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Calorimetric Measurements of the Effect of 330-MHz Radiofrequency Radiation on Human Erythrocyte Ghosts

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Irreversible changes in the heat capacity of human erythrocyte ghost suspensions due to the effect of 330-MHz radiofrequency radiation (at a specific absorption rate of approximately 9 mW/g) were detected by the method of scanning differential microcalorimetry. Using the data obtained from the analysis of infrared spectra of air-dried films of erythrocyte membranes, it can be postulated that the observed microcalorimetric changes are connected with the local interaction of electromagnetic radiation with the channel-forming portion of band-3 protein.

Key words: 330-MHz radiofrequency radiation, human erythrocyte ghosts, scanning differential microcalorimetry, infrared spectra

INTRODUCTION

Life on earth has evolved in the presence of electromagnetic fields. In the last 50 years there has been an exponential growth of man-made electromagnetic fields, associated with communications systems that now blanket the earth and with a vast and ever-increasing network of electric power distribution systems. It is, therefore, curious that although many of the most important fundamental observations of physical effects of nonionizing electromagnetic radiation were made more than 100 years ago, knowledge of its biological effects has remained minimal. Now research on the biological interaction with natural and artificial electromagnetic fields is of increasing interest among biologists.

This paper demonstrates that an application of one of the most sensitive methods for measuring changes of conformational states, ie, microcalorimetry [Steim, 1974], could be useful in investigation of the interaction of electromagnetic fields with biological membranes. It is an old method applied to a new problem. Its origins can be traced to pioneering work by Le Chatelier in the nineteenth century. Like any thermodynamic approach, differential scanning microcalorimetry cannot itself provide direct information at the molecular level. Therefore, we also took advantage of the infrared spectroscopic method, which allows one to identify, characterize, and

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quantify specific chemical groupings and to elucidate their interactions with other nearby bonds.

MATERIALS AND METHODS

Human erythrocyte ghosts were prepared according to the method of Dodge et al [1963]. Finally, the ghosts were dispersed in 5 mM sodium phosphate buffer, pH 7.4, to a concentration of 5 mg protein/ml. Protein determinations were prepared according to the method of Lowry et al [1951].

Suspensions of the human erythrocyte ghosts were exposed to 330-MHz radiofrequency radiation. A capacitor-type irradiation chamber was used: a silica chamber (20 × 20 mm with a 5-mm gap) filled with membrane suspension was placed between two copper plates connected to a generator (model 13-20, 200–820 MHz, 10–1,000 mW) set at 330 MHz, continuous wave. The volume of the suspension exposed was 2 ml. The specific absorption rate (SAR), determined using time-temperature profiles, was found to be 9.2 mW/g. Temperature was measured with a noninteracting temperature probe [Bowman, 1976]. The temperature in the sample chamber was 0.8 °C hotter after 20 min of radiofrequency irradiation than without it. All experiments were carried out at 20 °C.

The calorimetric scans were performed with the Privalov's high-sensitivity microcalorimeter [Privalov et al, 1975; Privalov and Khechinashvili, 1974] with a scan rate of 1 °C/min. The volume of the sample pan was 1 ml.

An infrared spectrometer (Karl Zeiss DDR model UR-20) was employed. Solid films of erythrocyte ghosts were prepared by applying about 0.7 mg membrane protein (in aqueous suspension) on 0.5 × 1.5-cm area in the center of a CaF_2-plate and drying it in air at approximately 20 °C. Once dried, the films were strongly adherent to the plate. In order to have the films reproducibly located in the optical path, all manipulations of the films were performed with plates in their plateholders. All chemicals were of analytical grade.

RESULTS

Figure 1 shows the results of heat capacity measurements on the erythrocyte membranes in 5 mM sodium phosphate, pH 7.4. Each of the many peaks is believed to be due to a localized structural transition induced by thermal stress. The origins of these transitions are discussed in detail elsewhere [Brandts et al, 1977; Brandts et al, 1978; Snow et al, 1978]. The A-transition (maximum at approximately 46 °C) in the main corresponds to the thermal inactivation of spectrin. The B_1-transition (maximum at approximately 52 °C) reflects the integral protein-lipid interaction in the membrane. The B_2-transition (maximum at approximately at 57 °C) corresponds to the thermal denaturation of the outer–membrane part of band-3 protein, and the C-transition (maximum at approximately 63 °C) corresponds to the thermally induced conversion of the transmembrane fragment of the same protein.

