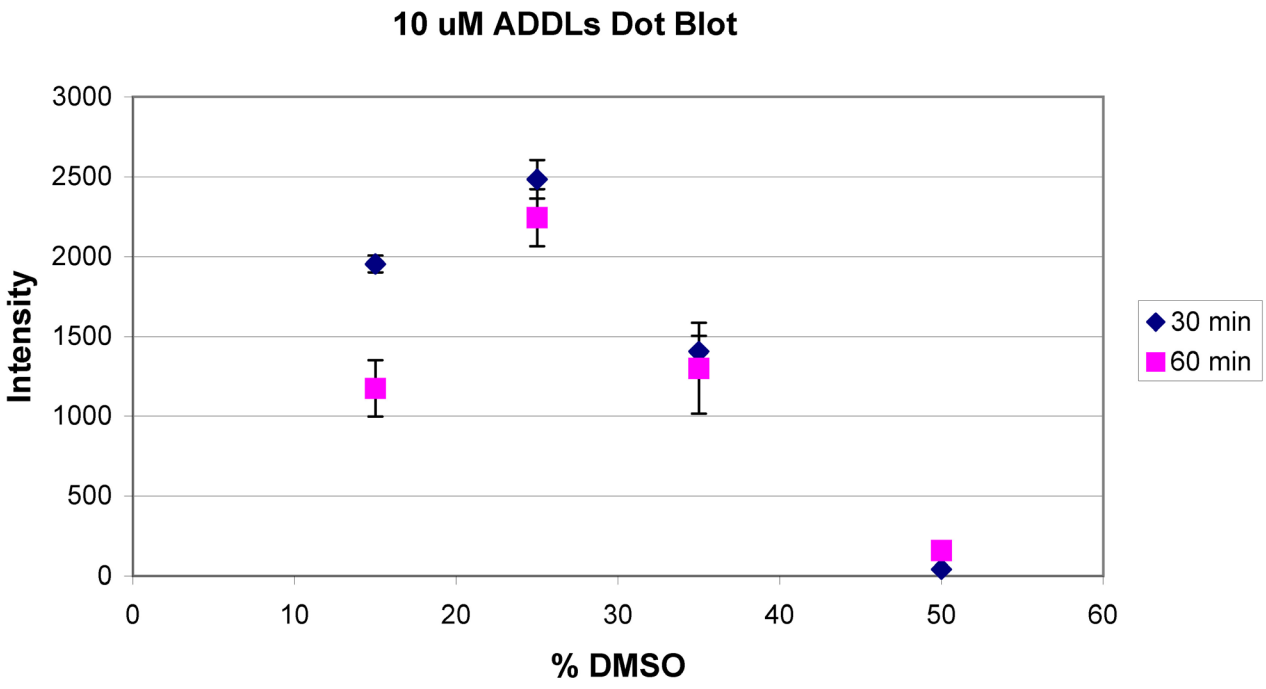

Figure 2. Graph displaying the optical intensity of the 10 µM ADDL dot blot over time with varying concentrations of DMSO.

a similar pattern to the 10 µM solution: In both cases, a lower amount of DMSO yields a decrease in ADDL concentration as time increases, while the opposite is true at higher DMSO concentrations.

Again, similar results were seen in the 100 nM and 10 nM solutions (Figures 3 and 4, respectively). Generally, a higher percentage of DMSO produced a lower concentration of ADDLs. Also, as with the 1 µM and 10 µM solutions, Figures 3 and 4 show that at concentrations of DMSO under 50%, the ADDL concentration decreases with time.

Again, as the amount of DMSO increases, the difference between ADDL concentration at 30 min and 60 min decreases.

Figure 5 shows an example of the actual data given by the software program for 10 µM ADDLs in 25% DMSO. As previously mentioned, the program determines an optical intensity, which corresponds to a concentration of ADDLs. This figure is included to demonstrate the data actually obtained from the program.

Discussion

There seem to be two separate implications present in the data from this study. The first is that DMSO decreases the amount of oligomerization; this can be seen in the fact that a higher amount of DMSO led to a lower ADDL concentration in most cases. The second implication is that DMSO is capable of reversing ADDL formation, as seen in decrease in ADDL concentration from 30 min to 60 min in much of the data. There are plausible explanations for both of these occurrences.

