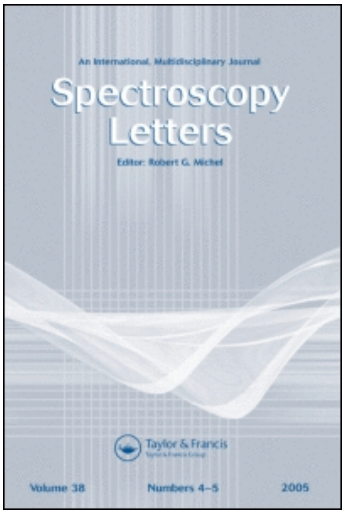

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Characterization of Proteins in Mouse Blood by Fourier Transform Infrared Spectroscopy

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Abstract: The Fourier transform infrared (FTIR) spectra of blood plasma and blood cells from two control female mice and four bred female mice were obtained using the blood component sample films formed on polyethylene IR cards. The protein level in each component was found to remain relatively constant for the control mice. Spectral differences due to variations in protein contents, however, were observed for the pregnant mice. The results show that the FTIR-IR card method can be simple and effective at probing total blood protein changes.

Keywords: FTIR, mouse blood proteins, mouse pregnancy

INTRODUCTION

Blood is a mixture of two components: cells and plasma. Plasma is the liquid portion of the blood. Blood cells like red blood cells float in the plasma. Also dissolved in plasma are electrolytes, nutrients and vitamins, hormones, clotting factors, and proteins. Plasma distributes the substances it contains as it

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circulates throughout the body. The cellular portion of blood contains red blood cells (RBCs), white blood cells (WBCs), and platelets. The RBCs carry oxygen from the lungs; the WBCs help to fight infection; and platelets are parts of cells that the body uses for clotting. Total blood protein measurements in plasma and cells can reflect nutritional state, kidney disease, liver disease, and many other conditions. If total protein is abnormal, further tests must be performed to identify which protein fraction, and then which specific protein, is abnormal.

Fourier transform infrared (FTIR) spectroscopy, which is used to measure the vibrational modes of functional groups of molecules, is sensitive to molecular structure, conformation, and environment. In recent years, efforts have been made to apply FTIR spectroscopy to quantify those important biochemical components in biological fluids.[1–3] In a recent study, we described a FTIR analysis of mouse urea content in dried urine samples using IR transparent polyethylene cards as the sample substrates.[4] The IR card method effectively eliminates the water IR absorption problem, which often prevents the FTIR quantification of these biological fluids.

The purpose of this study is to demonstrate the sensitivity and reliability of the FTIR-IR card method for the determination of the protein contents in mouse blood components and their variations due to physiologic and biochemical changes. The FTIR spectra of plasma and cells are obtained from two control female mice and four bred female mice after being paired with their mates for a period of 4 weeks. Spectral differences, especially those due to alterations in protein content, are expected for the pregnant mice because the pregnancy is anticipated to induce physiologic and biochemical changes.

MATERIALS AND METHODS

Mice

Out-bred laboratory mice, Mus musculus, strain CD-1 were fed rodent lab chow and maintained on a 12-hr light/12-hr dark cycle. Six female mice from two litters, 5031 and 5052, were chosen for this study. The three mice from each litter came from the same set of parents. Two mice from each litter, labeled "A" and "B," were used for the pregnancy study, and the third mouse, labeled "C," was used as the control. At the start of the 3-week study, each of the "A" and "B" mice, 5031A, 5031B, 5052A, and 5052B, was paired with a male mouse in a separate cage. The mating was also controlled carefully to prevent any possible inbreeding. Each female mouse was weighed daily on a top-loading balance (Denver Instrument XE-410D, Denver, CO, USA).

KSCN Solution

A 100-mL volume of KSCN solution was prepared by dissolving 4.00 g of potassium thiocyanate (p.a. grade, ACROS, Hanover Park, IL, USA),

KSCN, in deionized water using a 100-mL volumetric flask. The solution was stored in a refrigerator when it was not in use. Solutions were returned to room temperature before application.