All of these transitions are thermodynamically irreversible, ie, they have not been observed during repeated heating [Snow et al, 1981]. Changes in the complicated thermograms in our experiments can take different forms—the displacements of elementary bands of thermoabsorption along the temperature scale, different changes of intensity of these bands, etc—therefore it will be informative to establish the shapes

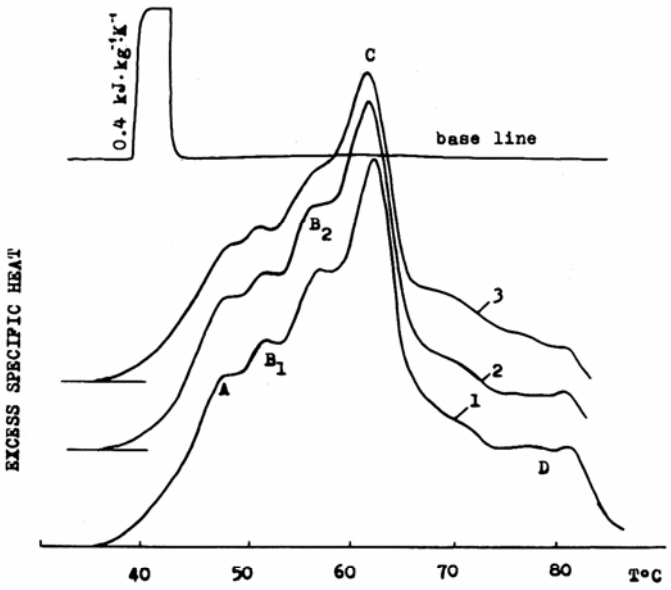

Fig. 1. Temperature dependence of specific heat of the human erythrocyte ghost suspensions: 1, intact membranes; 2, irradiated membranes (after 5 minutes); 3, irradiated membranes (after 30 minutes). The different starting levels for these curves are experimental results and not offsets for convenience.

of each elementary band. For this purpose we have used the "sequence annealing method." The main point is that the suspension of erythrocyte ghosts was placed in the measurement cell of the microcalorimeter and heated from 20 °C to a temperature 1.5–2 degrees higher than the temperature of the maximum for the first transition (A-transition). The sample was cooled to 20 °C and then was heated to a temperature exceeding by 1.5–2 degrees the temperature of the maximum for the B_1-transition. The suspension was again cooled in the microcalorimeter cell and the process was repeated for each transition in succession. As a consequence, we measured the curves presented in Figure 2. If curve 2 is substracted from curve 1 we should get the initial portion of the curve and the temperature of the maximum for the A-transition. Substracting curve 3 from curve 2, we obtained the initial portion of the curve and the temperature of the maximum for the B_1-transition. Repeating this procedure we can obtain the corresponding data for each transition. The portion of the curve after the maximum was constructed for each transition by assuming symmetry relative to the temperature of the maximum. Most likely, curve 6 in Figure 2 represents melting of immobilized lipids at erythrocyte ghosts after the denaturation of all proteins. This thermal transition is reversible. To obtain coincidence of the experimental curve of thermoabsorption and the curve obtained by the addition of the elementary contours of thermoabsorpiton derived by "sequence annealing," we altered only the intensity of the last ones. The results of decomposition of the complicated contour of thermoabsorption derived by this method and the errors of this decomposition are presented in Figure 3. The elementary contours of the main thermotransitions of erythrocyte membrane suspensions obtained according to this method before and after irradiation are presented in Figure 4.

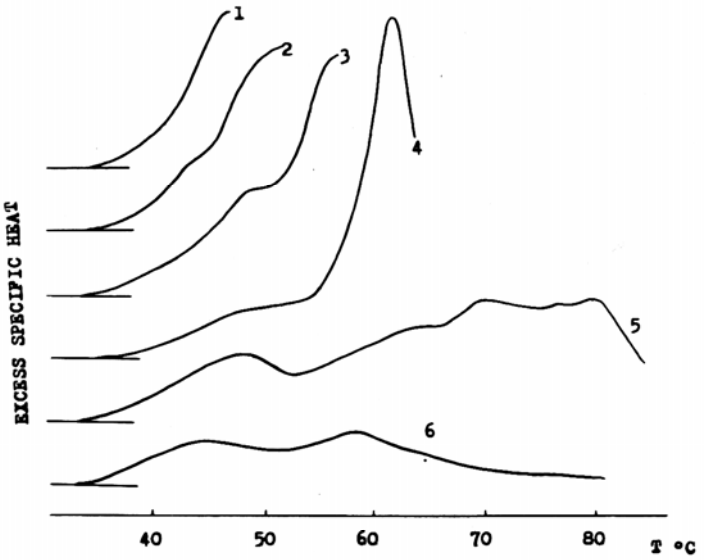

Fig. 2. Demonstration of the sequence "annealing" of thermally induced transitions in the suspension of human erythrocyte ghosts. The curves are displaced along the ordinate for clarity.