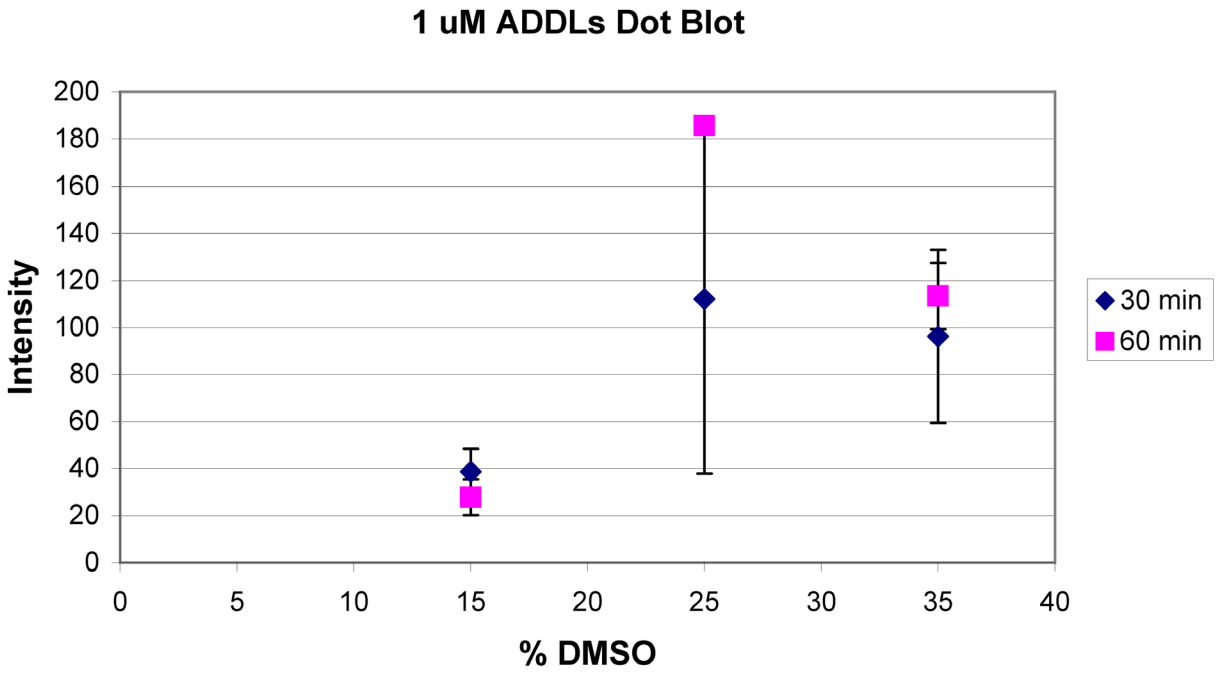

Figure 3. Graph displaying the optical intensity of the 1 µM ADDL dot blot over time with varying concentrations of DMSO.

The fact that DMSO results in a slowing of ADDL oligomerization was expected. This has been seen previously and has been attributed to a change in the tertiary structure of amyloid-β when in DMSO.[13] However, there is another possible explanation for this slowing in oligomerization that involves the fact that oligomerization occurred in a combination of DMSO and PBS. It has been shown that DMSO in an aqueous environment has a tendency to encourage the formation of ice-like water clusters, which are more highly structured than water, making them more stable and thus preferred.[17] These clusters may affect biological systems as a result of the change in the structure of the water; it has been hypothesized that this structural alteration could lead to changes in conformations and interactions of proteins and other molecules, as well as changes in ion chemistry, resulting in the solvation of molecules that have the ability to donate hydrogen bonds.[17] The possibility of DMSO/water mixtures affecting hydrogen bonds is of particular interest, since hydrogen bonds play an important role in the formation of ADDLs. Any or all of these effects could be responsible for the slowing of oligomerization seen when a mixture of DMSO and PBS is used. If DMSO does prevent ADDL formation by way of manipulation of the aqueous environment, this would indicate that oligomerization requires an aqueous environment. However, more studies must be done to conclude this.

The other effect seen in this study, the decrease in oligomerization as time increased, may be the result of powerful chemical interactions between DMSO and Aβ. The structures of DMSO and peptide bonds (Figure 6) play important roles in these interactions. The S=O bond of DMSO is a strong hydrogen

