Beer Consumption and Changes in Stability of Human Serum Proteins

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The aim of this study was to evaluate the influence of beer consumption (BC) on the functional and structural properties of human serum proteins (HSP). Thirty-eight volunteers (after coronary bypass) were divided into two groups: experimental (EG) and control (CG). Nineteen volunteers of the EG consumed 330 mL per day of beer (about 20 g of alcohol) for 30 consecutive days. The CG volunteers consumed mineral water instead of beer. Blood samples were collected from EG and CG patients before and after the experiment. Albumin (Alb), globulin (Glo), and methanol-precipitable proteins (MPP) from human serum were denatured with 8 M urea. Fluorescence and electrophoresis were employed in order to elucidate urea-induced conformational changes and structural behavior of proteins. The measured fluorescence emission spectra were used to estimate the stability of native and denatured protein fractions before and after BC. It was found that before BC the fractions most stable to urea denaturation were Glo, Alb, and MPP fractions. After BC in most of the beer-consuming patients (EG) some changes in native and denatured protein fractions were detected: a tendency to lower stability and minor structural deviations. These qualitative changes were more profound in MPP than in Alb and Glo. Thus, Glo is more resistible to alcohol influence than Alb, which in turn is more resistible than MPP. No serum protein changes were detected in patients of CG.

Keywords: *Beer consumption; human serum proteins; fluorescence; electrophoresis; denaturation*

INTRODUCTION

Alcoholic beverages were a food throughout the ages and are still an integral part of diets in most countries of Western civilization (*1*). At present, alcoholic beverages account for about 4 to 6% of the average energy intake (*2*). It was found that alcohol intake has a positive influence on coronary artery disease (CAD) (*3*−*7*). The rates of death from all cardiovascular diseases for men and women between the ages of 35 and 69 were 30 to 40% lower among those reporting consumption of one drink daily than the rates for nondrinkers (*8*). The above-mentioned and other similar investigations are some of the reasons for the increase of alcohol consumption. This is despite the common knowledge that alcohol consumption also has adverse effects. It was reported that alcoholic beverage consumption negatively influences protein metabolism (*9*, *10*). Some authors have found that acute ethanol dosage reduced synthesis rates of intestinal contractile proteins (*11*). The effect of ethanol on skeleton muscle and finding of an impaired synthesis of protein has also been described (*12*).

Normal conditions of human serum proteins are very important for pharmaceutics. Binding of new chemical entities to serum proteins is an issue confronting pharmaceutical companies during development of potential therapeutic agents. Most drugs bind to the most abundant plasma protein, human serum albumin (HSA), at two major binding sites.

The relationship between physicochemical characteristics of proteins was extensively investigated on bovine serum albumin as a model protein. The important role of this protein was detected, showing that albumin itself can act as an antioxidant (*13*, *14*). Oxidation of serum protein thiol groups in systems generates peroxyl free radicals (*13*, *15*).

The effect of alcohol consumption on human serum proteins has not been investigated. To the best of our knowledge, there are no reports showing the possible changes in human serum proteins in patients after consumption of alcoholic beverages. Therefore, we decided to study the influence of the most popular alcoholic beverage, beer, on functional and structural properties

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of human serum proteins (albumins, globulins, and methanol-precipitable proteins) in patients with coronary artery disease (CAD) and normal levels of serum lipids.

To investigate urea-induced conformational changes and the structural behavior of HSP, fluorescence and electrophoresis were used.

MATERIALS AND METHODS

Subjects. The study population was recruited from patients undergoing coronary bypass surgery (because of CAD) in the Institute of Cardiology in the University Medical Center, Rehovot, Israel. Male patients (38 in number) with normal levels of serum lipids were divided into two equal-number groups: experimental (EG) and control (CG). The diet for the 19 patients of the EG was supplemented once a day with 330 mL of Maccabee beer (about 20 g of alcohol) for 30 consecutive days. Patients of the CG (19 in number) consumed, instead of beer, 330 mL of the mineral water Netivot. Before and after completion of the experiment the serum samples of all patients were examined.

