The lability of rat-liver microsomes and ribonucleoprotein particles

That microsomes lose their ability to incorporate amino acids when kept at room temperature for a short period or at 4° overnight has been repeatedly observed. SACHS has noted that S-protein, an RNA-free fraction of cell sap, will partially stabilize microsomes[1]. To our knowledge, however, no kinetic study of the decay process has been carried out. In this paper we report the results of such studies on rat-liver microsomes and on active ribonucleoprotein particles obtained from the same source.

Once-washed microsomes were prepared from 200-g Wistar rats by a procedure based on the method of KELLER AND ZAMECNIK[2], and the modification of SACHS[1]. Sucrose (0.25 *M*) was used both for the homogenization step and for washing the microsomes. Ribonucleoprotein particles were prepared according to the procedure of RENDI AND HULTIN[3]. This method employs the detergents Lubrol WX (a polymer of ethylene oxide and a long-chain fatty alcohol, Arnold Hoffman and Co., Providence, R.I.) and sodium deoxycholate (Matheson, Coleman and Bell, Cincinnati, Ohio) to liberate particles from endoplasmic reticulum. The partially clarified mitochondrial supernatant obtained is layered onto a medium of higher density and centrifuged at 105,000 × *g*. This process separates the dense particles from the cell sap and dispersed reticulum.

The decay of microsomes or ribonucleoprotein particles was followed by pre-incubating suspensions in the desired medium and, after a variable period, adding the balance of salts, cell sap, radioactive leucine, and adenosine triphosphate-generating system necessary for incorporation. Incubation of the mixture (microsomes, 1 ml; ribonucleoprotein particles, 0.5 ml) was carried out at the same temperature as the pre-incubation for a standard period (20 or 30 min). The incorporation of radioactive leucine into protein was estimated in the usual way. Since in a given experiment all tubes contained the same amount of protein, no correction for self-absorption was necessary. The observed incorporations, were, however, multiplied by a factor such that the incorporation, i, for zero pre-incubation, $t = 0$, was equal to 100. This adjustment made it easier to compare the results of different experiments.

The results of a typical microsome-decay experiment at 37° are shown in Fig. 1a. The semi-log plots of such experiments show an initial curved region of fast decay which eventually approaches a straight line, *i.e.*, the decay process approaches the time course of a first-order reaction–simple exponential decay. Both the extent of the curved region and the slope of the linear region vary somewhat from experiment to experiment. The rate of decay is markedly affected by temperature; thus at 22° the incorporation was reduced by 16 % after 30 min as compared with 80 % at 37°. Similar decay curves were observed for ribonucleoprotein particles (7 mg protein/ml) in Tris buffer (0.035 *M*), Tris–KCl (0.05 *M*–0.2 *M*) and $MgCl_2$–KCl (0.01 *M*–0.2 *M*). The slope of the linear portion in these experiments was about half that found for microsomes.

If only a single species of labile particle capable of incorporation were present, the number of such particles decreased during pre-incubation according to first-order kinetics, and the observed incorporations were proportional to the number of active particles present at the start of the incubation, then a straight line would have been observed throughout the pre-incubation period. The form of the experimental curve

Abbreviations: RNA, ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane.

suggests that particles decaying at appreciably different rates are present. In Fig. 1a, selected to document fully the curved region, it is shown that the experimental points may be adequately represented by the sum of two first-order decay terms. The presence of two independently decaying species with half lives differing by a factor of

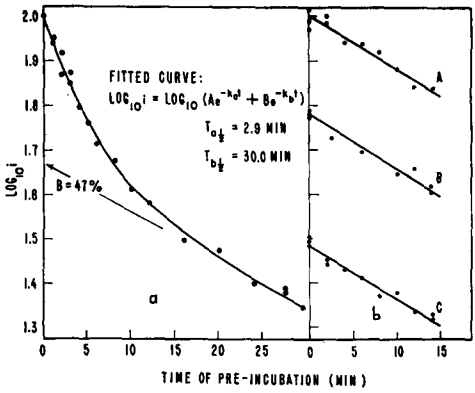

Fig. 1. (a) Microsome decay at 37°: microsome protein concentration, 1.5 mg/ml. (b) Microsome decay at 29° as a function of microsome concentration; microsome protein concentration A:B:C = 4:2:1, curve A, 5.2 mg/ml. The pre-incubation mixtures (0.8 ml) contained all the incubation constituents less cell sap, radioactive leucine, and 20 μmoles sucrose. Incubation mixture (1.0 ml): creatine phosphate (K^+), 20 μmoles; adenosine triphosphate (K^+), 1 μmole; creatine kinase, 140 μg(guanosine triphosphate (Na^+), 0.25 μmole; $MgCl_2$, 5 μmoles; KCl, 80 μmoles; glutathione (K^+), 5 μmoles; Tris–HCl buffer, pH 7.4, 30 μmoles; sucrose, 100 μmoles; radioactive, L-leucine, 0.1 μmole (200,000 counts/min; obtained from Nuclear-Chicago Corp.); and cell sap, 0.8 mg. All solutions were adjusted to pH 7.4 before mixing.

