Laboratory Exercise

Varying Iron Release from Transferrin and Lactoferrin Proteins. A Laboratory Experiment[S]

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Abstract

Iron metabolism is an important subject of study for undergraduate students of chemistry and biochemistry. Relevant laboratory exercises are scarce in the literature but would be very helpful in assisting students grasp key concepts. The experiment described here deals with different iron release mechanisms of two protagonists in iron metabolism: serum transferrin (Tf) and lactoferrin (Lf). Despite having very similar structures and iron-binding sites, Tf releases practically all its iron at pH 5.5 while Lf requires a significantly lower pH of 3. This difference in behavior is directly related to their respective biological functions as Tf blood-borne iron into the cell, while Lf competes with pathogens to sequester iron in biological fluids at more acidic pHs.

During this experiment, the students will carry out iron loading and unloading on both human Lf and Tf and monitor the iron release at different pHs using UV–Vis spectroscopy. With this simple approach, the students will discover the different patterns of iron release of Tf and Lf and how this variance in behavior relates to their biological functions. Furthermore, this laboratory practice can be expanded to allow students to investigate a variety of iron proteins. © 2017 by The International Union of Biochemistry and Molecular Biology, 45(6):521–527, 2017.

Keywords: *Biological chemistry; iron metabolism; proteins; laboratory instructions; hands-on learning/manipulative; UV/vis spectroscopy*

Introduction

Transferrins are a family of iron-binding glycoproteins that control the level of free iron in biological fluids [1]. Members of the family include serum transferrin, called simply transferrin (Tf); lactoferrin (Lf), found in secretions such as milk; melanotrasferrin, present in melanoma cells; and ovotransferrin, present in egg whites [2, 3]. All are monomeric proteins of 76–81 kDa consisting of two structurally similar lobes (termed N- and C-lobes) that each contains a single Fe^{3+} binding site. Due to the presence of two iron-binding sites, there are three known metallated forms for all Tfs, which differ in their iron-loading: apotransferrin (without iron), a monoferric form (one iron per protein) and a diferric or saturated holotransferrin form (two irons per protein). The iron content per protein can be calculated simply from the ratio between the iron and protein concentrations. These concentrations can easily be determined with UV–Vis spectroscopy through the absorbance of bands centered at 464 nm, which is the fingerprint of the iron coordination to the binding site of both Tf and Lf, and the band centered at 280 nm. The chemical environments of the two Fe^{3+} atoms coordinated to the protein are identical for the N- and C-lobes and consist of two phenolate oxygens from two tyrosines, one imidazole from a histidine, one carboxylate from an aspartate residue, and a synergistic adjacent bicarbonate or carbonate (Fig. 1) [4, 5].

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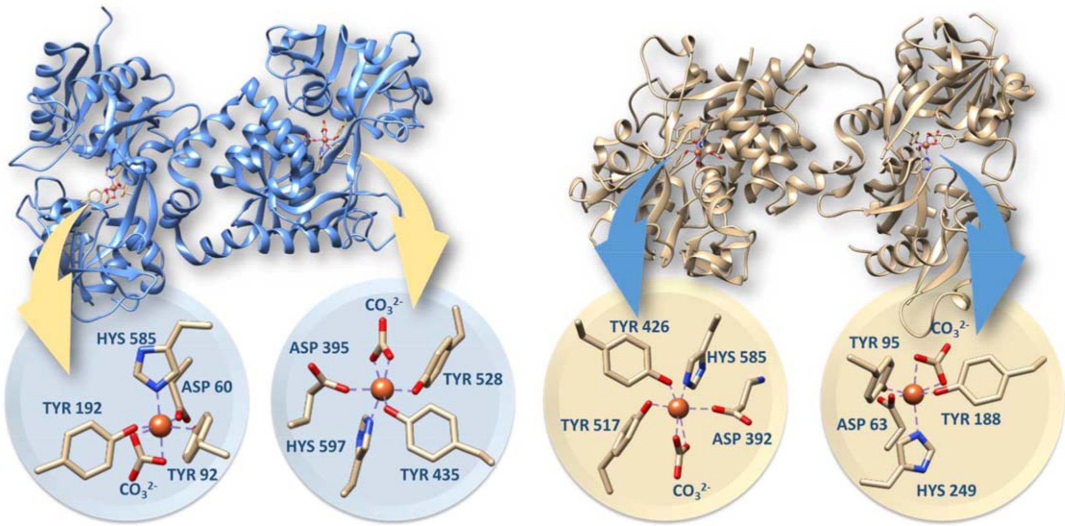