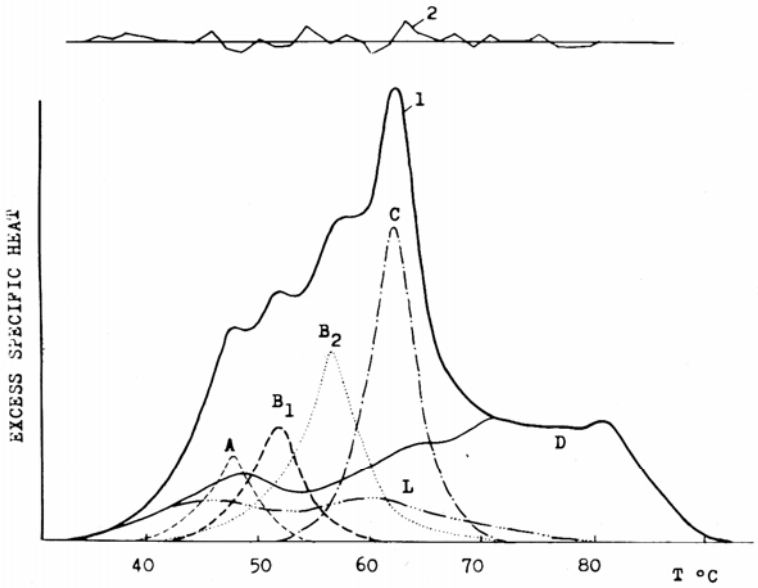

Fig. 3. Results of computer deconvolution of the heat capacity curve: 1, into components; 2, the error of deconvolution.

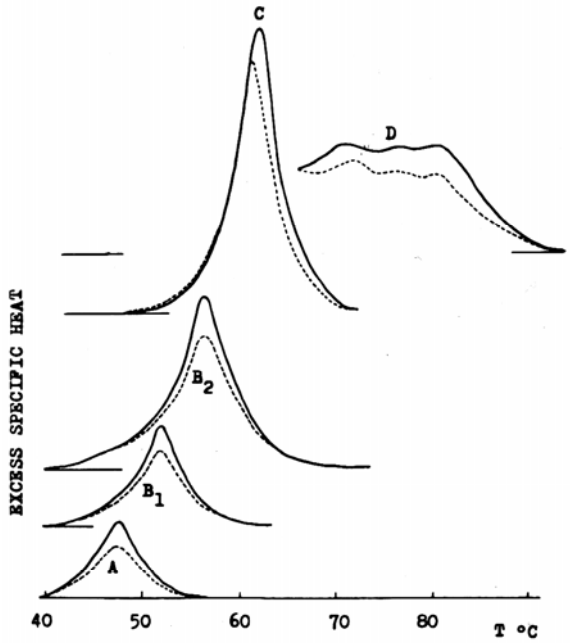

Fig. 4. Changes in heat capacity of the separate thermogram components after a 30-min irradiation of membrane suspension. Unbroken line, intact membranes; broken line, irradiated membranes. The curves are displaced along the ordinate for clarity.

There are three important points to be learned from Figures 1 and 4. 1. The deviation from baseline of the initial level of the specific heat depends on the irradiation period. 2. As the duration of irradiation increases, the total heat absorbed in the thermal conversion (the area under the curve) significantly decreases. 3. The temperature of the maximum for the C-transition is displaced toward lower temperatures, while for the rest of the transitions only a decrease of intensity occurs. All of these changes are irreversible (ie, are preserved in the suspension of irradiated erythrocyte ghosts at least 18 hours after irradiation) and approach the maximum change after 15 minutes of irradiation.

The 1,200–1,800 cm^{-1} and 2,600–3,800 cm^{-1} regions of the infrared spectrum of films of human erythrocyte ghosts before and after irradiation are reproduced in Figure 5. Figure 6 shows the infrared spectrum of erythrocyte ghost films before and after heating of the suspension to 85 °C. The amide I band (C = O stretching) is located at 1,654 cm^{-1}, the region associated with α-helical and/or random coil conformations of peptide chains [Miyasawa and Blout, 1961]. Other bands are N–H stretching (amide A) near 3,310 cm^{-1}, C–H stretching at 2,800–3,000 cm^{-1} (all membrane components), C–O stretching of ester groups of phosphatides with peak near 1,740 cm^{-1}, inplane N–H bending vibrations (amide II) with a peak near 1,545 cm^{-1}, CH_2 and CH_3 bending between 1,440 to 1,470 cm^{-1} from all membrane components, $CO^{-}{}_2$ stretching of ionized carboxyl groups at 1,400 cm^{-1} and amide III band (in-plane NH bending) with PO stretching of phosphatides near 1,250 cm^{-1} [Chapman et al, 1968]. The lipids make only a small contribution to the infrafred spectrum of the ghosts except in the region of the CH stretching frequencies near 2,850 cm^{-1} [Maddy and Malcolm, 1965]. The absorption at 1,590–1,720 cm^{-1} is

