Correlation between Absorbed Dose and Solubility of γ-Irradiated Bovine Bone

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Abstract

Results of studies on water solutions of irradiated bovine bone are presented in the paper. Irradiated bone becomes brittle and soluble in water. Studies on solubility, pH, electric conductivity and viscosity of bone solutions indicate that bone solubility depends on dose, time and temperature of dissolution. In the dose range of 10-100 kGy it is mainly that the inorganic phase of bone dissolves. The highest relative viscosity was measured at 70°C for the dose of 50kGy. For doses higher than 100 kGy, the mass of dissolved proteins (mainly collagen) is higher than the mass of the dissolved mineral part. Two local maxima of relative viscosity were found. The higher the dose, the more "flattened" the viscosity maximum.

Keywords: bone, dissolubility, γ-radiation, viscosity, conductance

Introduction

The γ-irradiated biological materials such as skin and bone change their physico-chemical properties. Bone belongs to the multiphase, non-homogenous materials. The mineral phase contains hydroxyapatite (HAP) and water. The organic phase is made up in about 30-35 % of dry bone. This phase contains about 90% of collagen [1]. Three components of bone, which are HAP, water and collagen differ in their physical properties. They form the complex structure of bone, which is distinct in its physical properties from the individual components. In spite of this, the radiative changes in biological materials are often characterised on the basis of the studies on the individual components [2-5].

A diverse studies on water solution of dissolved irradiate bone make it possible to precisely describe biological changes caused by ionising radiation [6-8]. In this study the bovine bone was irradiated in the air for a wide range of doses from 10 to 1000 kGy. Even though the temperature dependence of viscosity is the method accepted in studies on biological materials, except for [6-8] there is no available data on viscosity of irradiated, dissolved animal bone. Hence, the purpose of this study was to characterise the dynamics of the dissolution process of powdered bone using the temperature dependence of relative viscosity. The results of studies on temperature dependence of viscosity presented in the paper enable one to deepen understanding of the dissolution of irradiated bone and conclude on direct and indirect action of ionizing radiation.

Experimental procedures

The material used in the study, the irradiation process and the procedure of bone dissolution are described elsewhere [6-8]. Bone samples of mass of about 5g each, were γ-irradiated (^{60}Co, average photon energy of 1,2MeV) in the air. Irradiation details were described earlier [6-8]. Viscosity of water solutions of irradiated bone were measured in the shear rates range of 30-60s^{-1}, using a rotary-oscillating viscometer Contraves LS40, in the temperature range of 25-95°C. At the given temperature which was controlled with the accuracy of ±0.1°C, the viscosity was measured twice, during increasing and decreasing shear rate.

