Deconstructing the Mechanism behind Poly(vinyl alcohol) – Amino Acid Hydrogel Formation

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Statement of Purpose:

Poly(vinyl alcohol) (PVA) based hydrogels have a long history in medicine and have been made using a freeze-thaw method or by chemical cross-linking using reactive, sometimes toxic reagents. A novel approach to PVA based hydrogels was discovered in our lab in 2002 by mixing PVA and amino acids (AA) under aqueous condition. However, the mechanism of the gel formation was not well understood. Hydrogen-bonding between the amine [NH2] and carboxylic acid [COOH] groups of the AA and the hydroxyl group [OH] was originally thought to be the main effect responsible for gel formation. This original hypothesis failed to explain our experimental observations. Here we propose and test a new hypothesis based on the Hoffmeister Series of water structuring and destructuring molecules.

Methods:

PVA was obtained as a gift form Kuraray Inc. (Mowiol® 28-99LA, average molecular weight 50, 066 Da, >98% hydrolyzed) and precipitated in acetone. Pure glycine, L-proline, L-lysine, L-serine, L-alanine, L-lysine(HCl), β-alanine, 5-aminovaleric acid, L-leucine, L-isoleucine, L-phenylalanine, L-valine, L-trypotophan and L-threonine were purchased from Sigma-Aldrich. Sodium chloride, potassium chloride, sodium sulfate, sodium bicarbonate, sodium iodine and urea were purchased from Fluke. Nanopure water was used for all experiments.

A 10 wt% solution of PVA in water was used for all experiments. First, the solubility of each amino acid in water was measured at room temperature 25 ^{O}C. The amino acids were separated into two groups: those with solubility lower than 10 mg/mL (L-leucine, L-isoleucine, L-phenylalanine, L-valine, L-trypotophan, and L-threonine), and those with solubility higher than 10 mg/mL (glycine, L-proline, L-lysine, L-serine, L-alanine, L-lysine(HCl), β-alanine, 5-aminovaleric acid). Saturated solutions were used for AAs of low solubility. Amino acids with high solubilities were tested at several concentrations, starting at 10 mg/mL and increasing in increments of 10mg/mL to their saturation point. At each concentration, PVA [OH] : AA [NH2] and [COOH] functional group (FG) ratio was used to determine the amount of amino acid and PVA needed in each sample. The ratios tested were 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, and 1:12 (ratio 1:12 only applied to L-proline and L-lysine). Gel formation for each sample was recorded immediately after mixing and samples were monitored for 30 days. The same experimental setup was used to investigate PVA-salt hydrogel formation, with PVA [OH]: salt ion ratio in place of PVA:AA functional group ratio.

Differential scanning calorimetry (DSC) was performed on a Netzsch Thermo DSC200 over a temperature range from 30^{O}C to 300^{O}C at a scan rate of 5^{O}C per minute under nitrogen. The melting temperature was defined as the onset point of the endothermic melting peak by DSC.

Results / Discussion:

Only amino acids with solubilities equal to or greater than glycine exhibited substantial gel formation. Final hydrogel properties varied with AA composition. As shown in the Figure 1a, both 1-phase and 2-phase gels can be formed. Gelation experiments showed the AA:water ratio to be the critical factor determining the number of phases present in the final gel. The minimum AA:water ratios required for both 1-phase gel and 2-phase gel formation are shown (Figure 1b). Similar gel formation of PVA was shown for the stabilizing ions in the Hoffmeister series, while NaI and urea, the two destabilizing ions tested, did not form gels.

Elemental analysis of fresh and leached hydrogels showed over 98% of AAs leach from the gels yet the gels remain stable. Furthermore, DSC data indicated that the hydrogel structure was not AA specific, which suggested that AAs are not critical to the final hydrogel structure.

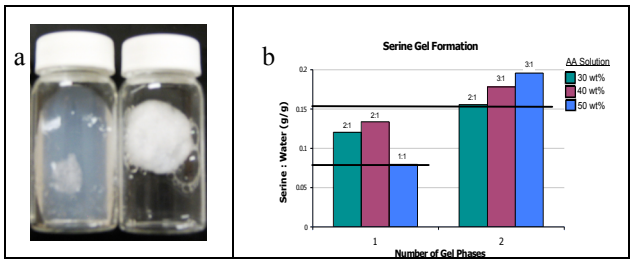

Figure 1: (a.) 1-phase (left) and 2-phase (right) PVA-AA hydrogel, (b.) typical PVA-AA-water hydrogel system, showing ratio of AA to water (y-axis) is a key element in 1-phase versus 2-phase gel formation (ratios above each data point indicate ratio of AA:PVA).

Conclusions:

PVA-AA gel formation relies upon all three components of the PVA-AA-water ternary system. The high concentration of amino acids in solution results in water being pulled away from PVA, allowing the unhydrated hydroxyl groups of PVA to hydrogen bond with each other. A 2-phase gel is formed when the amino acid solution is near its saturation point, which means the amino acid monomers are in a "thirsty" state, pulling large amounts of water away from PVA. A 1-phase gel forms when the amino acid solution is less concentrated, but still having the ability to complex or structure the water molecules. The reaction occurred at a much slower rate for 1-phase gels. The amine group and the carboxylic groups are acting as stabilizing ions just as the Hoffmeister series predicts. These novel PVA gels will be used in our research for tissue engineering applications.

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