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Hormonal and Nutritional Effects on the Binding of ^{125}I-Labeled Anti-Serine Dehydratase Fab′ to Rat Tissue Polysomes†

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ABSTRACT: With ^{125}I-labeled Fab′ specific for rat liver serine dehydratase it has been possible to localize polyribosomes synthesizing the enzyme under several different environmental conditions. Evidence is presented to show that, following the administration of amino acids in vivo, the relative synthetic capabilities of free and membrane-bound polyribosomes synthesizing serine dehydratase vary with time. Early during the period of induction of the enzyme by administration of amino acids or by feeding a high protein diet the majority of the newly synthesized enzyme is derived from membrane-bound polyribosomes. Later in the induction process an increasing proportion of the enzyme is synthesized by the free polyribosomes. Subcellular localization studies clearly show that serine dehydratase is synthesized by both subclasses of hepatic membrane-bound polyribosomes, the loose and tight membrane-bound polyribosomes, as well as by the free polyribosomes. It was found that the membrane-bound polyribosomes are the preferential sites of synthesis of the majority of serine dehydratase molecules in the Morris hepatomas 5123C and 7800. It is concluded that the synthesis of the enzyme, serine dehydratase, in rat liver is not discretely compartmentalized in either class of free or membrane-bound polyribosomes. Rather, the relative proportions of the serine dehydratase synthesizing polyribosomes within these two classes of polyribosomes can vary depending on the metabolic and physiologic state of the liver cell.

Considerable evidence (for recent review see Shires et al., 1974) has accumulated which demonstrates the presence of at least two distinct classes of polyribosomes in eukaryotic cells, those free in the cytoplasm and those attached to the endoplasmic reticulum. The membrane-bound polyribosomes have been proposed to be the sites of synthesis of proteins to be exported from the cell (Palade, 1956), whereas the free polyribosomes are considered to be preferentially involved in the synthesis of proteins destined for intracellular use (Birbeck and Mercer, 1961). However, exceptions to this generality have been reported in the case of two intracellular enzymes, NADPH[1]-cytochrome *c* reductase (Ragnotti et al., 1969) and serine dehydratase (Ikehara and Pitot, 1973), the synthesis of which has been shown to occur on both free and membrane-bound polyribosomes.

Identification of polyribosomes synthesizing a specific protein has been achieved by radioimmunochemical techniques which involve the binding of specific antibody molecules to polyribosomes (Warren and Peters, 1965; Taylor and Schimke, 1974; Ikehara and Pitot, 1973; Palacios et al., 1972; Konijn et al., 1973). Taking advantage of the sensitivity and specificity of this type of assay, we have investigated the intracellular localization of the synthesis of a specific intracellular enzyme, serine dehydratase, during modulation by various stimuli in vivo.

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[1] Abbreviations used are: SDH, serine dehydratase; Fab′, the monovalent antibody fragment obtained by papain digestion of the IgG molecule (see Porter, 1959); IgG, 7S immunoglobulin; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; EDTA, ethylenediaminetetraacetic acid; STKM, 0.44 M sucrose, 20 mM Tris-HCl (pH 7.4)–25 mM KCl–5 mM $MgCl_2$.

Materials and Methods

Materials and Animals. In the previous publication (McLaughlin and Pitot, 1976), information is supplied for the manufactured products and for the animals which were used in the experiments described herein.

In those experiments where animals were administered amino acids by gastric intubation, an equimolar mixture of arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, and tryptophan (100 μg/ml) was prepared in water.

Methods. Preparation of total as well as free and membrane-bound polyribosomes, preparation and iodination of anti-serine dehydratase Fab′ and nonimmune Fab′, construction of sucrose density gradients, and determination of ^{125}I-labeled Fab′ binding to polyribosomes was accomplished as described in the previous publication (McLaughlin and Pitot, 1976). All analytical methods which we used are also listed therein.

Preparation of Serine Dehydratase from Rat Liver. Rat liver serine dehydratase was purified by the method of Inoue et al. (1971). The crystallized enzyme preparation had a specific activity of 365 enzyme units per mg of protein. One enzyme unit is defined as that amount of enzyme which oxidizes 1 μmol of NADH per min assayed by the method of Pitot and Pries (1964). The reported molecular weight of the enzyme is 64 000 (Nakagawa and Kimura, 1969). Knowing the specific activity of the purified enzyme and its molecular weight, we estimate the catalytic constant (Mahler and Cordes, 1971) of serine dehydratase to be 2.3×10^4 mol of NAD^+ product formed per mol of enzyme per min. The enzyme has two reactive sites (Nakagawa and Kimura, 1969). Therefore, the turnover number (Mahler and Cordes, 1971) is estimated to be 1.2×10^4.

