DC Electrical Conductivity in Studies on Solid–State Proteins

L. Kubisz and D. Hojan-Jezierska

Department of Biophysics, Poznan University of Medical Sciences, Fredry 10, 61-701 Poznań, Poland

In structural studies on biological materials, among other methods, electrical techniques are used widely. Temperature dependence of electrical conductivity is a method permitting studies on denaturation, glass transition and water release — processes, which occur in solid–state proteins. Variations of amplitude and temperature of the peak on the recorded thermogram make it possible to draw conclusions about thermal stability and physicochemical processes occurring in the studied biological material. The shape of experimental curve is material–related and depends upon its "history". The paper is based on experimental results obtained mainly for collagen.

PACS numbers: 82.35.Lr, 82.35.Pq, 87.14.Ee

1. Introduction

Processing and utilization of biological materials for medical purpose and production of new biomimetic materials, necessitate knowledge of their structure and physico-chemical properties. Many of structural studies on biological materials have been carried out by means of X-ray diffraction and electron microscopy. Electric techniques in structural studies are applied in a wide frequency range but as Barnes wrote "... it is surprising how incomplete our knowledge is today of the effects of direct current (DC) and low frequency voltages and currents on biological systems" [1]. Particular attention is paid to the response of protein to the increase in temperature, therefore important are techniques providing studies on thermal transitions [2–5]. Temperature dependence of DC electric conductivity (σ) is used in studies, which are carried out on polymeric materials, polymer-metal compounds, inorganic semiconductors [6, 7] and variety of biological materials [8–12]. Moreover, the method permits to find the glass transition temperature from the slope changes of the logarithm of electrical conductivity with reciprocal absolute temperature plots [13].

Studies on biological materials such as bones, hemoglobin, elastin, collagen by means of electric conductivity-temperature relationship (σ–T) can provide useful information on phase transitions and water removal [3, 10, 11, 14–17]. Electric conductivity is sensitive to any change in chemical composition, structure of studied material and also permits to state whether the observed changes are permanent or temporary [18]. Measurements of σ–T are applied in studies on polymers and complexes as well [19].

2. Studies on thermal denaturation of proteins

One of the most important proteins is collagen. In most animals it constitutes about 20–30% of the total protein content. However, collagen molecules in solution denaturates close to the upper limit of the maximum body temperature of animal species, from which the collagen is extracted [20], the solid–state collagen is characterized by relatively high temperature of denaturation. The range of denaturation temperatures is an outcome of the degree of collagen crystallinity, water content and the presence of other substances such as hydroxyapatite (HAP). Usually denaturation of collagen is observed in the temperature range of 450–520 K, but denaturation of bone collagen was also found at 428 K [3, 10, 21, 22]. Changes in the thermal stability of collagen can be shown either by an increase or a decrease in the temperature of denaturation [23–26].

At enough high temperatures, the σ–T characteristic enables studies on thermal decomposition of protein. Thermal decomposition is usually related to decomposition of amino acids constituting a given protein and is observed at temperatures higher than denaturation temperature.

3. Effects of water content on electrical conductivity

Heating of collagen leads not only to structural changes known as denaturation but it leads to water release and thermal decomposition. At a fixed temperature, conductivity of hydrated collagen should tend towards a maximum value with increasing water content. Water release is a complex process, because water molecules participate in the structure of collagen macromolecule at different ways. Generally, water associated with proteins is divided into three types: structural, bound and free water released subsequently during heating. The structural water, about 0–0.07 g/g, is incorporated in the collagen structure and its release is coupled with thermal denaturation of collagen macromolecule. The term bound

water, 0.07–0.25 g/g, refers to water molecules tightly bound to specific sites in collagen chains filling in the spaces between molecules. The bound water–protein interaction is not as strong as in that of structural water. The term free water refers to the water content higher than 0.45 g/g. According to Nomura, in the range 0.25–0.45 g/g both, free and bound water are absorb. Free water is fixed preferentially between the microfibrils and constitutes an interfibrilar matrix gel. Clusters formed by free water molecules are large enough to behave as ice [27, 28]. Bound water is located in the interhelical region within collagen fibers and is bound to polar groups and peptide bonds and works as a plasticizer [28, 29].

The release of both free and bound water occurs below denaturation [28]. It is well described fact that hydration level influences electric conductivity of collagen. The increase in water content increases electric conductivity whereas the activation energy of charge conduction process is reduced [17, 30–34].

