Protective Coatings

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Egg White Varnishes on Ancient Paintings: A Molecular Connection to Amyloid Proteins**

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Abstract: *For about 400 years, egg white was used to coat and protect paintings without detailed understanding of its molecular properties. A molecular basis is provided for its advantageous properties and one of its protective properties is demonstrated with oxygen transport behavior. Compared to the native secondary structure of ovalbumin in solution of circa 33% α-helix and β-sheet, attenuated total reflection–FTIR (ATR-FTIR) spectra showed a 73% decrease of α-helix content and a 44% increase of β-sheet content over eight days. The data suggest that the final coating of dissolved ovalbumin from egg white after long exposure to air, which is hydrophobic, comprises mostly β-sheet content (ca. 50%), which is predicted to be the lowest-energy structure of proteins and close to that found in amyloid fibrils. Coating a synthetic polytetrafluoroethylene membrane with multiple layers of egg white decreased oxygen diffusion by 50% per layer with a total decrease of almost 100% for four layers.*

Varnishes are solutions of natural or synthetic resins in organic solvents that dry or cure when spread on a surface. The dried films are solid and relatively transparent. They are used to change the gloss, unify the gloss of the surface, saturate colors, and protect the surface of a painting. According to the composition of the solution, the films exhibit varying qualities of gloss, protective ability, flexibility, and durability. The variety of varnishing materials is as diverse as the choices of paint media and techniques used throughout the history of painting. By the early Renaissance, a variety of materials were developed for use as painting varnishes, ranging from egg white to resin. Numerous synthetic varnishes have also been developed that provide a wide array of surface characteristics. Although synthetic varnishes were popular, they have different properties than natural varnishes. Sometimes a gray, glassy layer was found on paintings that was insoluble in organic solvents as well as in water.

Cornelia Peres quoted from writings of the eighteenth and nineteenth-century that egg white varnishes were used as provisional varnishes.[1] They were applied to freshly painted pictures before the paint was dry enough to allow proper varnishing. The purpose was to give the surface an even sheen and to wet out matte areas to make the painting presentable for an exhibition or sale. In a letter to his brother, Vincent van Gogh wrote the following:

"My dear Theo, My warmest good wishes for good health and peace of mind on your birthday. I should have liked to send the painting of the Potato Eaters for this day, but although it's coming along well, it isn't quite finished yet. Yesterday, I took it to a friend of mine in Eindhoven who is doing some painting. In about 3 days' time I'll go back over there and give it some egg white and finish off a few details. I believe that The Potato Eaters will turn out well—as you know, the last few days are always tricky with a painting because before it's completely dry one can't use a large brush without running a real risk of spoiling it. Ever yours, Vincent".[2]

Until the early sixteenth century, egg white was used in book illumination as both medium and varnish.[3] In the seventeenth century, painters began to use it as a varnish. In the eighteenth century, egg white varnishes were generally accepted, although later in the nineteenth and twentieth centuries egg white varnish lost its popularity. Protein substances, such as egg yolk, glair, casein, animal glue, and their mixtures with oils, resins, gums, and so on have been used as binding media since very early times.[4] When properly used, their stability in various atmospheric conditions is said to be exceptional as compared with the yellowing and brittleness of aged oils and resins. This may be the reason why ageing processes of oils and resins are widely discussed, while those of proteins have been overlooked. The most commonly used techniques to analyze the binding media of ancient paintings are ATR-FTIR, mass spectrometry (MS), gas chromatography (GC), and 1D or 2D nuclear magnetic resonance (NMR) spectroscopy.[5]

Herein, we investigated egg white proteins at a hydrophobic interface to determine their structural transformations (using ATR-FTIR) and oxygen diffusion properties.[6] When adsorbed on hydrophobic surfaces, their protective properties against environmental deterioration owing to structural change and oxidation were analyzed. Secondary protein structure,[7] particularly β-sheet, is the major contributing factor in decreasing oxygen diffusion rate owing to oxygen absorption, with subsequent swelling of the protein layer.