Chemicals. Urea, sodium dodecyl sulfate (SDS), and all other reagents used for this study were reagent-grade chemicals from Sigma. All reagents were used without further purification. Deionized distilled water was used throughout.

Methods. Serum proteins before and after BC were separated into three groups by 2 M ammonium sulfate (Alb), 4 M ammonium sulfate (Glo), and methanol-precipitable proteins (MPP). Then, the precipitated fractions were dialyzed against water and freeze-dried. Protein assays were performed by the Lowry method (*16*), and human serum albumin (HSA) was used as a standard. The protein concentration corresponded to the absorbance less than 0.1 in a 1-cm path length. This range of concentration guarantees a linear increase in the relative fluorescence intensity. Absorbance values were measured using a Uvikon 930 UV spectrophotometer (Kontron AG Instruments, Zürich, Switzerland). Intrinsic fluorescence measurements of proteins were done using a Model FP-770 Jasco-Spectrofluorometer (Japan Spectroscopic Co., Ltd., Hachioji City, Japan). Proteins were dissolved in 0.01 M phosphate buffer, pH 7.2. For fluorescence measurements, protein solutions were 0.15 mg/mL. Fluorescence emission spectra were determined at excitation wavelengths (nm) of 274 and 295 and recorded from the excitation wavelength to wavelength of 450 nm (*17, 18*). A thermostatically controlled cell holder kept the temperature of the samples at 30 °C.

Urea Treatment. Treatment of serum proteins involved the addition of denaturants to the protein solutions in concentrations (M) such as: urea − 0, 2, 4, 6, and 8. Denaturation was determined after incubation of protein with denaturants for 1 h. All data were determined in triplicate for all experimental conditions.

Electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to Laemmli (*19*) in 8% gels. The protein samples were applied at a rate of 15 μL per lane. The gels were stained in a 0.1% Coomassie brilliant blue. The scan of the gel was done using Bio-Rad Multi-Analyst TM/PC, version 1.1. Dalton Mark VII-L (SDS-7) with molecular weights in kDa (14.2, 20.1, 24.0, 29.0, 36.0, 45.0, and 66.0) was used.

RESULTS AND DISCUSSION

In all patients after BC a change in protein amounts was not found (data not shown). To reveal the possible qualitative changes, serum proteins of both groups of patients were applied to one-dimensional SDS-gels (Figure 1). The proteins migrated as four bands at apparent molecular weights of 16, 36, 45, and 66 kDa. Some minor differences in the serum protein bands were detected in the range between 45 and 80 kDa in 12 out of 19 patients from EG. These additional bands appeared after BC in lanes 3, 5, and 7. The highest amount in all protein fractions was concentrated between 36 and 45 kDa. Our results are in complete agreement with the results of others (*20*), and correspond to the molecular weights of human serum albumin of 66 kDa, to methanol-precipitable fraction (fibrinogen-like) of 340 kDa, with chains of α-66, β-52, and γ-46 and γ-globulin of 150 kDa.