10 (2.9 min and 30 min) is therefore postulated. In agreement with this, the form of the curves for the time course of incorporation into microsomes at 37° may be accounted for by assuming linear incorporation into two species of decaying particles.

Measurements of the rate of sedimentation, at 20°, of lubrol–deoxycholate ribonucleoprotein particles were made with a model E Spinco ultracentrifuge fitted with Schlieren optics. Two major components with sedimentation coefficients of the order of 50 and 70 were observed in the following media (pH 7.8, protein concentration 5 mg/ml): Tris–lubrol (0.02 *M*–0.2 %), Tris–lubrol–KCl (0.02 *M*–0.2 %–0.2 *M*), Tris–$MgCl_2$ (0.02 *M*–0.0001 and 0.002 *M*). These two species may correspond to the components C (63S) and B (83S) found by HAMILTON AND PETERMAN[4] in rat-liver microsomes (sedimentation coefficients corrected to zero protein concentration). It is possible that these two kinds of particles are both capable of incorporation and that they decay at different rates.

The rate of microsome decay was found to be independent of microsome concentration over a 4-fold range of dilution (Fig. 1b). For this experiment, a temperature at which it was easily possible to follow the initial rate of decay was selected; 29° instead of 37°. Were the decay due to an enzyme released from the microsomes into the suspension medium, then the more dilute solutions would be expected to show a reduced rate of decay. To account for the observed results the enzyme, *e.g.* ribonuclease, would have to be attached to the ribonucleoprotein particle in such a manner that the enzyme could attack ribonucleic acid associated with its own particle but could not attack that of other particles. It seems more probable that the decay of

the particles should be ascribed to denaturation[5,6] or to spontaneous chemical changes. In this connection it may be mentioned that incubation, in 0.5 *M* NaCl, of ribonucleoprotein particles at 27° in pH-7 buffer for 24 h did not release measurable amounts of acid-soluble oligonucleotides (see ref. 7).

Thiols, *e.g.*, glutathione or mercaptoethylamine, which are required for transfer of amino acids from soluble ribonucleic acid to microsomal particles[8] markedly affect the stability of these particles. Mercaptoethylamine (0.012 *M*) in the presence of Tris buffer (0.05 *M*, pH 7.4) increased the rate of decay of ribonucleoprotein particles at 37° about 3-fold over the rate in Tris buffer alone. The reason for this effect is unknown.

The above results provide kinetic evidence for two species of ribonucleoprotein particle which decay at different rates. On ultracentrifugation two main components were observed, and it is possible that these represent the two species in question.

We wish to thank Dr. R. C. WARNER for the ultracentrifuge runs and Dr. S. OCHOA for his interest in this work. This work was aided by grants from the National Institute of Arthritis and Metabolic Diseases (Grant A-1845) of the U.S. Public Health Service; The Rockefeller Foundation; and the Jane Coffin Childs Memorial Fund for Medical Research.

The first author is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research. The second author is a Summer Research Student, 1959.

Department of Biochemistry, New York University School of Medicine, New York, N.Y. (U.S.A.)

KENNETH RALPH HANSON*
JOHN KASTOR
RENZO RENDI

1 H. SACHS, *J. Biol. Chem.*, 228 (1957) 23.
2 E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 221 (1956) 45.
3 R. RENDI AND T. HULTIN, *Exptl. Cell Research*, 19 (1960) 253.
4 M. G. HAMILTON AND M. L. PETERMAN, *J. Biol. Chem.*, 243 (1959) 1441.
5 K. LAIDLER, *Chemical Kinetics of Enzyme Action*, Oxford University Press, Oxford, England, 1958.
6 M. A. LAUFFER, *Proc. IV Int. Cong. Biochem.*, Pergamon Press, Vol. VII, 1958, p. 167.
7 D. ELSON, *Biochim. Biophys. Acta*, 36 (1959) 372.
8 D. NATHANS AND W. C. HÜLSMANN, *Federation Proc.*, 19 (1960) 347; unpublished results in this laboratory (R. RENDI).

* Present address, Connecticut Agricultural Experiment Station, New Haven, Conn. (U.S.A.).

Received August 15th, 1960