A Novel Technique to Characterize Conformational State of the Proteins: p53 Analysis

Saad Abdullah, Mauro Serpelloni, Giulia Abate and Daniela Uberti

Abstract As the technology is advancing, biotechnologist and pharmacologist seems more interested and focused towards the development of innovative sensing solution/technology capable of evaluating proteins without any limitations of time and cost which were encountered/offered by conventional/traditional methods such as ELISA used for protein quantification. To allow continuous monitoring and attain protein sample information in a non-invasive way, spectrophotometry might be considered as an alternate method which analyzes different conformational states of proteins by closely observing the variation in optical properties of the sample. The work presented studies p53 protein conformational dynamics and their involvement in various pathophysiological and neurodegenerative disease/disorders using the spectrophotometer-based method. By utilizing the technique of spectrophotometry, investigations were carried out on three samples containing varied molecular state of p53 (Wild p53, Denatured p53, and Oxidized p53), to detect the difference in light absorption. Overall, this proposes the possibility of a simple, non-invasive and optical based method capable of detecting and identifying different structural states of p53 while overcoming the complexities offered by the conventional procedures.

Keywords Spectrophotometery · p53 · Protein · Absorbance spectrum
Infrared spectra · Protein detection

1 Introduction

The advancements in technology represent an active research area for the study of protein conformational dynamics which in turn can give valuable information regarding certain chronic diseases, like diabetes, cancer, and neurodegenerative disorders.

Therefore, efforts are made to combine newest achievements in the fields of material science, mathematics, engineering, and bioinformatics to design and develop a non-invasive technique for the purpose of investigating various protein conformational states [1]. Since a strong correlation exists between protein conformational states and biological functions [2], biomedical research in the domains of neurology and generiatics focuses at an early stage diagnosis of pathology via reliable identification of biomarkers, i.e., proteins [3].

Among the proteins, which experience specific conformational states to play a role in specific biological functions, the tumor suppressor phosphoprotein p53 is undoubtedly the more studied, due to its high conformational flexibility and the complexity of its biological functions. p53 is usually regarded as the 'guardian of the genome' or the 'cellular gatekeeper', and its significance is highlighted by the discovery of p53 mutations in more than 50% of all human tumors [4]. In fact, p53 plays a vital role in cellular responses to stressors by activating different cellular strategies that involved cell cycle arrest, DNA repairing or apoptosis depending on the intensity of the toxic stimuli. On the other hand, p53 is involved in physiological functions, such as regulation of metabolic pathway, redox homeostasis, and control of immune system [5]. p53 protein represents an interesting redox-sensitive protein involved in different pathophysiological processes, ranging from cancer to neurodegenerative diseases. It is located at the crossroads of complex networks of stress response pathways. Several extracellular or intercellular stresses evoke cellular responses directly or indirectly through activation of p53-redox modulation [6–8]. Many studies demonstrated that the interplay among p53 and Reactive Oxygen or Nitrogen Species (ROS/RNS) is crucial for the cellular fate.

The ability to identify the transitions from wild type to mutated one introduces an extremely promising starting point for developing new therapeutic approaches. Pertinent literature has revealed that the alterations in the conformational states of p53 protein are also associated with the onset of neurodegenerative diseases [9]. Considering all this, the ability to successfully and accurately discriminate different conformations of p53, possibly correlated with specific loss or gain of function, is of significant interest. Currently, biochemical assays (e.g., ELISA) provide information related to quantification of the protein only, but not to the conformational state of this protein.

Aiming to combine sensitivity with non-invasiveness and ease of methodology, spectrophotometry represents one of the most promising, widely used, analytical procedures in biochemistry. This method is based on the two laws of light absorption by solutions, namely Lambert's Law and Beer's Law which states: "the amount of energy absorbed or transmitted by a solution is proportional to the molar absorptivity of solution and the concentration of solute [10]." Beers Lambert law is mathematically expressed as:

$$A = eLc \tag{1}$$

where, A is the absorption, e the molar attenuation coefficient, L the path length and c the concentration of solution.

A literature review conducted shows the application of this concept used for analysis of proteins. The research study conducted by Zhou et al. [11] demonstrates discrimination of different conformational states pH dependent of BSA protein on the basis of difference in absorbance wavelengths. Similarly, infrared spectroscopy was used in [12] to measure different conformational states of protein.

In consideration to the results obtained with other protein, we develop a new methodology that utilizes spectrophotometry to characterize specific and characteristic absorbance spectrums related to different p53 protein conformational state, hence identifying its conformational states which can give new insight in different pathophysiological conditions. Therefore, an experiment was designed to detect the three structural states of p53 proteins namely wild type, oxidative and denatured state, obtained by following a protocol extensively described in the literature [13–15].