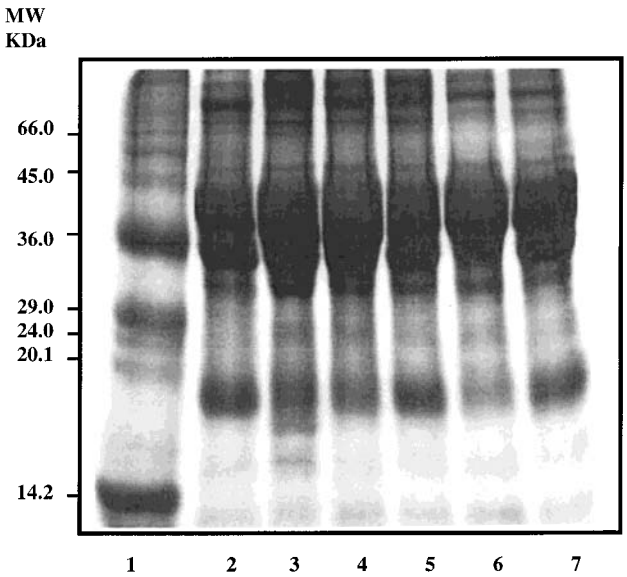

Figure 1. SDS-polyacrylamide gels of human serum albumin (Alb), globulin (Glo), and methanol-precipitable proteins (MPP) before and after beer consumption (BC) in 8% gel. 1, molecular marker (14.2, 20.1, 24.0, 29.0, 36.0, 45.0, and 66.0 kDa); 2, Alb before BC; 3, Alb after BC; 4, Glo before BC; 5, Glo after BC; 6, MPP before BC; 7, MPP after BC. Coomassie-stained gel. To each lane was applied 15 μL of protein.

SDS−PAGE did not reveal any major differences between protein fractions, therefore fluorescence was used for further studies.

The spectra exhibited a tryptophan-characteristic intrinsic fluorescence with maximum (nm) excitation at 295 nm (Figure 2). The emission peak centered for Alb before BC at 337 nm and after BC at 334 nm and showed a decrease in fluorescence intensity of 2.5 times (Figure 2, curves 3 and 6). At 274 nm (Figure 3, curves 3 and 6) the spectra showed peaks at 337.3 nm and 334.6 nm and a decrease in intensity as reported above for 295 nm.

The spectra of fractions that had been denatured with 8 M urea before and after BC are shown in Figures 4 and 5. The Alb fraction before and after BC (Figure 4, curves 3 and 6) at 295 nm showed peaks (nm) at 342.5 and 357.7, respectively, and a decrease in fluorescence intensity (FI) of about twice. For 274 nm (Figure 5, curves 2 and 6) two peaks appeared at 343.3 nm and 341.8 nm and showed the same decrease in FI as was shown at 295 nm. The Alb fraction at 295 nm represents in all spectra in Figures 2−5 an emission peak with a significant quantity of tryptophan. This fraction at excitation wavelength of 274 nm shows a shoulder at 305−308 nm which corresponds to that of tyrosine residues. Also, the emission wavelength at 357 nm corresponds to an unfolded protein structure where all tryptophan residues are situated in aqueous medium. The emission wavelength of 338−336.5 nm describes also a folded protein structure. Our results are in agreement with others who reported that in a urea-induced denatured state of Alb, a red-shift in the wavelength of maximum fluorescence occurred over