Collection of the Blood Plasma and the Blood Cell Samples

A tiny cut was placed on the tail of each mouse, and blood was collected into a Fisherbrand heparinized micro-hematocrit capillary tube (Fisher Scientific, Hanover Park, IL, USA). The tubes were then centrifuged for 30 min to separate the blood into plasma and cells.

Blood Plasma or Blood Cell/KSCN Solution

A plasma or cell solution was prepared by mixing plasma or cells with KSCN in a 1:3 ratio (v/v). The blood plasma and blood cell samples obtained ranged from 3.0 μL to 15.0 μL. Each liquid was measured using a 100-μL micropipette (Hamilton Co., Reno, NV, USA).

FTIR Spectroscopy

Samples were prepared by spreading the blood plasma or blood cell/KSCN solution evenly onto the circular area of the polyethylene film (Thermo Spectra-Tech ST-IR cards, Thermo Spectra-Tech, Waltham, MA). The samples were then air dried for 1 h. The transmission spectrum was measured using a Genesis II Fourier Transform Infrared Spectrometer (Mattson, Madison, WI, USA). A total of 32 scans were used at a resolution of 4.0 cm^{-1} with a signal gain of 20. The polyethylene substrate was used as the spectrum background, and the background spectrum was collected for each substrate before the blood plasma or blood cell/KSCN solution was placed on it.

RESULTS AND DISCUSSION

Figure 1 shows the typical FTIR transmission spectra of the corresponding mouse blood plasma and the blood cells. The peak at 2060 cm^{-1} is due to the absorbance of the internal standard, potassium thiocyanate (KSCN). This absorption serves to normalize the spectra, compensating for random variations in film thickness.

It can be seen that the overall appearances of both the blood plasma and the blood cell spectra are dominated by protein absorptions,[5] not surprisingly as protein concentration is expected to be much higher than any other blood constituent in each blood component. The most intense absorption band in

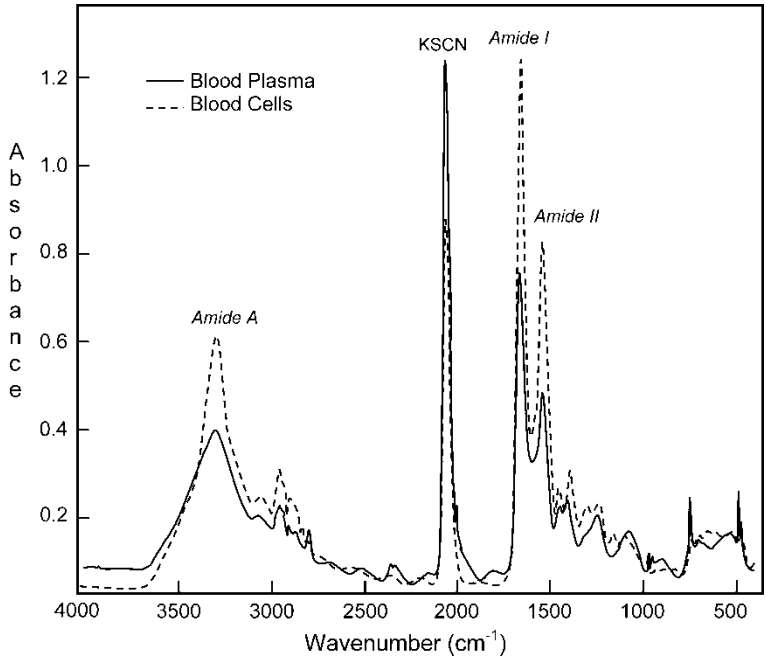

Figure 1. Typical FTIR transmission spectra of the corresponding mouse blood plasma and the blood cells.