FIG 1 *X-ray structure of $TfFe_2$ (left, Protein Data Bank 3V83) and $LfFe_2$ (right, Protein Data Bank 1LFG) showing the iron coordination sites [3]. [Color figure can be viewed at wileyonlinelibrary.com]*

Tf plays a crucial role for life, as it uptakes iron from the absorption centers in the duodenum and white blood cell macrophages, and transports it to all tissues. Although the iron bound to Tf is $<0.1\%$ (4 mg) of total body iron, it constitutes the most important pool of iron with the highest turnover rate (25 mg/24 hr) [1, 2]. Tf is recycled more than 10 times a day to supply the 20–30 mg of iron needed for the over 2 million new erythrocytes produced every second by the bone marrow. Tf binds iron tightly but reversibly (binding constant, $K = \sim 10^{20}\ M^{-1}$) [6]. The affinity of Tf for Fe^{3+} is extremely high but decreases progressively below neutral pH.

In contrast, Lf possesses various biological functions including antibacterial, antiviral, and antiparasitic activities [7]. High Lf levels are found in colostrum, milk, and in most mucosal secretions, including uterine fluid, vaginal and nasal secretions and tears [8]. Lf's affinity for iron is extremely high, double than that of Tf [2]. This extraordinary iron affinity undoubtedly determines part of its functionality. Lf's comparatively closed conformation with respect to that of Tf explains the higher iron affinity since, once sequestered, iron atoms cannot easily be transferred to another molecule by direct competition.

Lf is considered to form part of the immune system because its uptake of iron prevents the growth of pathogenic microorganisms. Because iron is a key nutrient for pathogenic microorganisms, which require the metal to survive and replicate, life to some extent can be considered a battle for iron in which the host must deprive undesirable guests of iron to combat the infections they cause. Lf's role in the immune system directly relates to its iron affinity: while apolactoferrin (Lf without iron) inhibits the growth of a large number of pathogenic bacteria, hololactoferrin (iron-saturated Lf) exhibits significantly lower inhibitory activities towards these pathogens [9].

The different functions that these two proteins carry out *in vivo* are to a large extent explained by the pH dependency of their iron uptake/release mechanisms. Tf is more sensitive to pH fluctuations than Lf. In fact, iron release into the cells is triggered by a slight decrease in pH. When Tf binds to receptors on the cell's surface to undergo endocytosis via clathrin-coated pits, a proton pump ATPase acidifies the endosome to pH 5.5, triggering Fe^{3+} release [3]. In comparison, Lf is less sensitive to pH changes, and is able to take up and retain the iron atoms even in the range of pH 3–4. Higher stability at acidic pH enables Lf to compete for iron even in the harsh environments present during bacterial infections [9].

Previous laboratory experiments have used iron chelators to study iron removal [6]. Here, we present an experimental procedure to illustrate in a clear and easy way the different behaviors of the Tf family proteins to release iron when subjected to decreasing pH. Students can learn from these experiments how two proteins of the same family, Tf and Lf, with very similar structures and essentially identical iron binding sites, have different patterns of iron release and how their different behaviour relates to their function: while Tf is the iron-transport protein that liberates iron at intracellular pH (5.5), Lf is an iron-sequestering protein that protects the host from pathogens in specific highly acidic biological fluids. By dialyzing both iron-saturated proteins (prepared by the students themselves) against decreasing pH media and collecting UV–Vis spectra, the iron release processes can be monitored and analyzed. At the end of the experiment, the resulting

apoproteins can be isolated and reused in another laboratory session.