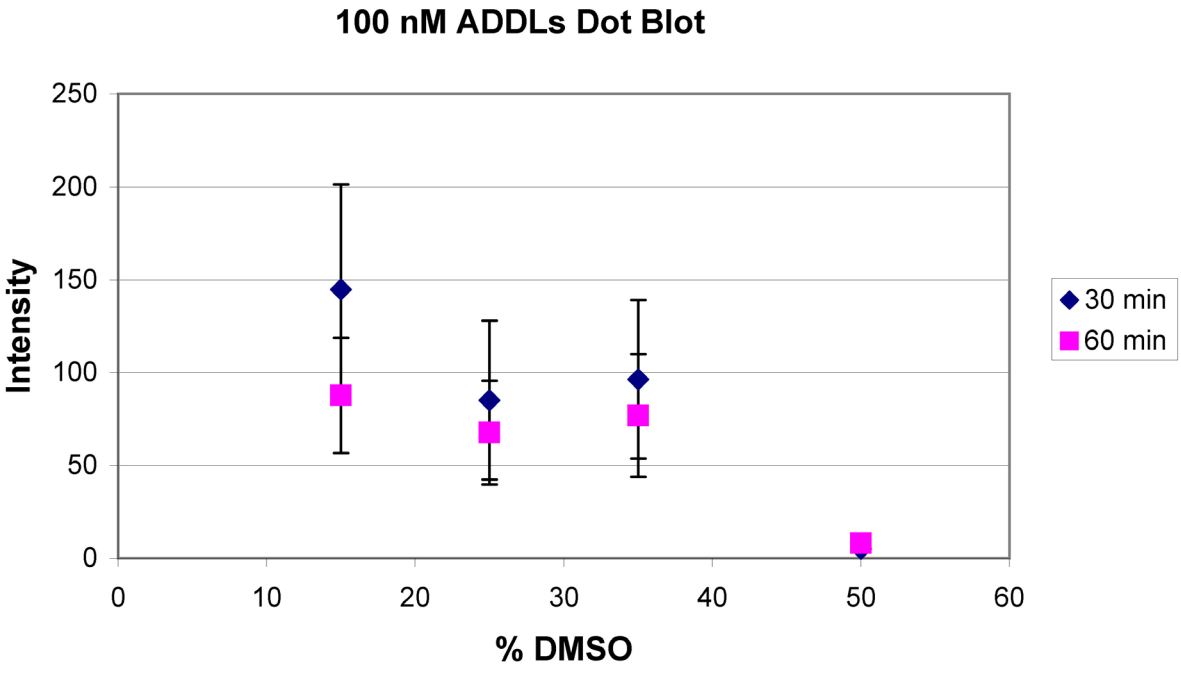

Figure 4. Graph displaying the optical intensity of the 100 nM ADDL dot blot over time with varying concentrations of DMSO.

bond acceptor and, as such, competes with C=O groups to form a hydrogen bond with the H-N of the amino acid peptide chain.[18] If the S=O from DMSO is able to prevent the bonding of Aβ monomers, ADDLs will not form. In addition to preventing hydrogen bond formation, DMSO seems to have the ability to break hydrogen bonds; in DMSO, C=O-H-N bonds are often broken to form S=O-H-N.[18] Furthermore, some of these broken hydrogen bonds are not able to form new hydrogen bonds and thus remain unbound.[18] The ability of DMSO to break hydrogen bonds, and then to sometimes prevent rebinding, may explain the decrease in ADDL concentration with time frequently seen in this study.

Thus, the use of DMSO in an aqueous environment affects the oligomerization of Aβ in either one or any combination of three distinct ways: (1) by changing the structure of the water and thus interfering in protein interactions and possibly hydrogen bonding, (2) by breaking hydrogen bonds, and (3) by preventing the formation of hydrogen bonds between Aβ monomers.

Conclusions

In summary, this study has shown that the effects of using a combination of DMSO and PBS on Aβ oligomerization are far-reaching and significant. The presence of DMSO seems to cause two separate effects. The first is the modification of the structure of the water molecules, which in turn may affect the interactions of the individual Aβ molecules and perhaps the hydrogen bonding essential to ADDL protein formation. Second, DMSO could affect the hydrogen bonds involved in the oligomerization of Aβ by breaking the bonds and/or preventing their formation.

This new information is essential to furthering the ability to monitor the formation of Aβ into ADDLs. Using the findings of this study, further studies will utilize the LSPR nanosensor to explore the possibility of obstructing or reversing the oligomerization of Aβ. This knowledge could in turn become a method to find drugs that can treat or prevent Alzheimer's disease.