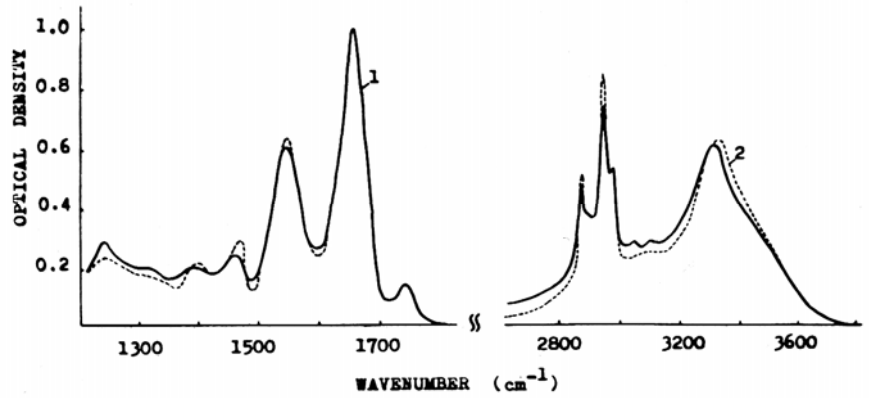

Fig. 5. The infrared spectrum of absorption of air-dried films of human erythrocyte ghosts (1) before and (2) after a 30-min irradiation.

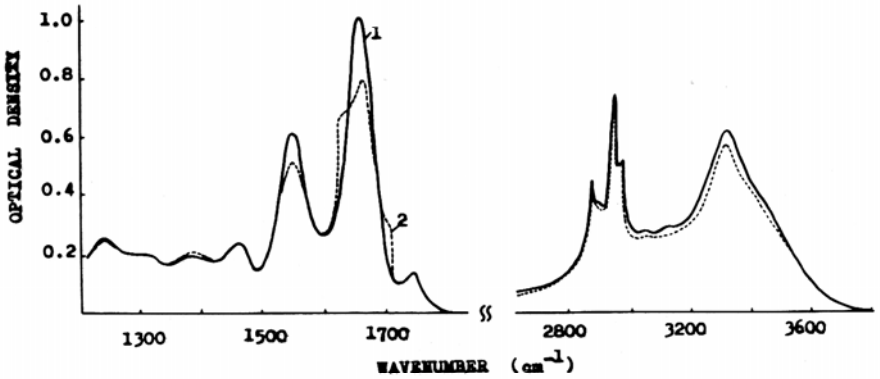

Fig. 6. The infrared spectrum of absorption of air-dried films of human erythrocyte ghosts (1) before and (2) after heating at 85°C.

attributed to carboxyl groups including neuraminic acid residues, side-chain amino-acid carboxyls and terminal amino-acid carboxyls.

All the characteristic amide modes are associated with the CONH grouping. All of them are, therefore, to some degree influenced by hydrogen bonding. The nature and magnitude of changes differ from band to band and depend on the particular type of molecular motion involved. It is a well-substantiated observation that the frequency of stretching vibrations is decreased and the frequency of bending vibrations is increased by hydrogen-bond formation [Susi, 1969]. The shift is generally larger for the stretching modes than for the bending modes. Again, the shifts involving proton-donating groups, such as OH and NH, are usually larger than the shifts involving proton acceptors, such as C = 0. For the data presented here, the most prominent frequency shift occurs in the region from 3,100 to 3,500 cm^{-1}, where OH, and NH stretching modes are observed.