proteins is the amide I peak, which is observed at 1659 cm^{-1}. Amide I is mainly associated with the C═O and C-N stretching vibrations and is also related to the backbone conformation. The next major absorption band is the amide II peak, which is found at 1545cm^{-1}. Amide II derives largely from the in-plane N-H bending, and the C-N and C-C stretching vibrations. Another prominent feature of the IR spectrum is the broad peak at 3298 cm^{-1}. This peak is referred to as the amide A peak. Amide A peak is due to the N-H stretching vibration, which does not depend on the backbone conformation but is very sensitive to the strength of amide hydrogen bond.[6] The two blood components contain different types and amounts of proteins. The major protein found in plasma is albumin.[7,8] The blood cell component, however, consists primarily of red-colored hemoglobin in the red blood cells.[8]

The total protein content in each component is estimated using the normalized amide I peak at 1659 cm^{-1} relative to the KSCN peak at 2060 cm^{-1} and therefore is dimensionless in this article. Local baselines are used for both the amide I peak height and the KSCN peak height measurements. It is found that the protein content in the blood cells is always higher than that in the plasma. This agrees with the fact that the blood cells contain more than 35%

hemoglobin, and the plasma is mostly water and the plasma total protein is less than 10%.[8]

For both control mice 5031C and 5052C, the total protein content was found to fluctuate over time and correspond with the mouse's weight change. Generally, the change was small and the value remained at a relatively constant level. Figure 2 shows the blood plasma and blood cell protein contents of mouse 5031C along with its weight over the observed period of time. The weight of mouse 5031C varies slightly and shows an overall decrease from 25 g to 23 g. Its protein contents in both the blood plasma and blood cells seem to increase as the weight increases and decease as the weight decreases. The protein content change is also less profound in the plasma than in the blood cells. For mouse 5031C, the blood plasma protein is 0.61 ± 0.16 and the blood cell protein is 1.50 ± 0.46; and for mouse 5052C, the plasma protein is 0.44 ± 0.22 and the blood cell protein is 1.98 ± 0.60.

Mice 5031A, 5052A, and 5052B have delivered 7, 8, and 6 healthy pups 27, 20, and 22 days after they were paired with their mates, respectively. Their total weight gain ranged from 12 g to 15 g. A regaining of weight was observed for each mouse right after its sharp weight drop due to the delivery. Although mouse 5031B was paired with its mate, it never got pregnant.

Figure 3 illustrates the plasma protein content, the blood cell protein content, and the weight of mouse 5031A during its 3- to 4-week pregnancy. It can be seen that the plasma protein content of the mouse decreases slightly. This might indicate that the protein albumin, the major constituent of the plasma, decreases slightly during the pregnant period. This observation

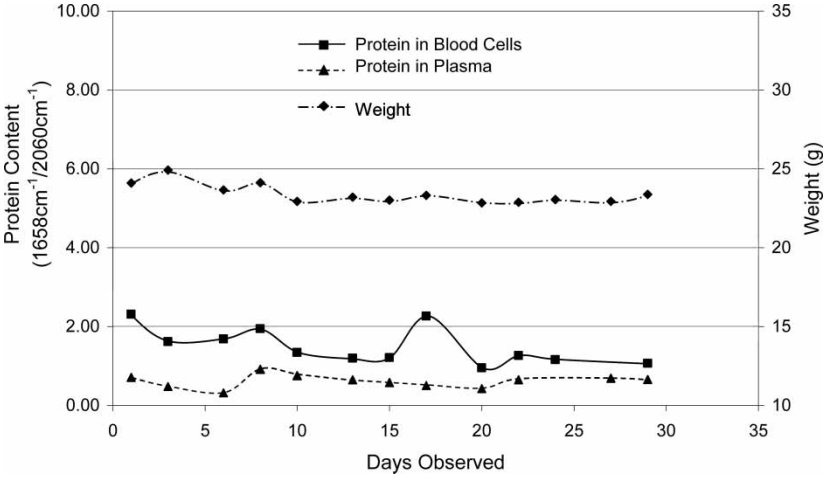

Figure 2. Blood plasma and blood cell protein contents of control mouse 5031C.

