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1.A

TECHNICAL PROGRESS REPORT C00-1714-5

Title: THE SOLID STATE ELECTRICAL PROPERTIES OF PROTEINS AND BIOLOGICAL SYSTEMS

A.E.C. Contract #AT-(11-1)-1714

MASTER

Period Covered by this Report: February 21, 1970 - February 28, 1971

a. Small Polarons in Organic and Biological Semiconductors:

Our previous calculations on the derivation of the compensation law of semiconductivity:

$$\sigma(T) = \sigma'_0 \exp[E/2kT_0] \cdot \exp[E/2kT] \quad (1)$$

were based upon the concept of charge tunnelling from an activated state (Kemeny & Rosenberg, J. Chem. Phys., 52, 4151, (1970)). This assumed, as reasonable values of the parameters, a triangular, intermolecular barrier of height = 6eV. and width at base of 3Å. This led to an effective mass of the mobile charge of 100 m_e , where m_e is the free electron mass. This large effective mass implies narrow energy bands in these materials. It is necessary to consider therefore, that the charge carriers may be small polarons. Small polarons of mass $m_p \sim 100 m_e$ are reasonable. However, since this mass is already derived on the basis of polaron tunnelling, we cannot use the same equations as in the previous calculation as this would involve taking tunnelling into account twice. We must therefore rederive the compensation law on the basis of small polaron theory alone. This we have done now, (Kemeny & Rosenberg, J. Chem. Phys. 53, 3549, (1970)). The polaron binding energy (W_p) is expressible in terms of the static and high frequency dielectric constants. Also, the semiconduction activation energy (E) is expressible in terms of these same constants. Therefore we may solve these equations for the polaron binding energy in terms of the activation energy, to derive the following very interesting result:

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$$E + 2W_p = E^{(o)} + 2W_p^{(o)} = \text{Invariant} \quad (2)$$

where E and W_p are the values at some effective dielectric constant, and $E^{(o)}$ and $W_p^{(o)}$ are the values at some initial state dielectric constant. The mobility of the polaron can be expressed in terms of a hopping integral, J_p , which is a function of the barrier height, width and activation energy. When this is done, the final form of the conduction equation takes the same form as the compensation law (eq. 1).

One new advantage accrues from this theory and that is that we may now associate the compensation law temperature, T_0 , with the Debye temperature, θ , as:

$$2T_0 \approx \theta \quad (3)$$

which is a more fundamental interpretation of the constant, T_0 . The derived formulas can be compared with the experimental data available, and give reasonably good agreement. It is likely, now, that the appearance of a compensation law in conductivity is a strong indication that the dominant charge carriers are small polarons (electronic).

b. Compensation Law for Protein Denaturation:

Our interest in the general phenomenon of a compensation law behavior has led us to investigate the theories of protein denaturation, as it appeared possible to explain the protein denaturation process as initiated by a small polaron formation.

The values of the activation energy (enthalpy) and activation entropy for denaturation of many different proteins are available in the literature. The denaturation rate constant, k_D is given by the absolute rate theory by:

$$k_D = \kappa \frac{k_B T}{h} \exp [\Delta S^\ddagger / R] \cdot \exp [-\Delta H^\ddagger / RT] \quad (4)$$

We have examined the $\Delta S^\ddagger$ and $\Delta H^\ddagger$ values for all available proteins and find that they are accurately correlated by the simple linear equation:

$$\Delta S^\ddagger = a\Delta H^\ddagger + b \quad (5)$$

If eq. 5 is put into eq. 4, this yields a compensation law. Thus, protein denaturation is a compensation law process. The values of the constants in equation (5) are:

$$T_c = 1/a = 330^\circ\text{K}, \text{ and } b = -64 \text{ Cal/mole}^\circ\text{K} \quad (6)$$

The close correspondence of T_c with T_o for protein conductivity, as derived by Eley ($T_o \approx 340 - 350^\circ\text{K}$), led us to try to develop the small polaron theory as the initiating step in protein denaturation. This has been done, and the paper describing this theory has been accepted for publication in the journal Biopolymers. However, there are still some problems with this theory, and much work remains to be done on it.

c. Protein Denaturation as the Cause of Thermal Death:

Again, pursuing this compensation law behavior one stage further, we have investigated the thermodynamic data on the killing rates in unicellular organisms at elevated temperatures. We have set three criteria for thermal death rate data to be used in this investigation: 1) the death rate must follow first order kinetics; i.e.

$$\frac{dN}{dt} = -k_D N \quad (7)$$

2) the thermal death rate constant must be exponentially dependent upon the temperature (Arrhenius plots must give straight lines) so that we can express the rate constant with eq. 4, as in protein denaturation. 3) The values of $\Delta S^\ddagger$

and $\Delta H^\ddagger$ so calculated, must be correlated by the compensation law of eq. 5.