DISCUSSION

The intensity of all thermally induced transitions was shown to decrease considerably after irradiation of erythrocyte ghosts (see Fig. 4). This result could be obtained by thermally denaturing about 20% of the protein membrane components during exposure. One can postulate that, due to "imperfections" in the method of irradiation, the preparation is locally overheated in some parts of the chamber. In this case, temperature must be at least 80–85°C because the same "annealing" of all elementary transitions occurs as is found at these temperatures. However, the experimental results obtained by infrafred spectroscopy do not support the assumption of interaction of the electromagnetic field with the substance via a direct thermal mechanism. As is seen in Figure 6, the action of heat on the suspension of erythrocyte ghosts leads to the appearance in the infrared spectrum of two additional bands at 1,630 cm^{-1} and 1,690 cm^{-1}. Such resonance splitting of the amide I band is the experimental criterion of appearance of the β-pleated sheet in the protein structure. A noticeable amount of β-pleated sheet has already been recorded when the erythrocyte membrane suspension is heated to 45°C, and along with denaturation of other protein components during a further temperature increase, the portion of this structure increases. The results presented in Figure 5 demonstrate quite different spectral changes. In the region of OH and NH stretching we observe a shift to higher frequency of the maximum for the amide A band of nearly 100 cm^{-1}, while the amide III band was slightly displaced to a lower frequency and, as noted above, these changes indicate that hydrogen bonds decreased. One can assume that the electromagnetic field affects the water bound to outer membrane components of the erythrocyte ghosts and this leads to a disturbance of hydrogen bond formation near the membrane surface. However, in our opinion, this interaction would be rapidly reversible while, as was mentioned, the changes recorded by us remained invariable for almost a day. Moreover, the intensity of the exposure field was not high; in order to get enough energy from the field for molecular rearrangements, it is necessary to assume concentrating of the field because of membrane heterogeneity, for example, due to the great difference in the magnitude of the dielectric constants of the membrane and its "holes"—channels.

If the latter case is assumed, then the electromagnetic field could affect the protein channel portion of band-3 protein, leading to disturbance of the intact hydrogen-bond formations of structured channel water that is reflected on the infrafred spectrum (see Fig. 5). Thus, the protein structure is slightly destabilized, which leads to the temperature shift of the maximum and the overall decrease in magnitude of the C-transition [Snow et al, 1981]. The change in the channel portion of band-3 protein was likely to initiate the change of protein-lipid interactions that had an influence on the infrared spectrum of erythrocyte membranes in the range of the CH stretching. This may be the cause of the other changes detected by the microcalorimetry.

We are far from thinking that this scheme is in full agreement with the observed effects. These investigations are being continued. Nevertheless, it should be emphasized that the experimental data obtained convincingly demonstrate the specificity of interaction of the weak radiofrequency electromagnetic fields with biological membranes.

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REFERENCES

Bowman BR (1976): A probe for measuring temperature in radiofrequency-heated material. IEEE Trans Microwave Theory Tech 43–45.

Brandts JF, Erickson L, Lysco K, Schwartz AT, Taverna RD (1977): Calorimetric studies of the structural transitions of the human erythrocyte membrane: The involvement of spectrin in the A transition. Biochemistry 16:3450–3454.

Brandts JF, Taverna RD, Sadasivan E, Lysco K (1978): Calorimetric studies of the structural transitions of the human erythrocyte membrane: Studies of the B and C transitions. Biochim Biophys Acta 512:566–578.

Chapman D, Kamat VB, Levene RJ (1968): Infrared spectra and the chain organization of erythrocyte membranes. Science 160:314–316.

Dodge JT, Mitchell C, Hanahan DJ (1963): The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Arch Biochem Biophys 100:119–130.

Lowry OH, Rosenbrough WI, Farr AD, Randall RV (1951): Protein measurements with the folin phenol reagent. J Biol Chem 193:265–275.

Maddy AH, Malcolm BR (1965): Protein conformations in the plasma membrane. Science 150:1616–1617.

Miyazawa T, Blout ER (1971): The infrafred spectra of polypeptides in various conformations: Amide I and II bands. J Am Chem Soc 83:712–719.

Privalov PL, Khechinashvili NN (1974): A thermodynamic approach to the problem of stabilization of globular protein structure: A calorimetric study. J Mol Biol 86:665–684.

Privalov PL, Plotnikov VV, Filimonov VV (1975): Precision scanning microcalorimeter for the study of liquids. J Chem Thermodyn 7:41–47.

Snow JW, Brandts JF, Low PS (1978): The effects of anion transport inhibitors on structural transitions in erythrocyte membranes. Biochim Biophys Acta 512:579–591.

Snow JW, Vincentelli J, Brandts JF (1981): A relationship between anion transport and a structural transition of the human erythrocyte membrane. Biochim Biophys Acta 642:418–428.

Steim JM (1974): Differential scanning calorimetry. In Fleischer S, Packer L (eds): "Methods in Enzymology," Vol 32, part B. New York: Academic Press, pp 262–272.

Susi H (1969): Infrared spectra of biological macromolecules and related systems. In Timasheff, SN, Fasman GD (eds): "Structure and stability of biological macromolecules." New York: Marcel Dekker, pp 575–663.