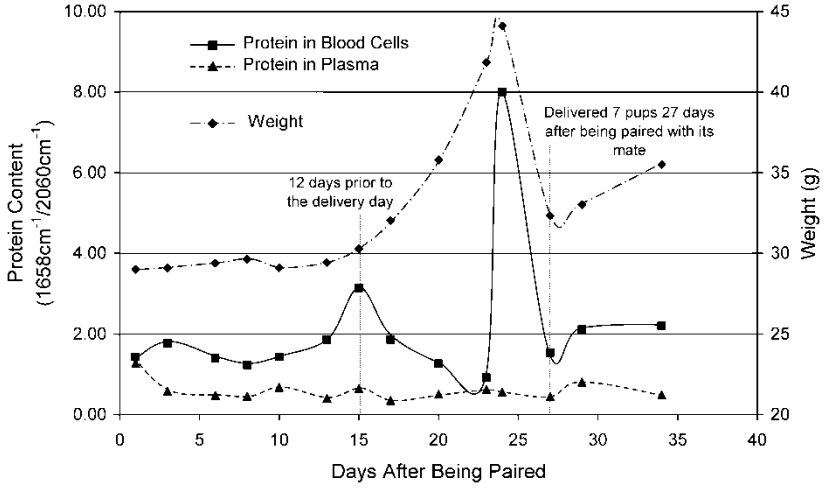

Figure 3. Blood plasma and blood cell protein contents of pregnant mouse 5031A.

also applies to the other two pregnant mice. The decrease in plasma protein concentration may be mainly due to an increase in fluid volume during the pregnancy.

Large variations in the blood cell protein, however, are observed for the pregnant mice, indicative of the changes in the red blood cell hemoglobin content during their pregnancies. All three pregnant mice showed a large increase in the blood cell protein content around 2 weeks before their deliveries: 12 days prior to the delivery day for the mice 5031A and 5052A and 14 days prior to delivery for the mouse 5052B. This may suggest that the physiologic, biochemical, and anatomical changes that occur at this stage of pregnancy require an additional amount of hemoglobin. The results also show that the blood cell protein varies right before the mouse's delivery: a sharp increase was observed for mouse 5031A, a slight increase for mouse 5052A, and a decrease, however, for mouse 5052B. Other than those changes, the blood cell protein fluctuates over time but remains at a relatively constant level. It is expected that the red blood cell volume and red blood cell mass both increase during a normal pregnancy for humans.[9] If the increases take place at the same rate, the hemoglobin content would stay the same. Otherwise, a change in the hemoglobin content might occur. Similar changes may occur during pregnancies of mice.

Figures 4a and 4b show the protein amide A peaks of mouse 5031A's blood plasma and blood cell spectra at the beginning of its pregnancy and at around 2 weeks before delivery. A left shift as large as 15 cm^{-1} can be seen in the plasma amide A peak. This left shift in the plasma amide A position is also present near, during, and right after the mouse's delivery. On the other hand, the blood cell amide A peak remains unaltered during