2 Methodology

2.1 Sample Preparation

In order to investigate the absorption of different conformational states of p53 protein, p53 wild type recombinant protein was exposed to different oxidant stressors to generate different redox-p53 products: (i) metal chelator agent that distrains Zn atom and induce the opening of the protein [16]; (ii) Fenton reaction, mainly mediated by the $OH\cdot$ derived from the decomposition of H_2O_2 in the presence of redox metals (Fe^{2+} and Cu^{+}) [17] generates a burst of oxygen radicals involved in protein oxidation. Thus, p53 recombinant protein was incubated for 1 h at 37 °C with the appropriate buffer: (i) 200 μM EDTA and 5 mM DTT (denatured p53); (ii) 10 mM H_2O_2 and 30 μM $FeSO_4$ (oxidized p53). PBS buffer solution alone was used as reference.

2.2 Spectrophotometer Testing

The spectrophotometry measurement was acquired at ambient conditions with Shimadzu UV-2600 system. The equipment has a measuring wavelength ranging from 185 to 1400 nm with a wavelength accuracy of ±0.3 nm. It used deuterium lamp and 50 W halogen lamp, which have a noise level of 0.00003 Abs RMS (500 nm) and adopted UV Probe application for operation of equipment.

The proposed method incorporates the use of spectrophotometer for recognizing the diverse conformations of p53 protein on the basis of distinct and distinguishing absorbance spectrum. The experimental setup employed for measuring the absorption of the three samples, containing the altered form of target protein, is shown in Fig. 1. For the purpose of holding altered form of p53 protein solutions 1 ml capacity quartz

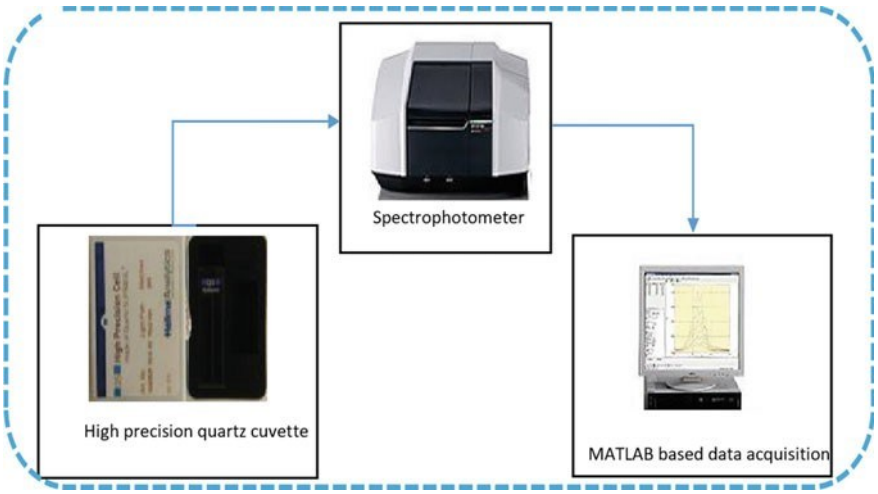


Fig. 1 Experimental setup

cuvette was used. The visual graphical trend of absorbance spectrum of each solution against the obtained data can be appreciated via MATLAB software.

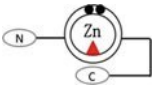
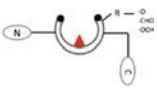
Quartz cuvette containing 1 ml of PBS buffer solution is set in the spectrophotometer to note the absorbance of the reference solution and to set the baseline for the experiment. The procedure is repeated with wide type, denatured type and oxidized states of p53 solution along with PBS as a reference solution to attain and record their absorbance respectively the experiment was repeated thrice to measure the repeatability of the results.

The absorbance data obtained from each solution prepared is then collected on which a MATLAB algorithm is applied to acquire a graphical absorbance spectrum for interpretation and obtain meaningful information related to absorbance of varied p53 protein conformational states.

3 Result

The different conformational states of p53 protein can be identified from their deviant absorbance spectrum obtained through spectrophotometer. The peak absorbance of different p53 protein solution are tabulated in Table 1. Furthermore, the absorbance region for each type of solution is highlighted in Fig. 2. The absorbance range for wild type p53 is identified to lie in the region of 205–206 nm whereas the major absorbance for denatured form of target protein is found within the region of 212–214 nm and shows a maximum peak at 213 nm. Also, a negative peak at around 235–245 nm can be appreciated for the denatured state of p53. On subtracting the reference absorbance spectrum of PBS buffer solution from the solution containing denatured kind of p53

Table 1 Absorbance wavelength of p53 Wildtype and p53 Denatured type and oxidized p53

Solution	Absorbance peak 1 (nm)	Absorbance peak 2 (nm)	Molecular orientation of p53
Wild p53	205	–	
Denatured p53	213	240	
Oxidized p53	230	274	

protein, a positive absorbance with a peak at 240 nm was observed. Moreover, for the oxidized p53 protein the absorbance region detected was found to be expressing in two regions (i) 225–235 nm with a peak absorbance at 230 nm and 270–278 nm with a peak absorbance at 274 nm.

It is evident from the obtained absorbance data shown in Table 1 and absorbance graphs indicated in Fig. 2 that absorbance varies with variation in molecular state of protein. Hence, the result obtained from spectrophotometer, enables to appreciate that each peculiar state of protein shows different absorbance spectrum thus allowing detection of various transitional state of p53 protein.