Experimental

It is advisable that the students work in pairs. The time required to complete the experiments is 3 hr, including the analysis of data, that usually takes 30 min.

Background

Tf and Lf are iron transport protein in mammals. They exhibit high Fe^{3+}-affinity. Both proteins contain two Fe^{3+} sites and can exist as apoproteins (no iron), monoferric (one iron per protein) and diferric proteins (two iron per protein). The iron content per protein in a sample can be calculated thanks to the UV–Vis bands of the bound iron to protein, centered at 464 nm and to the typical band of protein at 280 nm. From absorbance values at 464 and 280 nm and by the Lambert-Beer law (extinction coefficients are known), the concentration of *iron protein* and protein can be calculated. The ratio between *iron protein* and protein concentration is the iron content of the protein. Therefore it is possible by monitoring the increase or decrease of the band at 464 nm to follow the process of iron loading or iron removal from Tf and Lf.

Materials

Since the proteins can have different iron loads depending on their commercial sources and lots, it is highly recommended to start by removing all iron atoms to obtain the apoproteins and afterwards to saturate them with iron to obtain the holoforms. The color of the powdered Tf and Lf depends on their iron contents. Apoforms are white powders. An orange-red color appears and increases with greater iron content per protein. All protein forms are water soluble in a wide range of pH values (2–10).

For our experiment, human recombinant Tf (T3705) and human recombinant lactoferrin (L1294) were purchased from Sigma-Aldrich. Conventional dialysis bags of 12,000 MWCO (Molecular Weight Cut-Off) were used. Citrate-citric acid, TRIS-HCl, and TRIS-HCl/$NaHCO_3$ buffers were prepared following standard procedures.

Pre-Lab Preparation

For students without experience in dialysis processes, it is recommended that once the dialysis bag are prepared, they learn to handle them, fill them with water, close them with clips or knots and transfer the solutions from dialysis bags to vials. A short time (10 min) is enough for students to become acquainted with the techniques.

General Safety Precautions

All experiments should be carried out wearing safety goggles, gloves, and protective clothing. Although most of the reagents required for these experiments are not toxic, special precautions must be taken handling hydrochloric acid, sodium hydroxide, iron(III) nitrate, and citric acid solutions.

Hydrochloric acid (37%) is a strong acid that is toxic, corrosive, fatal if inhaled, irritant, permeator, and lung sensitizer. In contact with the skin it causes severe burns and damage in contact with eyes. Avoid inhalation of vapors and prepare the solutions under the fumehood. Sodium hydroxide is caustic. Contact with the skin causes severe skin burns and causes eye damage in contact with eyes. Iron(III) nitrate nonahydrate causes severe burns when in contact with skin and eye damage if in contact with the eyes. Citric acid is a mild acid that may cause serious eye irritation if in contact with the eyes.

All reactions are carried out at room temperature or 37°C.

Preparations of Solutions (30 min)

Before performing the experiment, the students must prepare several solutions, buffers, and the commercial protein solutions by the following procedures.

Preparation of 0.1 M sodium citrate/citric acid 0.1 M NaCl buffer at pH 3.0, 4.0, 4.5, and 5.5. A 1 M stock solution is prepared by adding 192.12 g of citric acid (251275, Sigma Aldrich), 294.10 g of sodium citrate tribasic dihydrate (S4641, Sigma Aldrich) and 58.4 g of sodium chloride (S7653, Sigma Aldrich) to 1 L of distilled water. Final working buffers (0.1 M sodium citrate/citric acid 0.1 M NaCl at pH 3.0, 4.0, 4.5, and 5.5), are prepared diluting 100 mL of the stock solution up to 500 mL with distilled water, pH adjusted (to 3.0, 4.0, 4.5, or 5.5) using HCl and NaOH 1 M and the final volume completed to 1 L with distilled water.

