Proc. Natl. Acad. Sci. USA
Vol. 74, No. 1, pp. 226–228, January 1977
Biophysics

Electronic properties of the casein–methylglyoxal complex

(semiconductivity/electron spin resonance/microwave dielectric measurements/electronic transference number)

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ABSTRACT **Measurements of the electron spin resonance, direct current conductivity, microwave permittivity, and electronic transference number are reported for the brown casein–methylglyoxal complex. Compared with normal white casein, the colored casein complex exhibits an increased electronic activity. This is considered to arise from the electron-accepting action of the methylglyoxal in separating electronic charge from an otherwise completely occupied electronic ground state of the casein macromolecule.**

The concept that electronic delocalization within protein molecules has significance regarding the underlying subtlety of many biological phenomena was explicitly stated in 1941 (1, 2). It was envisaged that the regular arrangement of peptide linkages in the proteins could result in the existence of electronic energy bands similar to those in elemental semiconductors. Theoretical molecular orbital calculations (3) and experimental studies (4) have supported the validity of this general concept, although it is clear that the energy gap between the so-called valence and conduction bands is too large for pure proteins to act as intrinsic semiconductors. However, this does not preclude the proteins' being able to exhibit a significant extrinsic semiconductivity, through the incorporation of an electron-accepting molecule in their structures, for example. Furthermore, because of the large energy band gap, the action of just one electron acceptor molecule in creating a positively charged "hole" in the protein molecule's valence band could be biologically significant, because there will be no masking effect arising from the intrinsic thermodynamic generation of such charge deficiencies.

We wish to report on the electronic properties of one such protein–electron acceptor entity, namely those of the casein–methylglyoxal complex.

Sample preparation

Ten grams of pure white casein powder (Fisher Scientific Co., Pittsburgh) was suspended in ten volumes of methanol containing 10% of the commercial (Aldrich Chemical Co., Milwaukee) neutralized 40% methylglyoxal solution. Such suspensions were incubated at 310 K for periods ranging from 16 hr to 6 days. The casein was then separated on a filter, washed with methanol and acetone, and dried in a vacuum desiccator overnight. In all cases the white casein assumed a vivid brown color, similar to that of blood-free liver.

Electron spin resonance (ESR) measurements

Free electron spin concentrations were determined using a Varian E-109 EPR system, and referenced against diphenylpicrylhydrazyl samples diluted in KCl powder. The brown casein–methylglyoxal complex exhibited a strong ESR signal centered at a value of $g = 2.005$ as shown in Fig. 1. Similar signals have been observed repeatedly by others (T. E. Cross, P. R. C. Gascoyne, J. R. Morgan, and H. A. Pohl) in this laboratory, not only for casein but also for methylglyoxal-treated serum albumin and methylamine, for example. A detailed account of these studies, to include solvent effects and hyperfine structure, is to be published at a later date. For the casein–methylglyoxal preparations studied here the free electron spin densities ranged from 6.2×10^{17} to 1.7×10^{18} spins g^{-1}, representing a concentration of from 0.4 to 1 free electron spins per casein molecule. Casein powder, which retained its white color after being incubated in methanol, washed in acetone, and desiccated, exhibited a much weaker free spin density of the order 8×10^{15} spins g^{-1}.

The structure of the ESR signal for the casein–methylglyoxal samples changed with increasing microwave power, and the peaks A and B of Fig. 1 had maximum intensities at differing power levels and saturated in dissimilar ways as shown by Fig. 2. This indicated that the peaks A and B were associated with two separate free radical species having different spin-lattice relaxation times. We believe that the ESR signals for the casein–methylglyoxal complex are associated with a bi-radical formed by an intramolecular charge separation within the covalently bonded casein–methylglyoxal complex, and possibly resulting from the creation of a relatively low-lying triplet state associated with the methylglyoxal molecule.

Electronic conduction measurements

Direct current resistivity measurements were made for normal white casein and the brown casein–methylglyoxal samples using spring-loaded copper electrodes in a massive brass conductivity cell whose atmosphere was maintained at a pressure of 0.13 Pa (1 μm Hg) over a solid CO_2 freezing mixture. The test samples took the form of discs compressed at 350 MPa (51,000 lb in^{-2}), with a diameter of 1.27 cm and nominal thickness 0.1 cm. With a Keithley 600 A electrometer, the resistivities of several casein–methylglyoxal samples were determined to be in the range 44–99 GΩ·m at 295 K. When 200 V were applied across the white casein samples, the steady-state currents attained were less than 0.3 pA, corresponding to resistivity values exceeding 50 TΩ·m. Measured in this way, the resistivity of the casein–methylglyoxal complex was some three orders of magnitude less than that of the white casein under the same atmospheric conditions.

