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Original Research Article

Effect of Human HSP90 on Secondary and Tertiary Structures of Core Protein of Hepatitis C Virus and HbsAg of Hepatitis B Virus

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HIGHLIGHTS

- The structure of recombinant protein is very important in vaccine complex with an adjuvant.
- The immunity of HCV core and HbsAg proteins could increase in complex with HSP90.
- Fluorescence spectroscopy and circular dichroism were used to determine the proteins structure.
- Structural data confirmed the hydrophobic properties if proteins changed in complexation with HSP90.

ABSTRACT

The secondary structure of recombinant proteins can change through complex formation with other proteins. Here, we have determined the spatial structure of two proteins, including core protein of hepatitis C virus and HbsAg of hepatitis B virus, without the effect of human HSP90 as well as with the effect of this recombinant chaperone. As a result, the increase in intensity from 297.5 to 346.64 was accompanied by different folding and being non-polar protein in complex with the chaperone. HbsAg protein, combined with HSP90, showed a reduction in the maximum peak wavelength from 385 to 369.07 nm. The property of protein of being non-polar and hydrophobic, as well as having an increase in intensity from 200 to 219, indicates the protein folding. The shift from 342 to 337 nm along with blue shift indicates hydrophobic properties and the removal of protein from the water environment.

Keywords:
Core protein
HbsAg
Circular dichroism

Introduction

The structure of the recombinant protein is always very important in vaccine complex with an adjuvant. In our previous project (Bandehpour et al., 2008), we have reached the increased immunity of HCV core and HbsAg proteins in complex with HSP90. HBV is a member of the hepadnavirus family prototype. The membrane proteins of HBV are encoded by the ORF S and located within lipid bilayer membrane of the virus. These proteins are involved in the binding to the receptors, the assembly of virus, and secretion. Three main proteins, L, M and S, were produced from one of the three genes, including pre-S1, pre-S2 and S (Liang, 2009). Six genotypes of HBV were divided into 9 subtypes from A to F in accordance with antigenic indexes of protein S. There was a difference of up to 4% between them. Hepatitis C is very similar to *pestiviruses* and *flaviviruses* with respect to the genomic and hydrophobicity profiles of polypeptides. This virus was divided into a separate strain from the *Flaviviridae* family. Hepatitis C has positive single-stranded RNA with 9,500 nucleotides. The first 191 amino acids formed the core protein with molecular weight 19 to 23 kDa (Knipe and Howley, 2013). At the same time, this protein entered the endoplasmic reticulum, and it was translated, and

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its signal peptide was isolated by peptidase located in the membrane and the mature form of core protein (179 amino acids) was released into the cytoplasm (Buratti et al., 1997).

In the present study, fluorescence spectroscopy and circular dichroism were used to determine the spatial structure of these two proteins, including core protein of hepatitis C virus and HbsAg of hepatitis B virus without the effect of human HSP90 as well as with the effect of this recombinant chaperone.

Materials and Methods

Materials

Core protein of hepatitis C virus, HbsAg of hepatitis B virus and the human HSP90 were previously expressed recombinantly and purified in our lab (Bandehpour et al., 2008). All other chemicals were supplied from Merck Chemical Company (Germany).

Determination of secondary and tertiary structures of core protein and HbsAg

The purified core and HbsAg proteins were investigated using Circular Dichroism Spectrometer (Model 215, company Aviv, USA). A few steps of dialysis with PBS, containing 4 M, 2 M, and 0.5 M urea, were used to remove urea from purified protein.

Tm determination of core protein and HbsAg

The folding of the protein structure was disturbed to study the chaperone effect of HSP90 on the proteins studied. For this purpose, heat was used. Tm is the temperature at which half the interest protein is deformed. Therefore, each of the proteins, core and HbsAg, was placed at temperatures 4 °C to 90 °C, and protein absorption was determined in the wavelength 280 nm with an interval of 5 degrees by spectrophotometer. The curve obtained from the temperature and absorbance (OD) was drawn for each protein. The Tm of protein is 4 to 5 degrees lower than the temperature at which the curve of protein is raised (Crowther et al., 2009). At first, 200 µg/ml of each protein was brought to the temperature of unfolding. Then, it was mixed with 10 µg/ml of HSP90, and incubated at 37 °C for an hour. The affinity chromatography was used to remove HSP90 from this mixture.