Preparation of TRIS-HCl 200 mM pH 6.8 buffer. Twenty-four grams of Tris(hydroxymethyl)aminoethane (252859, Sigma Aldrich) is added to 500 mL of distilled water, the pH is adjusted to 6.8 using HCl 1 M and the final volume adjusted to 1 L.

Preparation of TRIS-HCl 50 mM pH 6.8 buffer. Two hundred and fifty milliliter of the 200 mM stock solution of TRIS-HCl pH 6.8 is diluted up to 1 L to obtain a final buffer concentration of 50 mM.

Preparation of TRIS-HCl 50 mM/$NaHCO_3$ 20 mM pH 6.8 buffer. 1.68 g of $NaHCO_3$ (S5761, Sigma Aldrich) is added to 250 mL of the stock solution of TRIS-HCl 200 mM pH 6.8 buffer. The pH is adjusted to 6.8 using HCl and NaOH 1 M and the final volume adjusted to 1 L.

Iron citrate 4 mM pH 6.8. Iron citrate is freshly prepared according to a reported protocol [10]: 3.2 g of iron(III) nitrate nonahydrate (254223, Sigma Aldrich) is added to a solution of 6 g of sodium citrate tribasic dihydrate in 200 mL of water. The solution is heated at 37°C for 30 min while stirring. Then, 100 mL of the mixture is added to 500 mL of Tris-HCl 50 mM/$NaHCO_3$ 20 mM buffer and the pH is adjusted to 6.8 using 1 M HCl. Finally, the volume is adjusted with distilled water to 1 L. Keep in mind that

freshly prepared solutions are required for each protein loading in the next steps.

Preparation of Protein Solutions (60 min)

Human recombinant Tf (T3705, Sigma Aldrich) and human recombinant Lactoferrin (L1294, Sigma Aldrich) solutions were prepared by dissolving 10 mg of the respective commercial proteins in 1 mL of Tris-HCl 50 mM pH 6.8 buffer. Vigorous stirring should be avoided to prevent the formation of foam. As mentioned above, commercial Tf and Lf can show different iron loads depending on their lots and thus, it is highly recommended to prepare the respective apoproteins and afterwards saturate them with iron to obtain homogeneous preparations of the corresponding holoproteins, $LfFe_2$ and $TfFe_2$.

Preparation of apotransferrin and apolactoferrin. The iron of the commercial proteins is totally removed by following successive dialysis steps against 150 mL of 0.1 M citric acid/citrate 0.1 M NaCl buffer (pH 3.0) using a dialysis membrane with a MWCO of 12,000 Da. The iron desaturation of the proteins is carried out in the following steps:

1. Prepare two dialysis membranes of a MWCO of 12,000 Da by soaking them in distilled water for 15 min.
2. Transfer each protein solution into separate dialysis bags and place them in 1 L beakers containing 500 mL of 0.1 M citric acid/citrate 0.1 M NaCl buffer pH 3.0. Dialyze for 30 min while stirring to release the bound iron. Change the citric acid/citrate saline approximately every 10 min up to a total of three times, until the characteristic color iron-Tf/Lf (absorbance at 464 nm) is no longer observed in the solution of the dialysis bag (see next step). For both Tf and Lf, a typical band centered at 464 nm in the UV–Vis spectrum is the fingerprint of iron coordinated to the binding site due to the existence of a transfer charge band from tyrosine to Fe^{3+}.
3. Transfer the protein solution contained inside the dialysis membranes to a quartz cuvette and determine the protein concentration through the absorbance values registered at 280 nm by using the Lambert-Beer's law and the extinction coefficients listed in Table S4 (Supporting Information). It is crucial to dilute the samples 20 times before collecting these UV–Vis spectra as both proteins exhibit high extinction coefficients at 280 nm.
4. Once iron has been removed from the proteins and the protein concentration measured, dialyze the colorless-pale apoprotein solutions against 800 mL of Tris-HCl 50 mM buffer pH 6.8 for 30 min.