Egg white contains 11.1% (w/w) proteins and 87.3% (w/w) water, with other major constituents being lipids, carbohydrates, and minerals.[8] 54% of the total protein content is ovalbumin. This represents 6% of the total weight of egg white. Other major contributing proteins are ovomucoid (11%), ovotransferrin (12–13%), lysozyme (3.4–3.5%), and

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ovomucin (1.5–3.5 %).[9] Ovalbumin from chicken egg white (Sigma–Aldrich, St. Louis, MO 63103) was chosen as the model protein and polytetrafluoroethylene (PTFE, Sterlitech, Kent, WA 98032-1911) as the model surface (apolar mimic of fresh oil paintings) owing to its hydrophobic nature. However, extended periods of curing and ageing will cause oil paintings to become polar (B. Berrie, private communication). Ovalbumin from lyophilized egg white can be modeled well by pure ovalbumin, as can be seen by the similar second derivative ATR-FTIR (Magna-IR 550 Series II, Nicolet Instruments, Madison, WI) spectra (Supporting Information, Figure S1). Lyophilized egg white comprises 64–88 % ovalbumin, which explains why the two spectra correlate so closely. Figure 1 a shows the effect on secondary structure for one layer of egg white on a PTFE membrane over 24 h. Figure 1 b shows the effect of multiple layers of egg white on secondary structure content. This layer deposition was repeated four times on the same membrane to give four total layers of egg white coating. Each layer was applied by soaking the membrane in 50 mg mL^{-1} egg white solution for 24 h and drying it at room temperature for 24 h. The secondary structural content predicted by the second derivative of the ATR-FTIR spectra showed a decrease in α-helix content and an increase in β-sheet content over time and with number of coatings.[10,11] Upon adsorption and subsequent drying on the surface, the protein lost its native form within 24 h. The amide I stretch for the α-helix peak is from 1652–1654 cm^{-1} and the β-sheet peak is from 1631–1637 cm^{-1}.[10–12] We calculated that α-helix content decreased by about 73 % overall, while β-sheet content increased by about 44 % overall from the native structure of ovalbumin in egg white (ca. 33 % α-helix and β-sheet content). Similar hydrophobic surface-induced secondary structure changes were previously observed with lysozyme, another constituent protein of egg white.[13] An AFM image at the interface of a PTFE surface with adsorbed egg white is shown in the Supporting Information, Figure S4. Similar AFM images were used to measure the thickness of a layer of egg white. Average layer thickness as measured by AFM imaging was 73.5 + 37.9 nm. Amyloid fibrils were not observed.

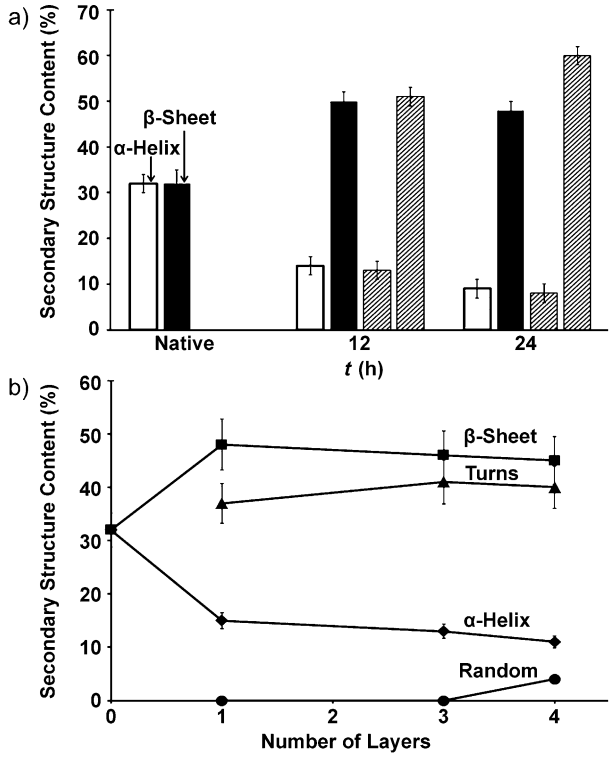

Figure 1. Secondary structure content a) over time and b) over number of layers, both calculated from second derivatives of ATR-FTIR data and native structure. a) Data for lyophilized egg white (solid bars) and pure ovalbumin (cross-hatched bars). b) Data for lyophilized egg white for 1, 3, and 4 layers. Note that in (b), no secondary structure percent is available for turns and random for the native structure of ovalbumin. Error bars shown in (a) and (b) are 10 % to account for error in the second-derivative method.