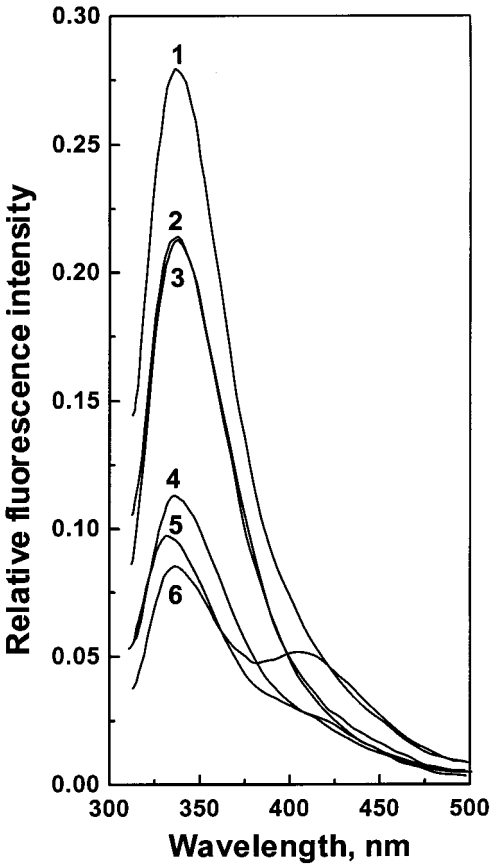

Figure 2. Change of native human serum albumin (Alb), human serum globulin (Glo), and human serum methanol-precipitable proteins (MPP) fluorescence spectra as a function of beer consumption (BC) at λ_{exc} 295 nm: 3, Alb before BC; 6, Alb after BC; 1, Glo before BC; 4, Glo after BC; 2, MPP before BC; 5, MPP before BC. All samples were dissolved in 0.01 M phosphate buffer at pH 7.2. The protein concentration was 0.15 mg/mL at 30 °C.

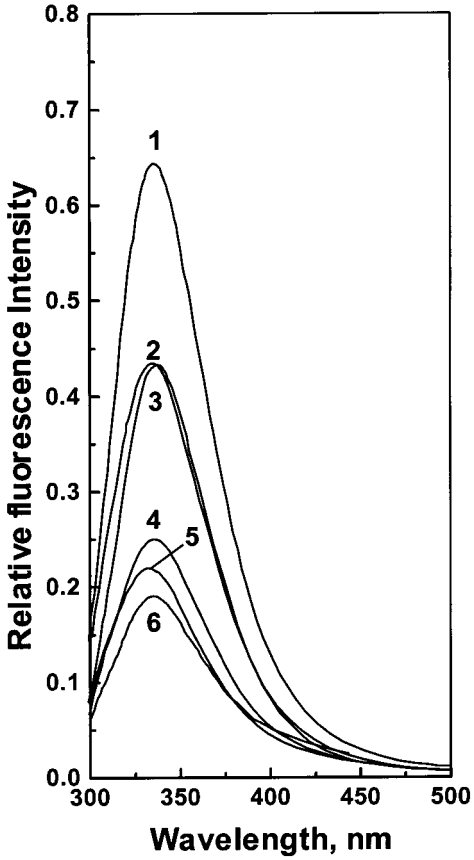

Figure 3. Change of native human serum albumin (Alb), human serum globulin (Glo), and human serum methanol-precipitable proteins (MPP) fluorescence spectra as a function of beer consumption (BC) at λ_{exc} 274 nm: 3, Alb before BC; 6, Alb after BC; 1, Glo before BC; 4, Glo after BC; 2, MPP before BC; 5, MPP before BC. All samples were dissolved in 0.01 M phosphate buffer at pH 7.2. The protein concentration was 0.15 mg/mL at 30 °C.

urea concentrations ranging from 4 to 6 M (*21*−*24*). This shift indicated that a structural change in domain II occurred simultaneously with the unfolding of domain III in this concentration range. In the presence of 8 M urea, a shift to a more polar environment takes place (336.5−345.5 nm). Our results are in agreement with others that albumin can withstand 8 M urea even at 44 °C with temporary loss of an α-helix, but without irreversible change (*25*−*29*).

Glo before BC showed an emission peak (336.0 nm) and a decrease in the intensity of about twice (Figure 2, curves 1 and 4). At 274 nm (Figure 3, curves 1 and 4) the peaks were at 335.5 and 334.9 nm, with a decrease in FI of 2.4 times. For the Glo fraction, the shift in the emission wavelength upon denaturation with urea was without quenching of tryptophan emission and increase of FI. So, tryptophan emission was not quenched after unfolding in the presence of water. Emission of Glo fraction at 295 nm was less intense than the combined emission of tryptophan and tyrosine at λ ex = 274 nm. Emission wavelength about 337 nm shows that proteins are in their folded structure and tryptophan residues have no contact with water. At λ ex = 274 nm, a clear shoulder at 309 nm that corresponds to the tyrosine residues in the protein fraction was observed. Addition of 8 M urea brings to the shift in emission wavelength from 337 to 346 nm an increase in intensity. Glo before and after BC (Figure 4, curves 2 and 1) showed the maxima of peaks at (nm) 346.4 and 355.9 with a shift to the left side of 9.5 nm and slight increase in the intensity. At 274 nm the peaks corresponded to 341.3 and 333.4 with a shift of 7.9 nm and a decrease in the intensity (Figure 5, curves 1 and 4).