Preparation of holo Tf and Lf [11]. The apoproteins prepared are loaded with iron until saturation by 30 min of dialysis at 37°C against 250 mL of a solution of iron citrate 4 mM (pH 6.8) using a dialysis membrane with a MWCO of 12,000 Da until the appearance of a characteristic reddish solution inside the dialysis bag. To obtain the desired holoproteins in their diferric form the following steps are taken:

1. Prepare two dialysis membranes of a MWCO of 12,000 Da by soaking them in distilled water for 15 min.
2. Transfer the previously prepared apoprotein solutions into different dialysis bags and place them in 1 L beakers containing 250 mL of the solution of iron citrate 4 mM in Tris-HCl 50 mM/$NaHCO_3$ 20 mM buffer pH 6.8. Dialyze for 30 min until the appearance of the characteristic reddish color inside the dialysis bag.
3. Remove the unbound Fe remaining inside the dialysis bag by transferring the bag to another 1 L beaker containing 250 mL of Tris-HCl 50 mM buffer pH 6.8 and dialyzing it for 30 min while stirring.
4. Transfer the holoprotein solutions contained inside the dialysis membranes to a quartz cuvette and determine the protein concentration and the iron amount per protein through the absorbance values registered at 280 and 464 nm by using the Lambert-Beer's law and the extinction coefficients listed in Table S4 (Supporting Information). Again, it is crucial to dilute the samples 20 times before collecting the UV–Vis spectra at 280 nm as both proteins show high extinction coefficients at this wavelength. No dilution is needed for the measurement at 464 nm.

To calculate the iron content per protein, use the following equation:

$$\frac{\text{Fe}}{\text{protein}} = \frac{\frac{A_{464\text{nm}}}{\varepsilon_{464}}}{\frac{A_{280\text{nm}}}{\varepsilon_{280}} * 20} \qquad (1)$$

Iron content per protein estimation. Iron and protein concentration values can be easily calculated by UV–Vis using the corresponding extinction coefficients listed in Table S4 (Supporting Information).

The iron contents per protein is calculated directly by UV–Vis spectroscopy from the relationship between the absorbance at 464 nm ($\varepsilon^{464} = 2{,}600\ M^{-1}$) and that at 280 nm ($\varepsilon^{280}$ Tf = 87,000 M^{-1} and ε^{280} Lf = 92,000 M^{-1}). The final iron/protein ratio values obtained for our experiment were 1.74 for Tf and 1.86 for Lf. These values were obtained from triplicate preparations, with $<8\%$ variation from the mean observed. Since the extinction coefficients of the proteins at 280 nm are higher than those at 464 nm, the proteins were diluted 20 times to collect the UV–Vis spectra at 280 nm. The ratio iron concentration/protein concentration directly provides the number of iron atoms per protein.

After completing these experiments and analyzing the protein iron contents after iron removal and iron loading, students' knowledge of the experiment and the theory behind it should be assessed.

Iron Release Experiments (60 min)

The iron saturated Tf and Lf solutions prepared previously were dialyzed for 30 min against 500 mL citrate-buffered saline 0.1 M at pH 5.5. by following this procedure:

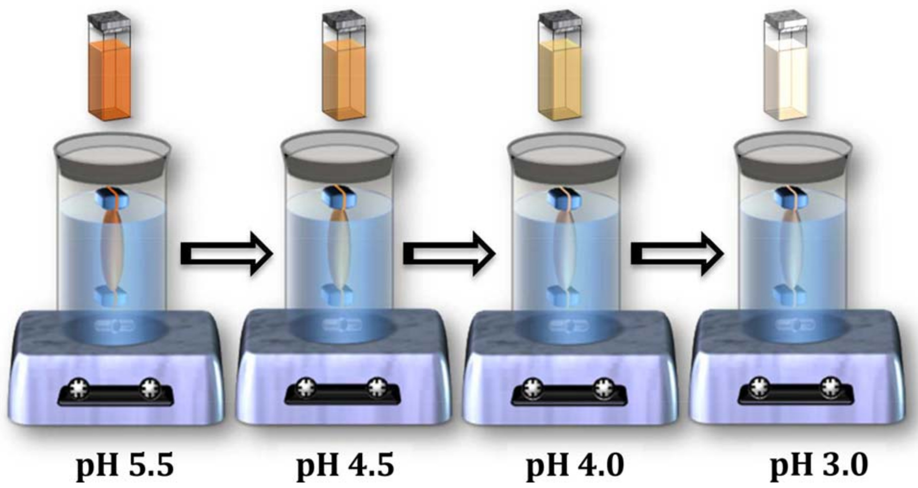

FIG 2 $TfFe_2$ and $LfFe_2$ were separately dialyzed 30 min against a solution at pH 5.5. After this time, UV–Vis spectra were collected and the proteins were transferred to a dialysis reservoir at pH 4.5. The operation was repeated at pH 4.0 and pH 3.0. [Color figure can be viewed at wileyonlinelibrary.com]

1. Prepare two dialysis membranes of a MWCO of 12,000 Da by immersing in water for 15 min.
2. Transfer each of the previously prepared reddish holoprotein solution into different dialysis bags and place it in a 1 L beaker containing 250 mL of 0.1 M citric acid/citrate 0.1 M NaCl buffer pH 5.5. During this experiment, dialysis membrane should be closed using dialysis membrane clamps to facilitate protein extraction and membrane reutilization. Dialyze for 30 minutes under stirring to release the bound iron.
3. After this time, the UV–Vis spectra of the dialysis bag solutions were collected and the protein concentration, as well as the iron content per protein (Tf and Lf), was spectrophotometrically determined as described above by transferring the holoprotein solutions contained inside the dialysis membranes to a quartz cuvette.
4. Again, place the protein solutions into different dialysis bags and repeat steps 2 and 3 for a next dialysis step against 250 mL of 0.1 M citric acid/citrate 0.1 M NaCl buffer pH 4.5. Dialyze for 30 min under stirring to release the bound iron.
5. Repeat step 4 by transferring the solutions to third and fourth dialysis reservoirs of citrate-buffered saline 0.1 M at pH 4.0 and finally to pH 3.0 (as described in Fig. 2).
6. At every pH studied, the iron content per protein was determined by UV–Vis spectroscopy from the relationship between the absorbance at 464 nm and that at 280 nm using Eq. (1).
7. After the last step, the resulting protein solutions show very low absorptions at 464 nm in the UV–Vis spectrum, which means that at pH 3.0, both proteins have released all (in the case of Tf) and practically all (in th case of Lf) their iron atoms. The resulting apotransferrin and apolactoferrin, with very low iron content, can be stored at 4°C and reused for another laboratory session.

Post-Lab Work (30 min)

Calculate the percentage of Fe retained by each protein with the decrease of pH using the UV data and the following equation:

$$\%\ \text{of Fe retained} = \frac{\text{Num.}\frac{\text{Fe}}{\text{protein}}(\text{pH})}{\text{Num.}\frac{\text{Fe}}{\text{protein}}(\text{initial})} \tag{2}$$

Percentage of iron retained per protein at different pHs is obtained dividing the calculated number of iron atoms per protein after each dialysis step by the initial amount of Fe bound to the protein at the beginning of the experiment.