Structural water occurs as water molecules between peptide chains and these water molecules form pairs of hydrogen bonds with the hydroxyl groups of hydroxyproline or between carboxyl groups in neighboring peptide chains [35]. The release of structural water is equivalent to the thermal denaturation, but the release of free- and bound water decreases the electric conductivity of a sample and is not connected with the thermal denaturation. The increase in hydration level leads to the decrease in the denaturation temperature whereas the opposite effect can be caused by the hydroxyapatite nanocrystals precipitated in the collagen matrix. The change in the free water and bound water content also can be caused by the action of chemical compounds and sometimes happens to be temporary.

4. Chemical agents effects on electrical conductivity of collagen

Free water and bound water are attached to specific sites in collagen. When the number of these sites decreases, the number of attached water molecules should decrease as well. Such a modification can be produced by a chemical agent preferentially bound instead of water. A chemical agent blocking specific sites in the collagen molecule should decrease both, the water content and the electrical conductivity of the material. Such effect was observed in the case of strong mutagenic and toxic compound, the 3-chloro-4(dichloromethyl)-5-hydroxy-2(5H)--furanone (known as MX). Due to its structure and electrophilic properties, the attachment of MX to specific sites in the collagen molecule instead of water molecules, changes in temperature dependence of electric conductivity were reported. However, the reduction in electric conductivity was noticed, it was also shown that MX had no effect on the structure of the collagen molecule itself. Moreover, the fall in activation energy of charge conduction process with time to values typical for non–treated collagen, suggested, that the influence of MX was a temporary effect [18, 36].

5. Ionizing radiation electrical conductivity of bone

Structural modifications of proteins are often performed under the action of ionising radiation. Irradiated protein, depending on the dose and other irradiation conditions, shows new physical properties, which depend upon the type of the dominant after–effect, that is cross–linking or degradation. The effect of irradiation of bovine Achilles tendon collagen and bone is manifested both in the change of the denaturation temperature and change in the electric conductivity of the irradiated material [8, 10]. Heating of the irradiated animal bone leads to liberation of the trapped charges and recombination of free radicals which contribute to electric current which flows through the sample. However, changes in the electric conductivity are not a simple measure of the absorbed dose of ionising radiation, the σ–T characteristic of irradiated bone can be used for the dose evaluation. The evaluation is based on the empirical Meyer–Neldel rule and consists in fitting of the experimental curve with arbitrary chosen curve: $\sigma = A\exp(T/t)$, at high temperature part of the experimental curve. The Meyer–Neldel modelled relationship: $\ln A = a + b1/t$, was linear ($r = -0.9714$) in the dose range of 0–100 kGy. The relationship can be applied to determine the dose of ionising radiation absorbed in bone [37].

6. Experimental details

All mentioned above processes could be studied during monotonic temperature increase. Variations of the amplitude and temperature of the peak of σ–T relationship make it possible to draw conclusions about thermal stability and physicochemical processes occurring in the studied biological material. The recorded themograms are characteristic for a given material and, therefore, they may be used for their identification [11, 12, 38]. The shape of experimental curve are not only material–related but depended upon its "history" [9].

In studies on temperature dependence of electrical conductivity the DC voltage, within the range of voltage–current linearity where Ohm's law is obeyed, is applied to a sample. Electric conductivity is calculated on the basis of the measured current, applied voltage and sample size. Monotonic heating of the sample induces changes in its size. Thus, the measured current is a sum of both, conduction and displacement current, and the latter is caused by a change in capacitance of the sample. Although the measurements of electrical conductivity carried out at monotonic heating are limited to the solid–state biological materials. Dry, solid state proteins are materials of relatively low electrical conductivity less than $10^{-6}\ \Omega^{-1}\ \mathrm{m}^{-1}$, which cause that electrode effects usually are negligible [39].

Heating, depending on the type of material, could be carried out up to 540 K. Recorded thermograms, due to complexity of the biological materials, usually revealed more than one peak. Separation and elimination of some peaks usually needed more than one heating run. In order to remove free water, samples should be heated to 380–390 K and kept at this temperature for an hour, than cooled down to the room temperature and heated again to appropriate temperature.

7. Conclusions

The method described in the paper seems to be useful for studies of biological materials. It is likely that further experiments performed for other biological materials and different experimental conditions will result in establishing this method as supplementary or preliminary, particularly is studies on solid–state biological materials. Finally, it is worth to emphasize the simplicity of the method and its low costs.

References

[1] F. Barnes, in *Handbook of Biological Effects of Electromagnetic Fields* (Eds: C. Polkow, E. Postow), CRC Press, Boca Raton, New York, Ch. 2 (1996).