We were quite surprised to find that all data we have uncovered so far on thermal killing of yeasts, bacteria and viruses do meet all three criteria. What is even more intriguing, is that the constants, T_c and b , for thermal killing are identical, within 1/2% accuracy, with the constants for the protein denaturation. The values of the constants are tabulated here for comparison.

TABLE I
COMPARISON OF COMPENSATION LAW CONSTANTS, T_c and b .

from:	$\Delta S^\ddagger = a\Delta H^\ddagger + b$	:	$T_c = 1/a$
	<u>$T_c (^{\circ}K)$</u>		<u>$b (\text{cal./mole } x^{\circ}K)$</u>
Proteins	329		-64.9
Virus	330		-64
Yeasts	325		-64.5
Bacteria	331		-65

From the close agreement between these constants, we are led to suggest that protein denaturation is the cause of thermal death in unicellular organisms. These data represent the first quantitative evidence available in support of such a hypothesis. We believe that this work opens up a completely new area for the investigation of thermal killing, and 'aging', in unicellular and other poikilothermic organisms.

It is not obvious how protein denaturation leads to death, or even how to identify any specific class of proteins whose denaturation is the causative agent of death. We are working now on a number of alternate hypotheses which couple

denaturation with death. These are: a) that there is a statistical distribution of concentration thresholds for an essential protein in a population of cells; b) that the stress on the cell to create these proteins at a rate sufficient to compensate the denaturation rate is the cause of death; c) that the stress on the cell to prevent the accumulation of protein debris kills the cell; and finally, d) that the denaturation of a single special protein molecule kills the cell. It is obvious that there are close similarities between these hypotheses and the various theories of aging that have been propounded. In its simplest terms, the four hypotheses we have given above correspond somewhat loosely to the theories known as a) the 'variable threshold'; b) the 'loss of vitality'; c) 'clinker'; and d) 'death gene' theories, respectively.

d. The Nature of the Charge Carriers in Biological Substances:

Our apparatus incorporating the mass spectrometer gauge to evaluate the types and quantities of gases evolved in our solid state electrolysis experiments has been constructed. Unfortunately, the student working on this system has been drafted and a new student has just begun to familiarize himself with the experimental details. He will begin tests with the apparatus in the near future. These studies have been described in our previous report.

e. The Effects of Adsorbates on the Semiconduction Properties of Proteins:

We have previously reported that the system hemoglobin-water produces a compensation law temperature $T_0 \approx \infty$. Other gases, such as H_2S , NH_3 , SO_2 . etc., when reversibly adsorbed on hemoglobin, however, do produce a compensation law behavior, with a $T_0 \approx 450^\circ K$. We are presently making simultaneous measurements of the adsorption isotherms, dielectric constant changes, and conductivity changes in hemoglobin crystals to determine whether there is a different pattern of effects on the controlling processes with these gases, from our previously measured

effects with hydroxylic solvents such as water, ethanol and methanol. As yet, we can find no significant differences between these two classes of gases to explain the different values of T_0 .

f. Ph.D. Thesis:

The following students were granted the Ph.D. degree in Biophysics with research theses supported by this contract.

Dr. Elliot Postow - 1970

Dr. Michael R. Powell - 1970

g. Approximate percentage of time Principal Investigator (Barnett Rosenberg) has devoted to this project:

June 1, 1970 - February 28, 1971 - 20%

March 1, 1971 - May 31, 1971 - 20%

Publications

Reporting on Work Supported by this Contract

1. Michael R. Powell and Barnett Rosenberg, "The Nature of the Charge Carriers in Solvated Biomacromolecules: DNA and Water". Biopolymers, 9, 1403 (1970).
2. Michael R. Powell and Barnett Rosenberg, "The Nature of the Charge Carriers in Solvated Biomacromolecules". Bioenergetics, 1, 493 (1970).
3. Elliot Postow and Barnett Rosenberg, "Dielectric and Semiconductive Behaviors of Solvated Biomacromolecules". Bioenergetics, 1, 467 (1970).
4. Gabor Kemeny and Barnett Rosenberg, "Theory of the Pre-Exponential Factor in Organic Semiconductors". Journal of Chemical Physics, 52, 4151, (1970).
5. Gabor Kemeny and Barnett Rosenberg, "Small Polarons in Organic and Biological Semiconductors", Journal of Chemical Physics, 53, 3549, (1970).
6. Gabor Kemeny, Barnett Rosenberg and R. C. Switzer, "Theory of Protein Denaturation Rates", Biopolymers, Accepted for publication, 1971.
7. Barnett Rosenberg, Gabor Kemeny, Robert C. Switzer and Thomas C. Hamilton, "Quantitative Evidence for Protein Denaturation as the Cause of Thermal Death", Submitted for publication, 1971.