Data Collection

The data obtained for each protein can be described in the following (Table 1). These are average values obtained

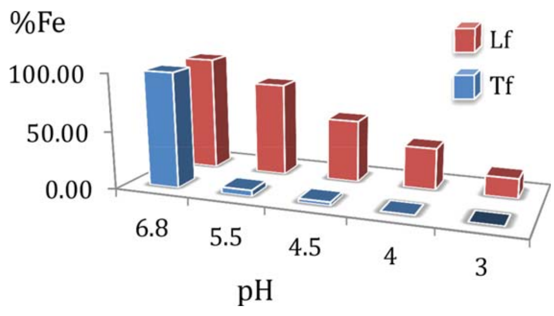

FIG 3 Representation of percentage of iron retained by Tf and Lf with the decrease of pH. The amount of Fe present in every protein at each pH value obtained from the UV–Vis data at 464 nm is divided by the amount of total Fe bound to the starting saturated Tf and Lf. Note that the iron from Tf is removed at a higher pH than that of Lf, which requires a very low pH to release iron. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 *Results obtained for each protein in a experiment done in a lab session with 5 student couples.*

Transferrin	*Initial*	*pH 5.5*	*pH 4.5*	*pH 4.0*	*pH 3.0*
Abs 280	0.376	0.366	0.362	0.374	0.368
[Tf] (M)	$8.64{\cdot}10^{-5}$	$8.41{\cdot}10^{-5}$	$8.32{\cdot}10^{-5}$	$8.60{\cdot}10^{-5}$	$8.46{\cdot}10^{-5}$
Abs 464 nm	0.395	0.033	0.013	0.002	0.000
[Fe] (M)	$1.52{\cdot}10^{-4}$	$1.27{\cdot}10^{-5}$	$5.00{\cdot}10^{-6}$	$7.69{\cdot}10^{-7}$	0.00
Fe/Tf	1.76	0.15	0.06	$8.95{\cdot}10^{-3}$	0.00
% Fe retained	100	8.58	3.42	0.51	0.00
Lactoferrin					
Abs 280	0.463	0.460	0.459	0.467	0.469
[Lf] (M)	$1.01{\cdot}10^{-4}$	$1.00{\cdot}10^{-4}$	$9.98{\cdot}10^{-5}$	$1.02{\cdot}10^{-4}$	$1.02{\cdot}10^{-4}$
Abs 464 nm	0.486	0.344	0.242	0.169	0.084
[Fe] (M)	$1.87{\cdot}10^{-4}$	$1.32{\cdot}10^{-4}$	$9.31{\cdot}10^{-5}$	$6.50{\cdot}10^{-5}$	$3.23{\cdot}10^{-5}$
Fe/Lf	1.86	1.32	0.93	0.64	0.32
% Fe retained	100	71.24	50.23	34.48	17.06

from the experiment done in a lab session with 5 student couples, after rejecting maxima and minima values. The error was never above 8%.

Data Presentation

The values of the absorbance at 464 nm obtained from the UV–Vis spectra plotted against the pH show the different patterns of iron release of Tf and Lf. The results are better visualized if represented as the percentage of Fe retained by each protein with the decrease of the pH (Fig. 3). This can be easily achieved by dividing the amount of Fe present in every protein at each pH value—from the UV–Vis data at 464 nm—by the initial amount of total Fe bound to the starting saturated Tf and Lf.

Post-Lab Questions

1. Represent as a column graph the percentage of Fe retained versus the pH.
2. Before performing the UV–Vis measurements at 464 nm, no dilution of the proteins was required, but at 280 nm a 20-fold dilution was necessary. Explain this based on UV–Vis spectroscopy.
3. During the iron reconstitution experiments, sodium bicarbonate ($NaHCO_3$) was added to the buffer but it was not used during iron removal. Explain the reason. Would it be possible to load the proteins with iron in absence of bicarbonate?
4. If both proteins are structurally similar, why do they show different patterns of iron release as a function of the pH? Does this have any biochemical meaning?

Specific Learning Outcomes

1. Iron is so crucial for mammals that they have developed a protein family to transport it to all cells. Students can learn about the extraordinary design of Tf and Lf to uptake/remove iron depending on pH.
2. This experiment demonstrates the usefulness of UV–Vis spectroscopy in monitoring important biological iron processes or information such as the iron contents of proteins. This technique will be useful for further work with metalloproteins.
3. The students learn to use dialysis procedures to load and unload metals from a biomolecule. Dialysis is traditionally used for separating small and large molecules as well as changing the pH to a protein solution. From this experiment, students can extend the use of dialysis to further proposals.
4. This experiment is a good scenario to practice the Lambert-Beer law for calculating iron content in a protein by the ratio between iron and protein concentrations.