Next, the effect of adsorbed and dried egg white on oxygen gas diffusion rate was investigated. A custom glass setup with two chambers was used for this experiment (Supporting Information, Figure S2). Briefly, oxygen was fed into the bottom chamber and nitrogen was fed into the top chamber simultaneously and oxygen diffusion was measured over time. Figure 2 a shows the results of oxygen transport experiments with a control PTFE membrane and with multiple layers of adsorbed egg white. The measured oxygen gas (Airgas, Albany, NY 12205) concentration on the permeate side is plotted against time. The data show that increasing layers of adsorbed egg white continued to decrease the rate of oxygen diffusion as well as final permeate concentration until it reached zero with four added layers. Using the linear section of the data for each layer (lines), normalized diffusivity (D/D_0) was calculated for each layer (inset in Figure 2 a). After four layers and an exponential decline, the normalized diffusivity approached zero with a value of 0.009.

The ATR-FTIR data (Figure 1) indicate that as the number of layers as well as drying time increased, the native α-helix content decreased from 33 % to 9 % and from 33 % to 8 % and the β-sheet content increased from 33 % to 48 % and from 33 % to 60 %, for egg white and pure ovalbumin, respectively. From this data and based on previous research,[13] we conclude that a two-step process occurred during the secondary structure rearrangement: 1) The α-helix is converted into turns and random and 2) the turns and random are converted into β-sheet structure.[13] From the oxygen diffusion results (Figure 2), it can be seen that increasing layers of egg white continued to decrease the oxygen diffusion rate and final permeate concentration. A decrease of about 50 % oxygen concentration was observed for each added layer of egg white. After four layers were added to the PTFE membrane, the rate of oxygen diffusion dropped to zero. We surmise that the initial entrance region (lag shown as first three circular points) was due to rearrangement of the protein layer from oxygen absorption and represents the time it takes for the oxygen front to move through the protein/membrane layers. The more stable β-sheet structure is likely able to stack the protein tighter than α-helix structure. We believe this leads to absorption of oxygen, causing physical swelling of the egg white coating. To test this proposal, a membrane coated with one layer of egg

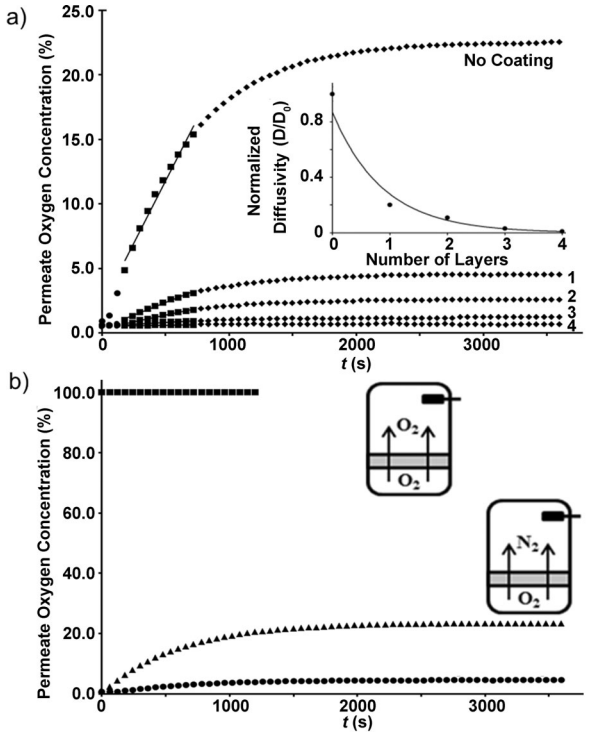

Figure 2. Oxygen diffusion data a) over time and b) with and without conditioning. The numbers next to each curve in (a) correspond to the number of layers of egg white coating. Inset: the normalized diffusivity versus the number of layers of egg white coating, where D_0 is the diffusivity measured through an uncoated membrane. The equation of the exponential decline was $y = 0.87\,e^{-1.136x}$ with an R^2 value of 0.990. b) The effect of conditioning on one layer of egg white coating. ● Without conditioning; ▲ after conditioning by pre-exposure of the coated membrane to pure oxygen for 20 min, followed by the normal experiment described previously; ■ data collected during oxygen conditioning that are included to show that the system was not leaking and maintained an oxygen concentration of 100% during conditioning. These results were reproduced in triplicate.