Dielectric and conductivity measurements were also made at a frequency of 9.9 GHz using a microwave resonant cavity perturbation technique that overcomes effects of electrode and inter-molecular defects (5). Relative dielectric constant and resistivity values of the order 2.2 and 20 Ω·m., respectively, were obtained for both the white and colored casein–methylglyoxal samples for measurements made in a dry nitrogen atmosphere. We believe that hydration effects may have masked any differences in the electronic properties of the white and colored

Abbreviation: ESR, electron spin resonance.

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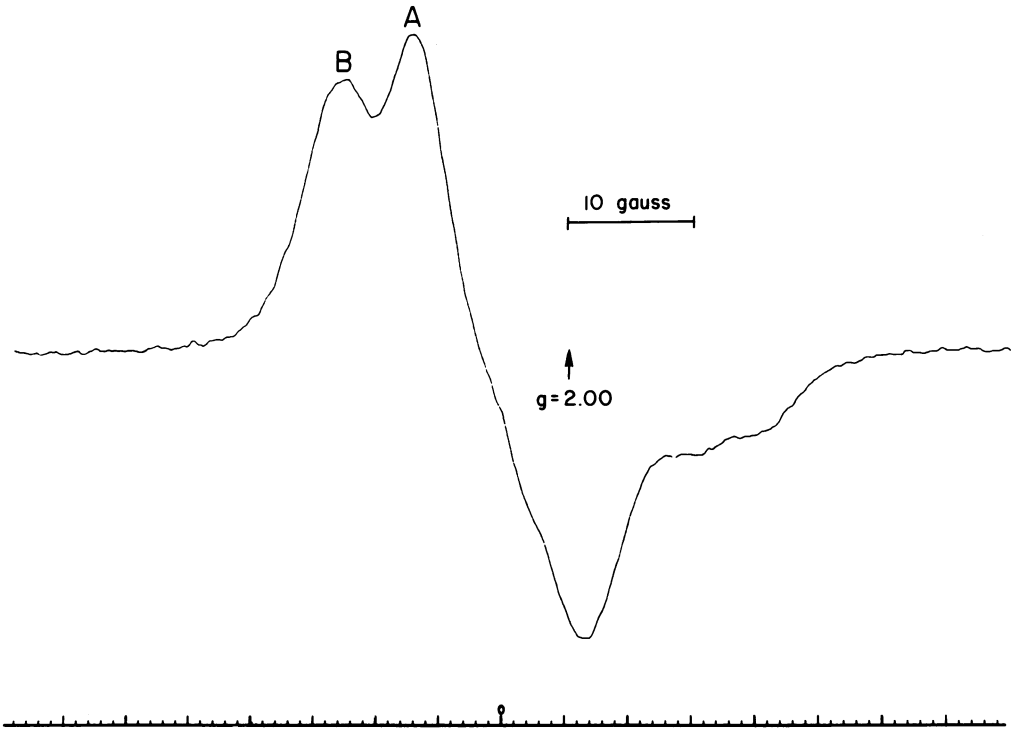

FIG. 1. The electron spin resonance signal for the casein–methylglyoxal complex, corresponding to a low microwave power level of 1.5 mW. The peaks A and B are referred to in Fig. 2.

casein samples at this microwave frequency. Future studies should use humidity-controlled resonant cavities.

Evaluation of electronic transference number

The question now arose as to whether the increased dc conductivity of the casein–methylglyoxal complex, compared to the white casein, resulted from an increase in electronic delocalization or from ionic conduction associated with solvent or other impurity effects. We have attempted to answer this question using a simple electrochemical technique described by Liang (6) for the determination of the electronic transference numbers of solid electrolytes.

Silver iodide was used by Liang as the electron-sensitive indicator for his studies on solid electrolytes. We have chosen to use the compound $RbAg_4I_5$ because of its stability and exceptionally high ionic conductivity for Ag^+ ions at room temperature (7). According to the concepts outlined by Liang (6), when an electric current is passed from left to right in the test cell illustrated in Fig. 3, silver will be deposited at the interface between the test sample M and the $RbAg_4I_5$ layer only if electrons are mobile in M. The amount of silver deposited will be proportional to the electronic transference number of the test sample.