[2] I. Doman, G. Toth, T. Illes, D. Lorinczy, *Thermochim. Acta* **376**, (2001).

[3] L. Kubisz, S. Mielcarek, *J. Non-Cryst. Solids* **351**, 2935 (2005).

[4] E. Marzec, L. Kubisz, F. Jaroszyk, *Int. J. Biol. Macromol.* **18**, 27 (1996).

[5] A. Rochdi, L. Foucat, J.-P. Renou, *Food Chem.* **69**, 295 (2000).

[6] Y.L.A. Kady, *Physica B* **305**, 259 (2001).

[7] A. Shekeil, *Polymer* **40**, 2879 (1999).

[8] L. Kubisz, E. Marzec, F. Jaroszyk, *Polish J. Med. Phys.* **8**, 157 (2002).

[9] L. Kubisz, *Bioelectrochemistry* **53**, 161 (2001).

[10] L. Kubisz, S. Mielcarek, F. Jaroszyk, *Int. J. Biol. Macromol.* **33**, 89 (2003).

[11] L. Kubisz, H. Ehrlich, *J. Non-Cryst. Solids* **353**, 4497 (2008).

[12] A. Marcinkowska, et al. *Protetyka Stomatologiczna* **58**, 171 (2008).

[13] M. Serin, *J. Optoelectron. Adv. Mater.* **6**, 283 (2004).

[14] J. Behari, D.V. Rai, R. Jha, *Calcif. Tissue Internat.* **28**, 33 (1979).

[15] A.R. Liboff, M.H. Shamos, in: *Biological Mineralization.* (Ed: J. Zipkin), Wiley, New York 1973.

[16] J. Behari, S.K. Guha, P.N. Agarwal, *Connect Tissue Res* **2**, 325 (1974).

[17] R. Pethig, *Dielectric and electronic properties of biological materials.*, Wiley &Sons, Chichester, New York, (1979).

[18] L. Kubisz, P. Andrzejewski, *J. Biol. Phys.* **27**, 285 (2001).

[19] R Vijayalakshmi, et al., *Chem. Phys.* **64**, 222 (2000).

[20] P.L. Privalov, *Adv. Protein Chem* **35**, 1 (1982).

[21] P.L. Kronick, P. Cooke, *Connect Tissue Res* **33**, 275 (1996).

[22] R. Usha, T. Ramasami, *Thermochim. Acta* **338**, 17 (1999).

[23] M. Luessher, M. Ruegg, P. Schindler, *Biopolymers* **13**, 2480 (1974).

[24] E. Marzec, *Bioelectrochem Bioenerg.* **46**, 29 (1998).

[25] R. Pethig, D.B. Kell, *Phys. Med. Biol.* **32**, 933 (1987).

[26] A. Sionkowska, A. Kamińska, *Int. J. Biol. Macromol* **24**, 337 (1999).

[27] A.L. Nguyen, B.T. Vu, G.L. Wilkes, *Biopolymers* **13**, 1023 (1974).

[28] S. Nomura, A. Hiltner, J.B. Lando, E. Baer, *Biopolymers* **16**, 231 (1977).

[29] R. Pethig, *Annu. Rev. Phys. Chem* **43**, 177 (1992).

[30] G.H. Bardelmeyer, *Biopolymers* **12**, 2289 (1973).

[31] H. Maeda, E. Fukada, *Biopolymers* **21**, 2055 (1982).

[32] C.A. Miles, N.C. Avery, V.V. Rodin, A.J. Bailey, *J. Mol. Biol* **346**, 551 (2005).

[33] G.N. Reddy, S. Saha, *IEEE Trans. Biomed. Eng.* **31**, 296 (1984).

[34] V.P. Tomaselli, *Biopolymers* **13**, 2423 (1974).

[35] G. Ramachandran, *Int. J. Peptide Res.* **31**, 1 (1988).

[36] L. Kubisz, J. Nawrocki, *J. Mater. Sci. Lett.* **19**, 685 (2000).

[37] L. Kubisz, *Physica Med.* **20**, 153 (2004).

[38] L. Kubisz, E. Marzec, F. Jaroszyk, *Med. Biol. Eng. Comput.* **34**, 171 (1996).

[39] M. Davies, in: *Dielectric Properties and Molecular Bahavior* (Eds: N.E. Hill, W.E. Vaugham, A.H. Price, M. Davies), van Nostrand Reinhold, London, (1969).