white was pre-exposed, or conditioned, to 100% oxygen for 20 min. The experiment was then run as previously, with oxygen and nitrogen on each side of the membrane (Figure 2b). The conditioned membrane allowed all of the oxygen to diffuse through during the one hour experiment. This shows that the mechanism for blocking oxygen is primarily, but not entirely, owing to absorption in the protein layer, rather than a physical impermeable membrane effect. It is likely that the membrane swelled owing to additional absorbed oxygen, which also decreased the oxygen diffusion rate through the coated membrane.

Why was egg white used and what were its unique properties as a varnish for paintings? These questions have puzzled the art community for hundreds of years. We have now provided a scientific basis for an answer. According to Dobson and others, proteins can form amyloid fibrils under specified conditions.[14,15] This is true for ageing diseases, such as Alzheimer's disease, and now we show it for egg white varnish. We showed how the application of egg white to a fresh oil painting analogue (PTFE membrane) altered the native protein secondary structure of egg white, which reduced the oxygen diffusion rate. The main constituent protein of egg white, ovalbumin, converts from α-helix to β-sheet when painted and dried on the membrane surface. The β-sheet content of approximately 50%, which is close to that of amyloid fibrils (ca. 47%),[16] led to the ability of the membrane to swell and trap oxygen, thereby preventing it from diffusing through the membrane. Therefore, this is an absorption effect in combination with absorption/desorption and impermeability owing to the protein layer swelling. The combination of these effects preserves the integrity of the painting and prevents it from degrading. During the first year of the life of the painting, these effects would decrease the curing rate of the oil. This would be advantageous in that the upper layers of the paint may cure at a rate more similar to the lower layers of the paint.

Experimental Section

Egg white solutions were prepared at a concentration of 50 mg mL^{-1} lyophilized egg white (albumin from chicken egg white powder, 62–88%) in PBS solution (10 mM phosphate buffer, 2.7 mM potassium chloride, and 137 mM sodium chloride with pH 7.4 at 25°C). The solution was mixed under medium stirring with a stir bar for 30 min until the protein was dissolved.

Attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy measurements: Secondary structure components were quantified using the second derivative technique of ATR-FTIR spectra (Magna-IR 550 Series II, Nicolet Instruments, Madison, WI) of egg white adsorbed onto the PTFE coating.[10,11]

Atomic force microscopy (AFM; MFP 3D, Asylum Research, Santa Barbara, California) images of PTFE surfaces coated with adsorbed egg white were obtained. This was used to measure the thickness of each layer and for surface characterization. An average of 111 height measurements was used to calculate the average thickness of the egg white layer. The height difference was measured from many positions on the egg white layer, but at the same location on the PTFE membrane. PTFE structure is not uniform and therefore only one location was used to keep the height measurements consistent.

The oxygen gas diffusion test was performed on a custom made all glass setup comprised of two separate chambers that can be clamped at the center with a membrane placed in between them (Supporting Information, Figure S2). The top chamber has a volume four times larger than the bottom chamber. The bottom chamber was charged with industrial grade oxygen gas while the top chamber was simultaneously purged using ultra high purity nitrogen gas. The entire system was then sealed and oxygen gas concentration on the permeate side was measure using a PASPORT oxygen gas sensor (PASCO, Roseville, CA 95747).

Data analysis of the AFM data was carried out using IGOR 6 by measuring the difference in height of the membrane and protein layer directly from the AFM images taken. The analysis of the ATR-FTIR data was done using Wolfram Mathematica 8.0.1.0 and Omnic version 6.1a. The ATR-FTIR data was processed in Omnic using automatic baseline correct, normalizing by the highest peak, taking the second derivative (built-in processing function), and viewing the range from 1700 to 1600 cm^{-1}. The area of each labeled peak was measured (using the ranges indicated) and that was divided by the total area of the measured peaks.

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