Study of protein secondary structure with circular dichroism (CD)

The secondary structure of proteins was determined in the region of Far-UV spectra with a range wavelength 190 to 250 nanometres. With this technique, the percentage of alpha helix, beta sheet and irregular structures (random coil) were determined in the totality of the molecule. 200 µl of each protein, with a concentration of 200 µg /ml in PBS buffer, was used.

Study of third structure with CD

The near-UV spectrum (e.g. 250 to 350 nm) was used to study protein tertiary structure. For this purpose, some 400 µl protein, with the concentration of 1 mg/ml seen, was examined. Data obtained from the study of protein structure using Circular Dichroism Spectrometer, Model 215, was analysed using the software CDNN (Circular Dichroism Nerve Network).

Fluorescence spectroscopy

Fluorescence spectroscopy was performed using a fluorometer (Jenway 62 series). The amino acids containing the cyclic substituent, such as tryptophan, tyrosine and phenylalanine, had the property of fluorescence. These amino acids were excited in the wavelengths 260 to 300 nm, and they emitted a wavelength of 300 to 400 nm. For this purpose, 600µl of protein with concentration 1 mg/ml rose. The excitation wavelength was entirely in 280 nm, and the emission on the main in wavelengths 300 to 400 nm. According to information obtained by this method, the charts of each protein were stable, using Microsoft Office Excel 2007.

Results

Study of secondary structure of core and HbsAg proteins by CD

As observed in Fig. 1, the chart identified by brown colour was the chart of alpha helix for core protein after exposure to HSP90 in vitro. A maximum and two minima were observed in the wavelength range of 200 nm and the range of 220 to 225 nm respectively (Fig. 1).

Protein HbsAg (blue graph) and the others reviewed in the chart obtained from far UV did not show any particular structure. This might be the result of several forms of the protein or the being as the membrane protein. The types of oligomers (electrophoresis of protein in non-denaturation condition) were so caused so that the suitable range of this protein did not obtain the secondary structure of core protein. However, the alpha form of this protein was achieved in the mixed form with chaperone and separation with affinity chromatography.

Data obtained from the study of proteins with near UV were studied with software CDNN. In the spectrum obtained from the core protein and core /HSP, the signals detected showed the change of waving protein and the position change of aromatic amino acids in protein structure. No signal was observed for HbsAg protein in the mixed form with HSP. Therefore, HSP did not have

Table 1. Percentage of secondary structure in proteins Core and HbsAg.

structure / protein	α-Helix (%)	β-Sheet (%)	Beta-turn (%)	Random coil (%)	Total sum (%)
Core	15	31.2	18.1	45.2	109.5
Core + HSP [1]	10.3	37.8	14.7	39.4	102.1
Core / HSP [2]	4.4	56.5	17.1	32.3	110.3
HbsAg	13.8	50.2	21.6	32.1	117.7
HbsAg + HSP [3]	6	59.8	15.3	29.9	110.9
HbsAg / HSP [4]	4.8	59.2	18.9	28.6	111.6

[1] The core protein was combined with chaperon in vitro.
[2] The core protein was expressed at the same time of chaperon.
[3] HbsAg protein was combined with chaperon in vitro.
[4] HbsAg protein was expressed at the same time of chaperon.

the effect on correct folding of the protein (Fig. 1).

Percentages of α-helix, β sheet, turn, and random coil in proteins structure of core and HbsAg

The alpha helix showed 68.7% and 43.47% decrease in the secondary fabric of both proteins, core and HbsAg, in the mixed form with HSP90. About 20% increase was reported in beta-sheet structures. When the expression of HSP90 proteins core and HbsAg occur in at the same time of chaperone HSP90, the 29.4% and 34.8% decreases were observed in the alpha structure. In contrast, 55% and 20% increases were found in the beta structure. Random and third structures did not show significant changes (Table 1).

Fluorescence study of the structures of proteins core and core + HSP90

The proteins had three amino acids with fluorescent characteristics. They were tyrosine, phenylalanine, and tryptophan. The highest emission was observed in tryptophan. The absorption wavelength of this amino acid was higher than other amino acids. The energy released by phenylalanine and tyrosine often transferred to the tryptophan in the same protein. The excitation and emission of these proteins were studied by fluorometer respectively in the wavelengths of 280 nm and the range of 300–400 nm.