4 Discussions

For the purpose of investigating various molecular orientations of p53 protein, the technique of spectrophotometer was employed. The graph obtained using spectrophotometry approach shows optimum absorbance for wild form at 205 nm as appreciated from Fig. 2a. For denatured state of p53 protein, positive absorbance height is obtained at 213 nm, and a negative peak was found at 240 nm as indicated from Fig. 2b, c. Similarly, the two peak absorbance at 230 and 274 nm are shown in Fig. 2d were noted for oxidized form of target protein. Hence it is comprehensible from the results obtained that the technique of spectrophotometer can successfully identify various molecular orientations of the protein.

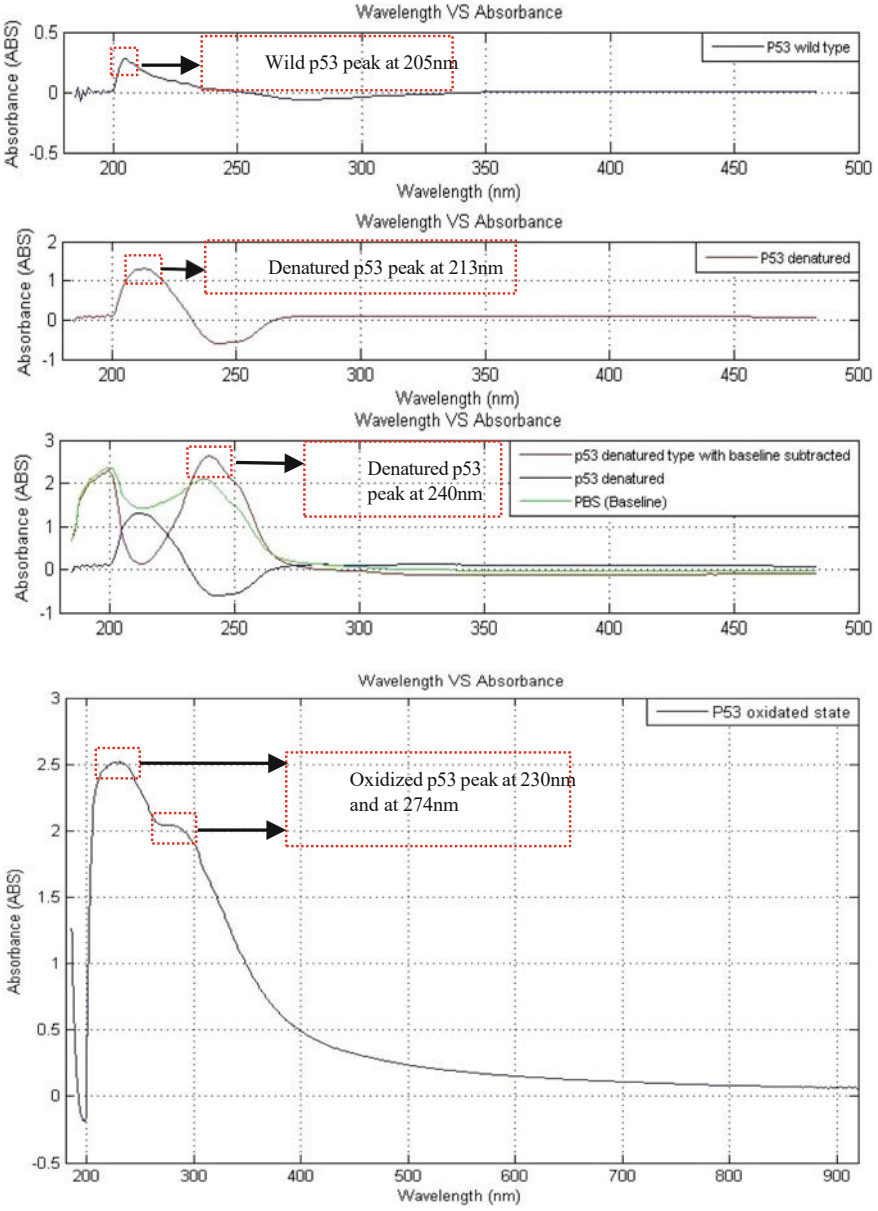


Fig. 2 a Absorbance graph of Wild p53. b Absorbance graph of Denatured p53. c Absorbance of denatured p53 with baseline subtracted. d Absorbance graph of oxidized p53

5 Conclusion

The proposed method of spectrophotometer yields distinguishable absorbance peaks for different p53 protein solutions thereby indicating the use of spectrophotometer technique as a novel and independent technology capable of detecting and distinguishing protein conformational states based on variations in absorbance spectrum. For wild p53, denatured p53 and oxidized p53 the absorbance peak wavelength noted are 205 nm, 213 nm and 240 nm, 230 nm and 274 nm respectively.

The positive results obtained from the technique of spectrophotometry has therefore proven to be a novel technological advancement in improving health care by enabling the identification and detection of different molecular orientations and modifications in protein structures hence assisting the clinicians to detect disease in its initial stages by identifying mutations and variations of biomarkers.

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