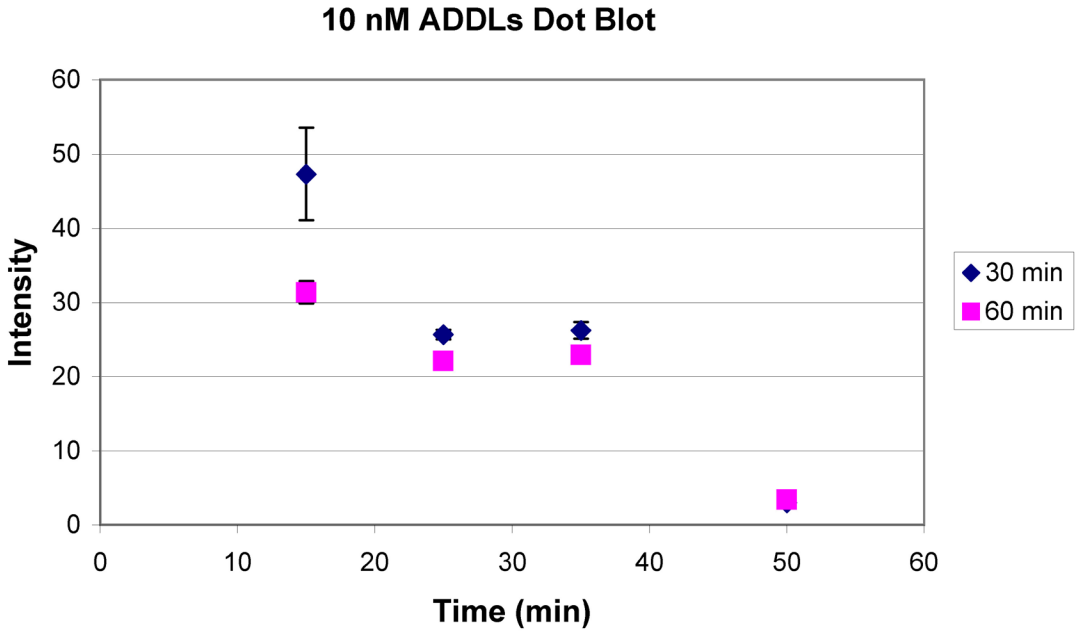

Figure 5. Graph displaying the optical intensity of the 10 nM ADDL dot blot over time with varying concentrations of DMSO.

References

(1) Alzheimer's Organization. www.alz.org/AboutAD/statistics.asp

(2) Selkoe, D. J.; Hardy, *J. Science* **2002,** *297,* 353–356.

(3) Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.; Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci.* **1998,** *95,* 6448.

(4) Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwyl, R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. *Nature* **2003,** *416,* 535.

(5) Chromy, B. A.; Nowak, R. J.; Lambert, M. P.; Viola, K. L.; Chang, L.; Velasco, P. T.; Jones, B. W.; Fernandez, S. J.; Lacor, P. N.; Horowitz, P.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Biochemistry* **2003,** *42,* 12749.

(6) Prussiner, S.; Rosenburg, R.; Di Mauro, S.; Barche, R. *The Molecular and Genetic Basis of Neurological Disease, 2nd ed.;* Boston: Butterworth-Heinemann, **1997.**

(7) Phelps, C.; Khachaturian, Z.; Trojanowski, J. Q. *Neurobiol. Aging* **1997,** *18,* S1–S2.

(8) Mattson, M. P. *Nature* **2004,** *430,* 631.

(9) Hardy, J.; Selkoe, D. *J. Science* **2002,** *297,* 353.

(10) Gong, Y.; Chang, L.; Viola, K. L.; Lacor, P. N.; Lambert, M. P.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci.* **2003,** *100,* 10417.

(11) Clark, C. M.; Xie, S.; Chittams, J.; Ewbank, D.; Peskind, E.; Galasko, D.; Morris, J. C.; McKeel, D. W. Jr.; Farlow, M.; Weitlauf, S. L.; Quinn, J.; Kaye, J.; Knopman, D.; Arai, H.; Doody, R .S.; DeCarli, C.; Leight, S.; Lee, V. M.; Trojanowski, J. Q. *Arch. Neurol.* **2003,** *60,* 1696–1702.

(12) Haes, A .J.; Chang, L.; Klein, W. L.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2005,** *127,* 2265.

(13) Shen, C. L.; Murphy, R. M. *Biophys. J.* **1995,** *69,* 640–651.

(14) Lambert, M. P.; Viola, K. L.; Chromy, B. A.; Chang, L.; Morgan, T. E.; Yu, J.; Venton, D. L.; Krafft, G. A.; Finch, C. E.; Klein, W. L. *J. Neurochem.* **2001,** *79,* 596–598.

(15) Georganopoulou, D. G.; Chang, L.; Nam, J.; Thaxton, C. S.; Mufson, E. J.; Klein, W. L.; Mirkin, C. A. *Proc. Natl. Acad. Sci.* **2005,** *102,* 2276.

(16) Lambert, M. P.; Velasco, P. T.; Chang, L.; Viola, K. L.; Fernandez, S.; Lacor, P. N.; Khuon, D.; Gong, Y.; Bigio, E. H.; Shaw, P.; De Felice, F. G.; Krafft, G. A.; Klein, W. L. *J. Neurochem.* **2007,** *100,* 23–35.

(17) Szmant, H. H. N.Y. *Acad. Sci.* **1975,** *243,* 20–23.

(18) Shanmugam, G.; Polavarapu, P. L. *Biophys. J.* **2004,** *87,* 622–630.

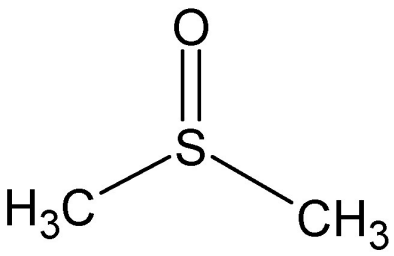

Figure 6. Structure of dimethyl sulfoxide (DMSO).