The compound $RbAg_4I_5$ was prepared following the procedure of Owens and Argue (7) by adding 9.39 g of AgI to 2.12 g of RbI, melting the mixture, and quenching in cold water. The product was ground into powder and pressed with silver mesh at 350 MPa into discs of diameter 1.27 cm and nominal thick-

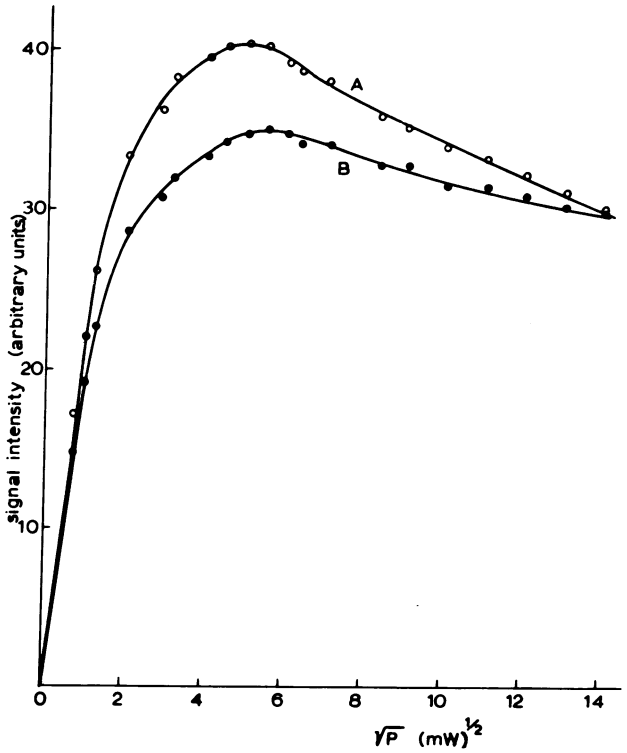

FIG. 2. The variation of the intensity of peaks A and B of Fig. 1 with the square root of the microwave power P.

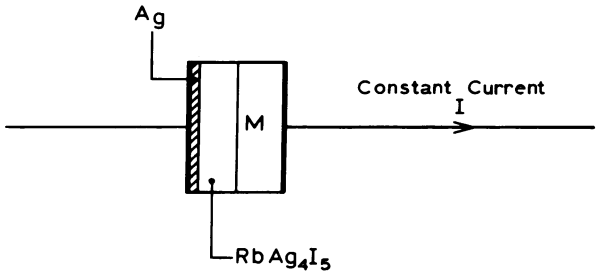

FIG. 3. The test cell arrangement for the determination of electronic transference numbers. The symbol Ag refers to the silver mesh electrode embedded in the $RbAg_4I_5$ disc, and disc M represents the test material.

ness 0.1 cm. The silver mesh was located so as to protrude through one major face of the disc and was thus able to act as a sacrificial electrode for Ag^+ ions. These discs were annealed at 438 K for 16 hr, after which their resistivity was found to be of the order 1 Ω·m. at 295 K.

The perylene–chloranil charge transfer complex was chosen as a test organic material, because its conductivity is considered to be essentially electronic (8, 9). Discs of $RbAg_4I_5$ and polycrystalline perylene–chloranil were pressed together at 440 MPa (64,000 lb in^{-2}), and placed in the conductivity cell. A constant "deposition" current of 1.5 nA was passed through this disc pair with the $RbAg_4I_5$ at the positive potential, and a Boonton 95A dc meter was used to monitor the voltage (about 890 mV) developed across the conductivity cell, this voltage being recorded using a Sargent Model SRL recorder. After 1 hr the two discs were separated and the method outlined by Liang (6) was adopted, whereby the surface of the perylene–chloranil disc formerly in contact with the $RbAg_4I_5$ was scraped to remove the deposited silver and the scrapings were incorporated into a compressed graphite disc. This graphite disc was pressed at 440 MPa onto a $RbAg_4I_5$ disc, and a constant "oxidizing" current of 1.5 nA was then passed through them. The polarity of this oxidizing current was such that the initial ionic current through the $RbAg_4I_5$ required the sacrificial electrode action of the silver scrapings incorporated into the graphite disc. A typical recording of the voltage developed across the conductivity cell during such deposition and oxidizing current runs is shown in Fig. 4. The rapid rise of the voltage during the oxidizing run indicated the end-point for the oxidation of the silver mixed with the graphite powder, and represented the increased potential required to dissociate other ions in order to maintain the constant oxidizing current. From the ratio of the total oxidizing charge to the total original deposition charge, a value of 0.95 ± 0.04 was obtained for the electronic transference number of the perylene–chloranil complex, as was to be expected for an essentially electronic conductor. Similar experiments using deposition and oxidizing currents of 2 μA gave electronic transference numbers of 0.98 ± 0.04 for graphite (an electronic conductor) and less than 0.05 for AgI (an ionic conductor). These tests verified the applicability of the technique outlined by Liang (6) and also indicated that deposition charges some twenty times less than those employed in this earlier work could be successfully used.