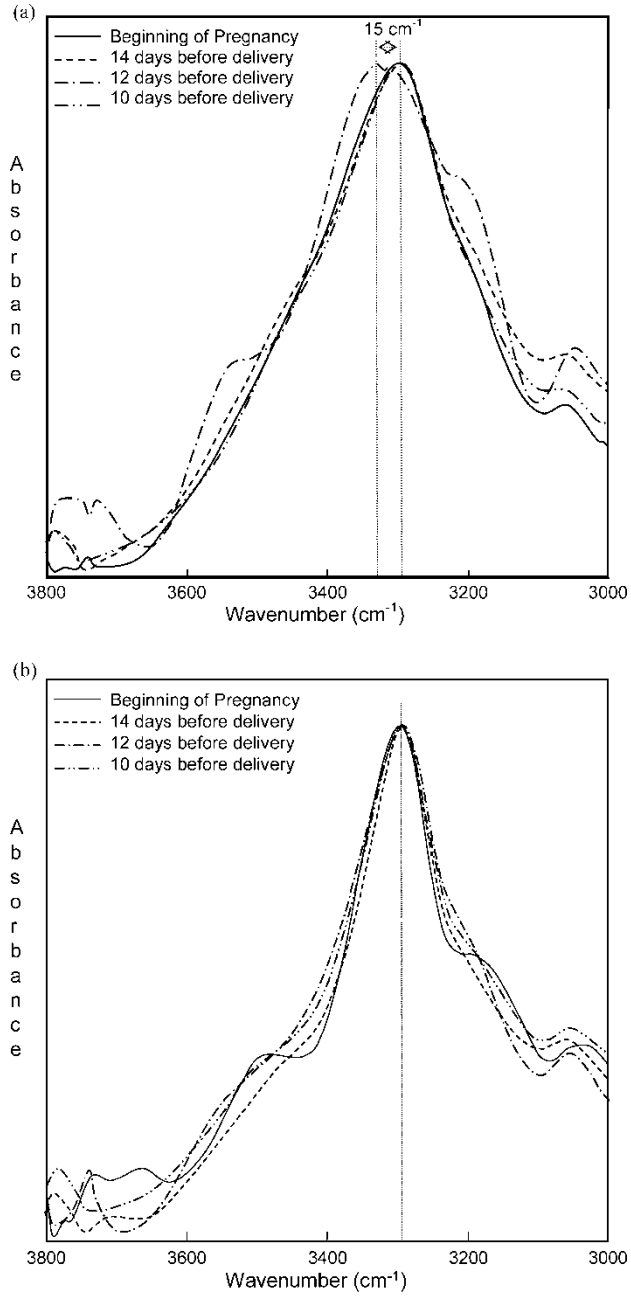

Figure 4. (a) The protein amide A peaks of mouse 5031A blood plasma spectra at the beginning of its pregnancy and approximately 2 weeks before delivery. (b) The protein amide A peaks of mouse 5031A blood cell spectra at the beginning of its pregnancy and approximately 2 weeks before delivery.

the pregnancy. Similar patterns are demonstrated by the pregnant mice 5052A and 5052B. A slight left shift in plasma amide A peak is also observed for the control mice 5031C (on the 17th day) and 5052C (on the 13th day), but to a much less extent. The large shift might imply a variation in the strength of protein amide hydrogen bonding due to changes in the plasma chemistries at different stages during the mouse's pregnancy. These shifts seem to occur at the same time when large fluctuations in the blood cell protein content are observed.

CONCLUSIONS

FTIR-IR card studies show that the total protein content in mouse plasma is lower than that in mouse blood cells, as indicated by the normalized amide I peak. For the control mice, the total protein in plasma or blood cells remains at a relatively constant level. Spectral differences due to variations in proteins, however, are observed for the pregnant mice during their pregnancies, especially around their deliveries and 2 weeks prior to their deliveries:

1. The blood plasma amide I peak shows a slight decrease, indicating the change in amount of the plasma major component, albumin.
2. The blood cell amide I peak stays at a relative constant level but shows large variations about 2 weeks prior to, right before, during, and right after the delivery, suggesting different amounts of hemoglobin required at these stages.
3. The plasma amide A peak is found to shift up to 15 cm^{-1} near, right before, and right after the delivery as well as about 2 weeks prior to delivery. This shift implies a large change in plasma chemistries affecting the level of protein amide hydrogen bonding.

Spectral variations of mouse blood components suggest that the FTIR-IR card method can be simple and effective at probing blood protein changes.

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