Further progress in our knowledge of the structure of fibroin will depend on attempts to establish unequivocally the existence of inter-chain bonds and to break these and isolate the component chains of the molecule.

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BIOPHYSICS

Dry Thermal Inactivation of Trypsin and Ribonuclease

IN the course of some recent work, it became necessary to obtain information on the dry thermal inactivation of trypsin and ribonuclease. A search of the literature indicated that very little work had been done on the dry inactivation of enzymes. In the present experiments, dry samples of these two enzymes were heated *in vacuo* at temperatures ranging from 160° to 200° C. The inactivation process was found to be irreversible. The values of the thermodynamic parameters ΔH and ΔS were also calculated from the survival data.

The vacuum heating chambers used in these experiments consisted of six small brass cups with thin (0·6-mm) copper bottoms. The cups were connected to a mechanical forepump through a vacuum manifold. With this arrangement, the pressure could be maintained at 40μ of mercury during the course of an experiment.

The enzyme samples were prepared by pipetting 0·02 ml. of a stock solution (1 mg/ml.) of the enzyme in distilled water on to a stainless steel disk. These disks were then placed in a desiccator and dried by fast vacuum pumping. After this operation, the disks were placed in the vacuum chambers and the chambers were evacuated.

The heating procedure consisted of immersing the cups in a constant-temperature bath for a known length of time. After the heating period, the cups were cooled by placing them in a cold-water bath (20° C). The temperature was measured by means of a thermocouple placed in the bath. A number of runs were made with a second thermocouple placed on the inside bottom of the cups. The purpose of these runs was to determine the time necessary for the temperature in the cup to rise to the bath temperature. This rise time was found to be short (the maximum observed was 200 sec at 210° C) and all heating times were corrected for it.

The activity of heated trypsin samples, with respect to a non-heated control, was determined by the usual casein digestion assay[1]. The protein from two disks was dissolved in 2 ml. of acetate buffer at *p*H 5·5. 1 ml. of this solution (20 μg/ml.) was added to the casein solution (10 mg/ml.) and incubated for 24 min at 37° C. The assay was standard in all other respects.

In the case of ribonuclease, only one disk was added to 2 ml. of acetate buffer, also at *p*H 5·5. 1 ml. of this solution (10 μg/ml.) was incubated with 3 ml. of a 1-mg/ml. solution of ribonucleic acid (RNA), in the same buffer, for 10 min at 25° C. The reaction was stopped by adding 1 ml. of a 25 per cent perchloric acid–uranyl acetate solution. The assay of Anfinsen was carried out on the supernatant liquid[2]. The enzymes and the RNA were commercial products obtained from General Biochemicals, Inc. The distilled water, used in making all solutions, was injection-grade water supplied by Baxter Laboratories, Inc.

Within the limits of experimental error, the survival ratio at any fixed temperature was found to be an exponential function of the heating time. This function had the form $A = A_0 \exp(-kt)$. The value of k, the rate of thermal inactivation, was obtained from the slope of the inactivation plots (on a semi-logarithmic scale) and the value of ΔF was calculated according to the theory of absolute reaction rates[3]. These data are summarized in Table 1.

Table 1

Enzyme	Temperature (° C)	k (sec^{-1})	ΔF (cal/mole)
Ribonuclease	161	$1{\cdot}04 \times 10^{-4}$	33,700
	170	$1{\cdot}94 \times 10^{-4}$	34,200
	192	$6{\cdot}00 \times 10^{-4}$	34,500
	204	$1{\cdot}04 \times 10^{-3}$	34,900
Trypsin	160	$1{\cdot}58 \times 10^{-4}$	33,300
	175	$2{\cdot}98 \times 10^{-4}$	33,800
	184	$5{\cdot}20 \times 10^{-4}$	34,100
	195	$8{\cdot}75 \times 10^{-4}$	34,300
	205	$1{\cdot}50 \times 10^{-3}$	34,500

As can be seen from Table 1, ΔF is a slowly increasing function of temperature. ΔF also satisfies the relation $\Delta F = \Delta H - T\Delta S$. Thus for trypsin, ΔH = 22,500 cal/mole; $\Delta S = -25$ cal/mole degree and, for ribonuclease, ΔH = 22,400 cal/mole and $\Delta S = -24$ cal/mole degree. Zero or negative values of ΔS have been observed in the past for the dry heating of biological materials[4]. Since the meaning of this behaviour is obscure, no physical significance has been attached to the values of ΔH and ΔS found in this work. They are considered as constants which characterize a given reaction.

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Influence of Electric Field on the Capacity of Phospholipid Membranes

VARIOUS bimolecular models of the cell membrane have already been made[1–3]. Such bimolecular films separating two aqueous solutions are formed by applying a solution of phospholipids to the hole in a 'Teflon' cup.

In the present investigation, we have attempted, using artificial phospholipid membranes, to investigate the electromechanical properties which apparently play an essential part in the activity of the cell membrane. Indeed, there is a potential difference of the order of 0·1 V across the resting excitable cell membrane. This produces substantial mechanical forces which compress the membrane. In the course of excitation, the electric field across the membrane decreases and the mechanical compression also falls. This, in turn, could lead to enlargement of the radii of the pores and an increase in the permeability of the cell membrane. In fact, the pores in cell membranes do enlarge in the course of excitation[4]. With the use of a phospholipid model, it became possible