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Denaturation process of laccase in various media by refractive index measurements

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ABSTRACT

In this work, we are interested in the denaturation process of a laccase from Tramates versicolor via the determination of the refractive index, the refractive index increment and the specific volume in various media. The measurements were carried out using an Abbe refractometer. We have shown that the refractive index increment values obtained from the slope of the variation of the refractive index vs. Concentration are outside the range refractive index increments of proteins. To correct the results, we have followed the theoretical predictions based on the knowledge of the protein refractive index from its amino acids composition. The denaturation process was studied by calculating the specific volume variation where its determination was related to the Gladstone-Dale and the Lorentz-Lorentz models.

1. Introduction

The characterization of proteins by dynamic, static light scattering and sedimentation is often related to the measurements of the refractive index of solutions where its determination is necessary to calculate several physical parameters [1–7]. For that reason, generally, an experimental compromise was followed to determine the refractive index. The experimental determination of the refractive index of polymer or protein solutions is based on the direct reading of the values obtained with refractometers and the index increments from the slope of the curve representing the variation of the refractive index vs. concentration or temperature. But, the problem is that the direct determination of the refractive index and the refractive index increments of protein allow us to obtain an exact or an approximated value and then an exact or approximated result.

In literature, little works are interested in the investigation of solutions the optical propriety and characterizing biopolymers vs. the knowledge of their refractive index. The refractive index of proteins is a physical parameter serving in various biophysics techniques and optical imaging [8,9]. It represents the consequence of the local polarizability of atoms and chemical groups due to the deformation of electron configurations of protein [10–12]. Therefore, for the best determination of this parameter, Doty et al. [13] have shown the importance of the composition, the density and the environmental factors. In the same way, Adair et al. [14] have considered that the refractive index of proteins or amino acids may be approximately determined from its elementary composition. They have also considered that by means of a good approximation, a consensus value can be used where the amino acids composition represents the determining factor to calculate the exact value of the refractive index increments of proteins [10], knowing that the most index increments of proteins vary between 0.173 and 0.215 ml / g [10]. Likewise, the exact value of the refractive index increments permits the determination of interesting parameter giving information on the protein behaviour in solution is that the specific volume. The specific volume determination of is usually related to several models such as the Gladstone-Dale [15,16] and the Lorentz-Lorentz models [17–20].

Herein, we are interested in the investigation of the optical properties of the laccase from the determination of its real refractive index, its refractive index increment and its specific volume. We have chosen the laccase from fungus Tramates versicolor because the fact that their optical proprieties and denaturation process are rarely investigated even their frequent use in biotechnology where their applications vary from organic synthesis, biosensors and surface treatment.

We have followed the Wiener model [10,21,22] to determine the refractive index increment and Lorentz-Lorentz model to determine the specific volume [17–20]. The values of the refractive index increments and the specific volume of laccase relative to various solutions are obtained from the amino acids composition in the solid state. In our knowledge, there are no subsequent studies who are interested in the determination of the refractive index, the index increment and the variation of the specific volume of laccase for different pH, chemical and organic denaturants and in the presence of ionic liquids.

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Table 1
Samples description.

Chemical name	Source	Purity	Purification method
Laccase	Sigma Aldrich	–	None
Sodium acetate	Sigma Aldrich	99%	None
Phosphate buffer	Sigma Aldrich	99%	None
[pyrr][F]	Synthesized	99%	DURP
[morph][F]	Synthesized	99%	DURP
NaOH	Sigma Aldrich	98%	None
HCl	Sigma Aldrich	37%	None

[pyrr][F]: pyrrolidinium Formate.
[morph][F]: morphilinium Formate.
NaOH: Sodium hydroxide.
HCl: Hydrochloric acid.
DURP: Distillation Under Reduced Pressure.

2. Experimental

All the chemicals and synthesized samples description are given in Table 1. We have chosen a Laccase from fungus Trametes versicolor (EC 1.14.18.1/CASRN ¼ [80498-15-3]) and has a molecular weight of 57 kDa. The laccase is of the highest purity grade available from Sigma-Aldrich and used without further purification. The used laccase is powder and its activity is superior to 0.8 U / mg. This enzyme contains 4 copper atoms distributed in type 1, type 2 and type 3 sites. Ultra-pure water which has a specific conductivity of about 0.65 mS / cm was used to prepare all concentrations. The solution preparations were based on the "Cold Method" technique. The Cold Method was expanded from the preparation polymer aqueous solutions. It consists in the dissolving the enzyme in cold water where the temperature does not exceed 4 °C. All concentrations are prepared from a stock enzyme solution. The solutions were stirred for 3 h. After agitation, the solutions are equilibrated for 24 h at 4 °C.