As observed in Fig. 2, drawn from the results obtained by Eclipse software, the decrease in wavelength (blue shift) in the tryptophan range was seen from 342 to 337 nm, as the pure form of core protein was compared with this protein in the mixed form with the chaperone. This result was so represented that this protein in the mixed form with chaperone had more hydrophobicity than the previous form.

The intensity also increased from 297.5 to 346.64, indicating the folding change of the protein in the mixed form with the chaperone (Fig. 2).

Fluorescence study of the protein structures of HbsAg and HbsAg + HSP90

According to the graph obtained from the study of HbsAg protein with the mixed form with HSP90 by Eclipse software, the decrease in wavelength was observed from 385 to 369.07 nm or blue shift in the chart of tryptophan. Non-polar and hydrophobicity of protein, as well as the increase in the intensity of the 200.021 to 219.581, indicated that there were changes in the folding of the protein (Fig. 3).

Discussion

HSP90 chaperones are used for the stability and activity of a diverse range of proteins (Zuehlke and Johnson, 2010). HSP90 had the monomer and homodimer states at 37 °C. In this condition, it showed a little tendency of binding to peptides. By raising the temperature to 60 °C, it formed oligomers and their binding affinity was increased (Thorne and McQuade, 2004). In this study, HSP90 was evaluated by electrophoresis on SDS-PAGE gel at 37 °C. As found in a study (Nakai et al., 2006), protein purified by affinity chromatography formed multimeric states. In this study, 191 amino acids of this protein were used. It is known that the total sequence of this protein influenced the formation of its spatial structure. Owing to S and poly-histidine peptides, affinity chromatography was used to purify proteins core and HbsAg. Poly-histidine sequences had the highest application in the preparation and purification of recombinant proteins. Doody et al. (2004) found that glycoprotein 96 is capable of forming twisting in MHC I and MHC II proteins and plays a role in antigen presentation. Kunkel and Watowich have investigated the biophysical characterization of the core protein of HCV virus. They found that this protein was present as

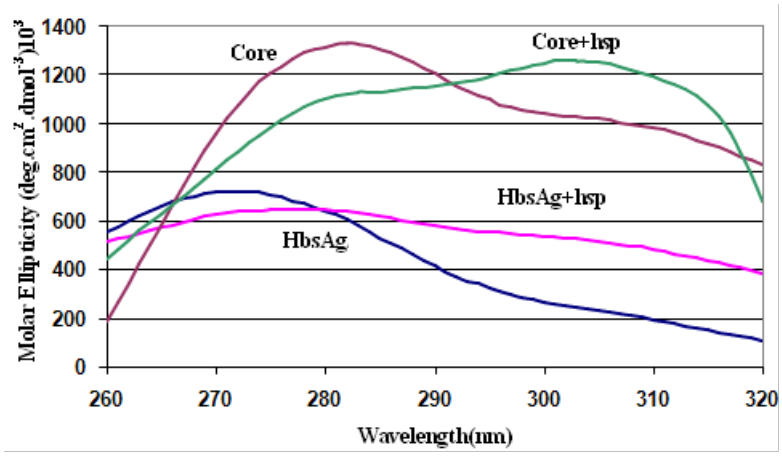

Figure 1. Near UV CD spectra of proteins Hbs and core in phosphate buffer pH 7.4.

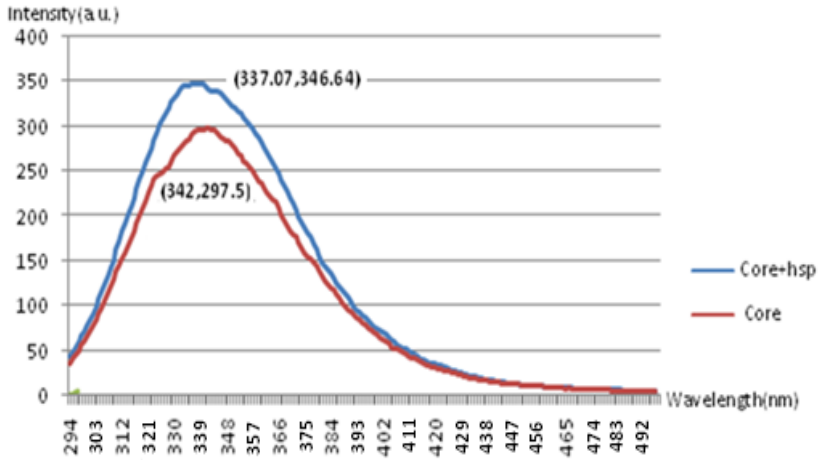

Figure 2. Fluorescence emission spectra of pure core protein (red) and the protein mixed with chaperon with ratio 5% (blue).