Results for the casein–methylglyoxal samples could not be so readily obtained because of their relatively high resistivity. The Keithley electrometer was used in the resistance measurement mode to supply constant deposition currents of the order 9.5 pA for 12 hr. Measurements of the oxidizing currents were made using AgI rather than $RbAg_4I_5$ as the electron-sensitive indicator. The higher resistivity of AgI allowed for the monitoring of the cell potential (about 12 μV), using an oxidizing current of 200 pA. The estimated oxidation end-point time of 28.2 min corresponded to an electronic transference number of 0.83. From four other measurements the following values were also obtained: 0.84, 0.81, 0.75, and 0.89. Although polarization and electrical noise effects resulted in an accuracy of measurement of no better than around 10%, these results were taken to indicate that the casein–methylglyoxal samples were essentially electronic conductors. The exceptionally high resistivity of the pure white casein samples prevented the determination of their electronic transference number.

Conclusions

The increase in ESR activity and dc conductivity of the brown casein–methylglyoxal complex over that of normal white casein gives support to the concept that the action of methylglyoxal is to desaturate the otherwise electron-filled ground states of the casein macromolecule. According to this viewpoint, the ESR signals reflect the partial or complete decoupling of electron pairs, one electron of which resides mainly in the methylglyoxal molecule. From other work in this laboratory we have come to consider that the methylglyoxal is covalently bound to the protein via a Schiff base (—C=N— linkage) and that the lone pair electrons of primary amines are the ones involved in the effective charge separation.

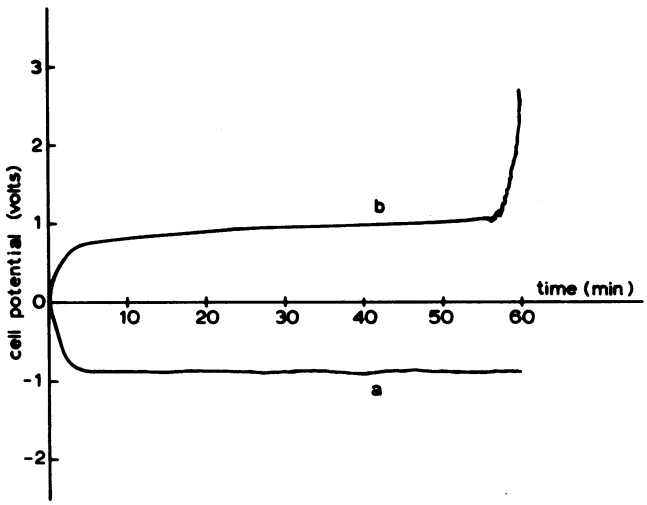

FIG. 4. The time recording of the cell potential for (a) the deposition current of 1.5 nA, and (b) the oxidizing current of 1.5 nA, for determination of the electronic transference number of a perylene–chloranil complex test sample.

The increase in electronic conductivity is taken to reflect the decrease in energy required to produce free electrons by separating the decoupled electron pairs completely, and the observed high electronic transference numbers imply that ionic impurity effects do not significantly complicate this interpretation.

According to concepts developed in this laboratory (10), the color, electronic desaturation, and activity of the biologically important structural proteins are intimately related. We believe that an increased knowledge of such relationships will have relevance to the full understanding of molecular disorders inherent in cancer, for example.

We wish to acknowledge the assistance of Jane McLaughlin and useful discussions with W. M. Arnold, P. R. C. Gascoyne, and Prof. T. J. Lewis.

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