In order to study the effect of pH, we have used buffer solutions for pH ranging from 2 to 9. To prepare solutions having pH < 6, we have used the Sodium Acetate of 0.1 M. For solutions having pH ranging between 6 and 8, we have used the Phosphate Buffer of 0.1 M.For the solutions having pH ≥ 9, we have used the Tris HCl of 0.1 M. The pH of the solutions was adjusted using the HCl and the NaOH.

The Guanidinuim Chloride (GdmCl) and the urea are used as chemical denaturants for concentrations ranging between 0.5 and 6 M. The phenol, the methanol and the acetonitrile are used as organic denaturants. The volumetric fractions added vary between 10% and 60% of the total volume of the laccase/water solution. All chemical and organic denaturants are Sigma-Aldrich products.

The Pyrrolidinium Formate ($C_5H_{11}NO_2$), and the Morpholinium Formate ($C_5H_{11}NO_3$), represent the ionic liquids used in this work. They are used for volumetric fractions ranging between 20% and 80% of the total volume of the laccase/water solution. The Pyrrolidinium Formate ([Pyrr][F]) and the Morpholinium Formate ([morph][F])are in their liquid state and have a hydrophilic character [23–25].

The Abbe refractometer is the instrument used to determine the refractive index of different solutions studied. The optical system relative to the refractometer is based on the measurement of the refraction limit at the interface between the prism having a refractive index of 1.7 and a liquid having a refractive index less than that of the prism. In this work, the Abbe refractometer has a wavelength of 589 nm. All values are measured with an accuracy of 10^{-4}.

All studied systems are reported at atmospheric pressure. All curves are treated using the software OriginPro (OriginLab/OriginPro 8.5, USA).

3. Theoretical background

To study the effect of solvents on the specific volume of laccase, first we have determined the partial specific volume, the refractivity per gram and the refractive index of the laccase in its solid state from the index data of amino acids related to laccase [26–29]. The refractive index relative to the chemical components is related to the molar refractivity R via the following equality [17]:

$$R = \frac{n^2 - 1}{n^2 + 2}\frac{M}{\rho} \quad (1)$$

For proteins, it was usually considered that $n = n_p$ and $R = R_p$. We recall that n_p is the refractive index of protein in its solid state, R_p is the refraction per gram defined as the weight average of the contribution from the individual amino acid R_a [10,26–30], ρ is the density and M is the molecular weight of the constituent. The refraction per gram R_p is given by:

$$R_p = \frac{\sum_a R_a M_a}{\sum_a M_a} \quad (2)$$

where, $\sum_a$ represents the summation over the number of the same amino acids. McMeekin et al. [26,27] have shown that the refraction per gram can be written as a function of the refractive index of protein in its solid state as:

$$R_p = \frac{n_p^2 - 1}{n_p^2 + 2} v_p \quad (3)$$

From Eq. (3), we can obtain the equation determining the refractive index n_p as a function of R_p and the partial specific volume of the protein v_p:

$$n_p = \sqrt{\frac{2R_p - v_p}{v_p - R_p}} \quad (4)$$

where, v_p is obtained from the amino acids composition using the following relation:

$$v_p = \frac{\sum_a v_a M_a}{\sum_a M_a} \quad (5)$$

Table 2 shows the obtained values of the refractive index of the laccase in its solid state, the refractivity per gram and the partial specific volume.

To determine the specific volume of protein and to follow the denaturation process, we have used the Lorentz-Lorentz model [18–20]. This model consists on the determination of the specific refractivity from the protein refractive index in its solid state. The specific refractivity according to Lorentz-Lorentz is given by:

$$R_{L-L} = \frac{n_p^2 - 1}{n_p^2 + 2} v_{sp} \quad (6)$$

For dilute protein solutions, Wiener [21,22] showed that the refractive index increment is related to the partial specific volume of protein by the equation:

$$\left(\frac{dn}{dc}\right)_{c\to 0} = \frac{3}{2} v_p n_m \frac{n_p^2 - n_m^2}{n_p^2 + 2n_m^2} \quad (7)$$

where, n_m is the refractive index relative to the solvent without laccase. Substituting Eq. (6) in Eq. (7), the refractive index increment is written as a function of the refractivity and the specific volume of the protein

Table 2
Numeric values of the specific volume variation obtained by using the UCSF Chimera 1.8.1 software for different pHs.

n_p	1.6255
R_p	0.2559 ml / g
v_p	0.7233 ml / g

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