Methanol fraction (MPP) at 295 nm showed the maxima at 337.8 and 331.7 with a shift of 6.1 nm and a decrease in intensity of 2.3 times (Figure 2, curves 2 and 5). MPP at 274 nm showed the peaks (nm) at 334.5 and 332.1 with a shift of 2.4 nm and a decrease in the intensity twice (Figure 3, curves 2 and 5). MPP before and after BC (Figure 4, curves 4 and 5) showed peaks at 343.6 and 340.6 nm and a decrease in FI of 1.7 times. At 274 nm the peaks were at 344 and 337 with a shift of 6 nm and a decrease in FI of 1.5 times (Figure 5, curves 3 and 5).

For MPP there is a clear shoulder at 308 nm at 274 nm that corresponds to tyrosine emission (even two distinct peaks at 305−309 and 336.5 nm). This means that the distance between tyrosine and tryptophan residues is enough to receive two emission peaks for these two residues. Emission wavelength at 336.5 nm describes the folded protein structure where tryptophan residues are situated in the interior of protein molecule.

Comparison of all these fractions before and after BC shows the same range of changes in Alb and Glo. Only in MPP was there registered a bigger shift than in Alb and Glo, and this fraction was more stable. The change in the intensity of all samples after beer consumption can be explained by the structural changes of proteins.

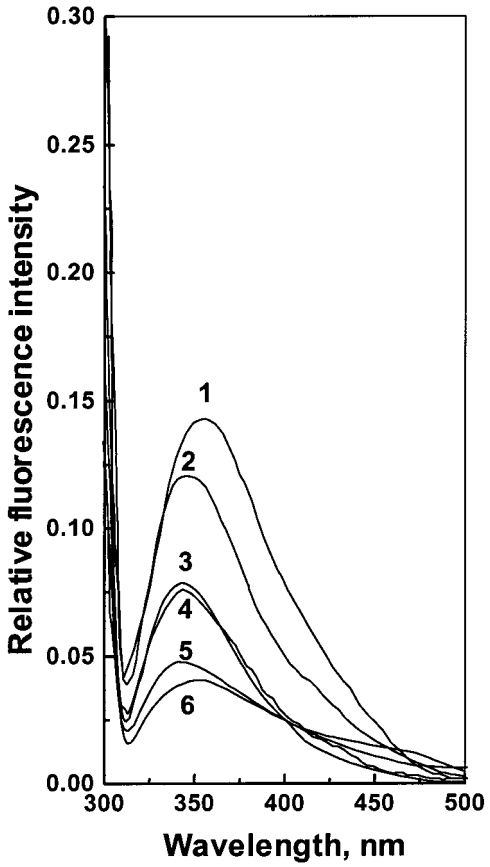

Figure 4. Change of denatured-with-8-M-urea human serum albumin (Alb), human serum globulin (Glo), and human serum methanol-precipitable proteins (MPP) fluorescence spectra as a function of beer consumption (BC) at λ_{exc} 295 nm: 3, human serum albumin with urea (AlbU) before BC; 6, AlbU after BC. 2, human serum globulin with urea (GloU) before BC; 1, GloU after BC; 4, human serum methanol-precipitable with urea (MPPU) before BC; 5, MPPU after BC. All samples were dissolved in 0.01 M phosphate buffer at pH 7.2. The protein concentration was 0.15 mg/mL at 30 °C.