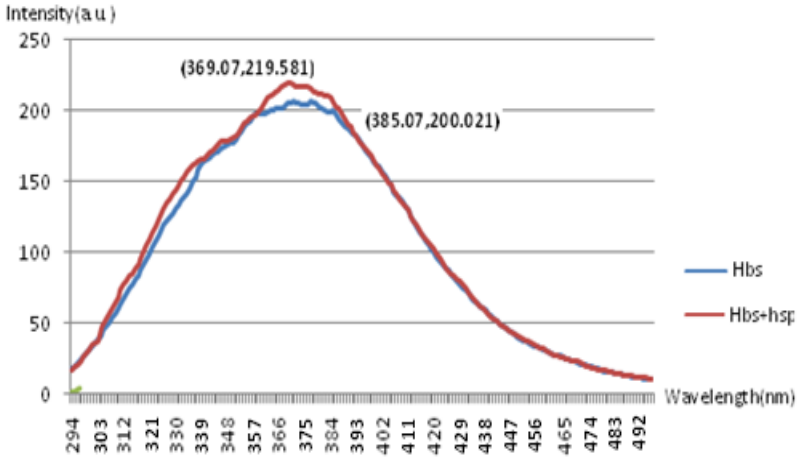

Figure 3. Fluorescence emission spectra of pure HbsAg protein (blue) and the protein mixed with chaperon with 5% ratio (red).

large multimers in physiological conditions. The study by Far-CD showed that this protein had the strong secondary structure (Kunkel and Watowich, 2004). Our review also indicates that the noise on charts Far-CD was consistent with this subject. The fluorescence spectroscopy and near-UV showed that the regions rich in tryptophan (amino acids 76 to 113) were ultimately located on the surface of the protein, and they were in contact with the solution. The curve obtained from near-UV of core protein showed a signal for tyrosine (285 nm). The signals of tyrosine and tryptophan(290–300nm) were observed in the curve of core/HSP, indicating that these proteins folded and the aromatic amino acids exposed to the solution. Regression analyses of the CD in protein secondary structure showed that there were 16% alpha helix, 29% beta-sheets as well as turns and 55% random twists in the studied protein. The results obtained from this study were consistent with our results. There were 15% alpha helix, 33.2% beta-sheets and 18.1% turns and 45.2% random twists. Some percentage of differences may be the result of changes in the sequence. Li et al. found that the heat, ions, vertex and adsorption increased the hydrophobic levels and aggregation of HbsAg protein. After these changes, the percentage of α-helix decreased from 48.2% to 34.4% and γ-turn increased from 29.6% to 38.7% (Li et al., 2007). We found that the secondary structure of proteins core and HbsAg, combined with HSP90, showed 68.7% and 43.47% reductions in alpha-helix structure. However, when they expressed at the same time, 29.4% and 34.8% reductions were observed in alpha helix structure. They showed 55% and 20% increases in beta-sheet structures. Random structures and turns show no significant changes. The comparison ofthe optimal effect of chaperones on protein structure indicated that the aromatic amino acids like tryptophan showed the reduction in the peak of fluorescence graph in pure form. The shift from 342 to 337 nm along with blue shift caused the protein to show hydrophobic properties and removed from the water environment. As a result, the increase of intensity from 297.5 to346.64 was accompanied with folding and being non-polar of protein in form mixed with a chaperone. HbsAg protein, combined with HSP, showed the reduction of peak wavelength from 385 to 369.07 nm. The non-polar and hydrophobic properties of protein, as well as the increase in intensity from 200 to 219, indicated the protein folding. The shift from 342 to 337 nm along with blue shift caused that the protein showed hydrophobic properties when removed from the water environment.

Conclusion

In the present study, the HSP90 that was introduced as adjuvant in vaccine formulation has a significant effect on core protein of hepatitis C virus and HbsAg of hepatitis B virus structures.

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Competing Interests

The authors declared that there are no competing interests**.**

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