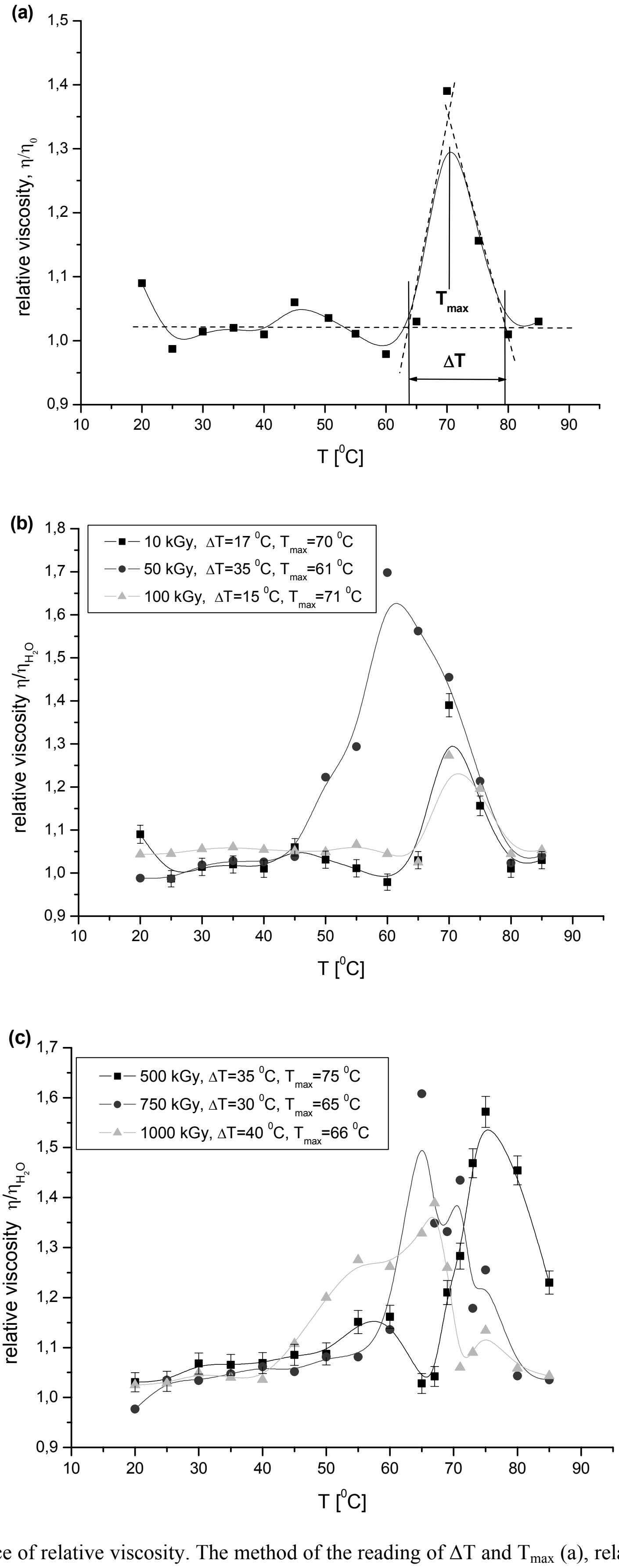

Fig. 1. Temperature dependence of relative viscosity. The method of the reading of ΔT and T_{max} (a), relative viscosity vs. temperature for doses of 10, 50, 100 kGy (b) and for doses of 500, 750, 100 kGy (c)

Results

The method of reading T_{max} of the maximum viscosity, and the temperature range ΔT where the changes in viscosity were observed are shown in Fig. 1a. The accuracy of temperature and temperature range estimation were ± 1°C and ± 2°C, respectively. The temperature dependence of the relative viscosity (η/η_0) is shown in Fig. 1b. The presented data concern the dose of 100 kGy.

The maximum viscosity of bone solutions irradiated with the dose of 10 and 100 kGy was observed at the temperature of 70°C. Temperature ranges found for the both doses were similar too. For the dose of 50 kGy the η/η_0–T curve was flattened out. The maximal relative viscosity was lower than for other doses and at lower temperature that is 63°C. The temperature range, which is a measure of the peak width, was bigger than for other doses.

The η/η_0–T curves for other doses that is for 500, 750 and 1000 kGy are presented in Fig. 1c. Increase in the dose widens the ΔT to 40°C. For better transparency, the measurement errors are shown only for one solution.

Discussion of results

As it was reported [6] the mass of the dissolved bone increases linearly with the logarithm of dose. It was stated that for the dose of 10, 25, 50 and 100 kGy the concentration of the mineral phase of bone was higher than the concentration of the organic /protein/ phase. The opposite situation was observed for doses higher than 100 kGy. Not only ionizing radiation does breaking of the peptide bonds in proteins and degradation of the polypeptides chains, it causes formation of new cross-linking bonds as well. According to many studies, cross-linking, dominates for doses lower than 100 kGy. Therefore, collagen irradiated with such doses is less soluble. A piece of information on degree of protein destruction is obtained from studies on temperature dependence of viscosity. The increase in viscosity of the dissolved bone with increasing temperature is most probably caused by the thermal denaturation of collagen which is the main protein of bone. The range of temperature interval /ΔT/ where the change in viscosity was observed is probably attributed to the dissolved homogenous protein. The increase in the ΔT signified the decrease in the protein homogeneity, that is proteins of different polypeptide chain lengths, different states of aggregation and different conformations are present in the solution. The shift of the T_{max} toward lower temperatures is the result of the increase in the number of broken bonds in polypeptide chain, and cross-linking bonds between different helices which form the collagen macromolecule while the helical structure of collagen is preserved. The above statements are based on the previous studies on electric conductivity, pH, and viscosity [6-8]. The highest value of the relative viscosity η/η_0=1.6±0.1) was obtained for bone irradiated with the dose of 50 kGy at the temperature T_{max}=(61±1)°C. Simultaneously the ΔT=35°C is bigger than for the doses of 10 and 100 kGy. The dose of 50 kGy probably contributes to formation of the biggest number of cross-linking bonds, among other to formation disulfide bridges, which stiffen collagen structure. The space structure of a such protein may be similar to spheroid. The longer the elispode the higher the viscosity [9]. Moreover, at 20°C, the pH=7.35, is higher than isoelectric point which indicates domination of the negative electric charge of molecules. Because electric charge appears in specific places on protein surface, the number of electrically charged places is connected with the number of broken bonds in polypeptide chain caused by γ radiation. The charged groups may attract water molecules which form the hydration envelope [10]. The higher the number of electrically charged places, the bigger the hydration envelope. As a result in a solution the diversity of molecules is present and causes big changes in the ΔT and high value of relative viscosity.

The increase in the dose over 100 kGy to 1000 kGy caused the rapid increase in the protein concentration in the solution [6]. The triple increase in concentration is caused by the domination of the degradation process. The consecutive maxima appear on the relative viscosity-temperature curve at the temperature of 40 °C and 85°C. Probably, individual maxima of relative viscosity correspond to group of macromolecules burdened with similar degree of damage. The mean value of pH=7.9 at 20°C increases to pH=8.0 at 80°C which in practice means not a large change in the concentration of H_3O^+ in the solution. The damage of protein is so big that even at lower temperatures the secondary and tertiary structure of collagen is destroyed.

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