Impact/Interest

All Tf proteins, including serum Tf and Lf, have the same basic polypeptide folding, comprising two homologous

globular lobes, each further divided into two domains. Likewise, all Tf proteins have essentially identical iron binding sites, one per lobe. Students can ask themselves what stimuli trigger iron release and why.

Despite virtually identical iron binding sites in both proteins, Lf retains its iron at significantly lower pHs. From the experiment, students can note that after 30 min at pH 5.5, Tf liberates practically 100% of its iron content while Lf maintains its iron atoms (81%) and requires a significant lower pH of 3.0 to release more iron (Fig. 3). The pH decrease induces iron release in both proteins, but the existence of cooperative interactions between the two lobes of Lf makes the binding of the first iron atom in the C-lobe stabilize the binding of a second iron atom in the N-lobe (9). These conformational changes explain the different iron release patterns observed in the laboratory demonstration.

This laboratory experience allows students to address the study of two crucial proteins in iron metabolism, a key topic in Biochemistry and Bioinorganic Chemistry. This demonstration introduces a simple method to illustrate differences in the iron biochemistry of Tf and Lf and encourages the students to appreciate the extraordinary designs in Nature: how living organisms have structurally similar proteins with very similar iron binding sites, but with such different iron release mechanisms that allow each protein to perform different biological functions.

References

[1] Gkouvatsos, K., Papanikolaou, G., and Pantopoulos, K. (2012) Regulation of iron transport and the role of transferrin. Biochim. Biophys. Acta 1820, 188–202.

[2] Lambert, L. A. (2012) Molecular evolution of the transferrin family and associated receptors. Biochim. Biophys. Acta 1820, 242–253.

[3] Chung, M. C. (1984) Structure and function of transferrin. Biochem. Educ. 12, 146–154.

[4] Haridas, M. I., Anderson, B. F., and Baker E. N. (1995) Structure of human diferric lactoferrin refined at 2.2 A resolution. Acta Crystallogr. Sect. D 51, 629.

[5] Nicholas, N., Nicole, C. E., Muse, O., Naoko, M., James, G., Evzen, B., Ashley, N. S., Olga, Z., Philip, A., Emad, T., Robert, W. E., Andrew, R. G., Anne, B. M., Alasdair, C. S., and Susan, K. B. (2012) Structural basis for iron privacy by pathogenic *Neisseria*. Nature 483, 53–58.

[6] Hamilton, D. H., Battin, E. E., Lawhon, A., and Brumaghim, J. L. (2009) Using Proteins in a Bioinorganic Laboratory Experiment: Iron Loading and Removal from Transferrin. J. Chem. Educ. 86, 969.

[7] Baker, E. N., and Baker, H. M. (2009) A structural framework for understanding the multifunctional character of lactoferrin. Biochimie 91, 3–10.

[8] Baker, E. N., and Lindley, P. F. (1992) New perspectives on the structure and function of transferrins. J. Inorg. Biochem. 47, 147–160.

[9] Orsi, N. (2004) The antimicrobial activity of lactoferrin: current status and perspectives. Biometals 17, 189–196.

[10] Faller, B., and Nick, H. (1994) Kinetics and Mechanism of Iron(III) Removal from Citrate by Desferrioxamine B and 3-Hydroxy-1,2-Dimethyl-4-Pyridone. J. Am. Chem. Soc. 116, 3860–3865.

[11] Carmona, F. Muñoz-Robles, V. Cuesta, R. Gálvez, N. Capdevila, M. Maréchal, J. D., and Dominguez-Vera, J. M. (2014) Monitoring lactoferrin iron levels by fluorescence resonance energy transfer: A combined chemical and computational study. J. Biol. Inorg. Chem. 19, 439–447.