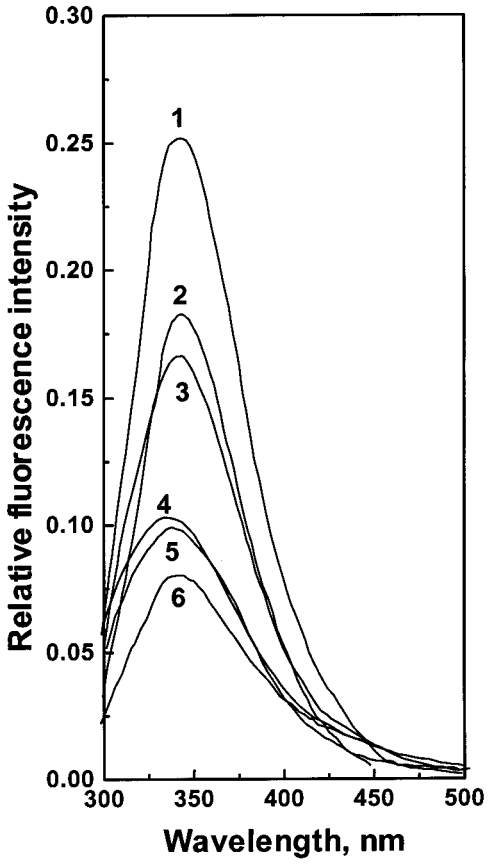

Figure 5. Change of denatured-with-8-M-urea human serum albumin (Alb), human serum globulin (Glo), and human serum methanol-precipitable proteins (MPP) fluorescence spectra as a function of beer consumption (BC) at λ_{exc} 274 nm: 2, AlbU before BC; 6, AlbU after BC; 1, GloU before BC; 4, GloU after BC; 3, MPPU before BC; 5, MPPU after BC. All samples were dissolved in 0.01 M phosphate buffer at pH 7.2. The protein concentration was 0.15 mg/mL at 30 °C.

Denaturation for Glo, Alb, and MPP with 8 M urea brings a shift in the emission wavelength. The fluorescence intensity gradually increased with the increase in urea concentration. The difference in the extent of denaturation between the protein fractions may be explained by the differences in amounts of amino acids and by the sulfur bridges existing in such proteins.

Denatured fractions after BC show that the most stable fraction is methanol. Comparison of the fractions before BC gives the following order: Glo> Alb > MPP.

These results are in agreement with the results of others, indicating that urea interacts with proteins by electrostatic forces, yielding a randomly coiled conformation in its unfolded state (*27*, *30*, *31*). The free-energy change of unfolding versus concentration of urea suggests that the variation of the electrical charge of proteins influences the final state of the unfolded form of the protein.

The effectiveness of a protein in stabilizing an interface is influenced by its amino acid profile, and its structure, hydrophobicity, and hydrophilicity ratio. Alteration of the native protein structure via urea treatment (8 M) increased the surface activity of the protein, as the modified protein decreased surface tension to a greater extent and at a faster rate than the modified protein. The more hydrophobic the protein, the greater its ability to reduce interfacial tension (*32*−*34*).

After beer consumption, a decrease in fluorescence intensity and a shift in the maximum of emission, which reflected unfolding of proteins, was observed. Intrinsic measurements show that the urea-denatured state before BC is relatively compact compared to that after BC. Similar changes were found in serum proteins in CAD patients with high levels of lipids (*35*). As was mentioned in the Introduction, normal conditions of human serum proteins are very important for pharmaceutics. The minor changes in serum proteins which were found in this investigation cannot be an obstacle in binding of drugs.

In conclusion, a short term of moderate beer consumption in CAD patients does not induce any quantitative changes in serum proteins. After BC, in most of the beer-consuming patients (EG), the qualitative changes were expressed in the lower stability and some minor deviations in the protein electrophoretic bands.

ABBREVIATIONS USED

Alb, albumin; BC, beer consumption; CAD, coronary artery disease; Glo, globulin; HSP, human serum proteins; MPP, methanol-precipitable proteins; SDS, sodium dodecyl sulfate; AlbU, human serum albumin denatured with urea; GloU, human serum globulin denatured with urea; MPPU, human serum methanol-precipitable proteins denatured with urea.

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