Immunochemical Techniques. Rabbit antiserum to serine dehydratase was prepared as described previously (Jost et al., 1968; McLaughlin and Pitot, 1976). The specificity of the anti-serine dehydratase IgG preparation was demonstrated by double diffusion in agar by the method of Ouchterlony (1968). A single precipitin band was obtained indicating a single reactive species (data not shown). Gels for immunoelectrophoresis were prepared as described by Clausen (1969). Immunoelectrophoresis was performed as previously described (McLaughlin and Pitot, 1976). Immunoelectrophoresis of purified serine dehydratase and a crude liver cell extract in agarose gel was followed by inoculation of the central trough of that gel with anti-serine dehydratase IgG and immunodiffusion for 2 days carried out at room temperature. The formation of a single precipitin arc and the absence of spur formation (data not shown) are indicative of a monospecific antibody preparation (Ouchterlony, 1968). Although the pH of the buffer (8.4) used to prepare the agarose gel and used in the reservoirs of the immunoelectrophoresis apparatus is quite basic compared with the isoelectric point of pH 6.7 for serine dehydratase (Nakagawa and Kimura, 1969), the enzyme protein migrates only a short distance if at all. Rat serum albumin migrated reproducibly toward the anode under identical electrophoretic conditions (McLaughlin and Pitot, 1976).

Antibody precipitation reactions were performed in conical 3-ml Pyrex centrifuge tubes. The source of antigen was the supernatant from a rat liver cell homogenate which had been centrifuged at 105 000g for 90 min in a Beckman 50 Ti rotor. This supernatant was heated at 37 °C for 30 min and again centrifuged for 90 min at 105 000g. This heat-treated liver cell supernatant contained serine dehydratase at a level of 18 units/ml. The immunoprecipitation reaction was initiated by adding a constant amount of anti-SDH (1 mg) IgG to varying amounts of heat-treated liver cell supernatant in a constant volume of 0.5 ml. Controls for the nonspecific precipitation of protein were constructed by incubating nonimmune IgG (1 mg) with varying amounts of the appropriate antigen. Incubation was for 30 min at 37 °C followed by 18 h at 4 °C. At the end of the incubation period, mixtures were centrifuged at 183g in the Sorvall GLC-1 centrifuge at 4 °C for 20 min. The supernatant was removed and assayed for SDH activity. The precipitates were washed twice with 0.5-ml aliquots of 50 mM Na_2HPO_4 (pH 7.6) in 0.15 M NaCl at 4 °C. The remaining supernatant washes were carefully removed and the protein content of the precipitate was determined. At the equivalent point, 120 μg of anti-serine dehydratase IgG precipitates 1 μg of the enzyme. The microgram quantity of serine dehydratase precipitated was estimated by knowing the enzymatic activity of the supernatant prior to incubation with the antibody preparation. For example, 2.0 units of enzyme is equivalent to 5.55 μg of serine dehydratase, assuming a specific activity of 365 enzyme units per mg of purified serine dehydratase protein.

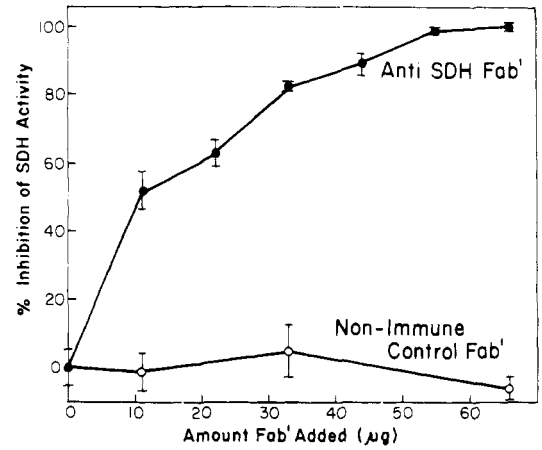

FIGURE 1: Inhibition of serine dehydratase (SDH) activity by anti-SDH and nonimmune Fab′. Anti-serine dehydratase Fab′, nonimmune Fab′, and rat liver 105 000g supernatant (S_3) were prepared as described under Materials and Methods. Aliquots of S_3 in triplicate (containing 0.17 μg of SDH) were incubated with either anti-serine dehydratase Fab′ or nonimmune Fab′ at 37 °C for 30 min. The enzymatic activity of serine dehydratase in the incubated samples was immediately assayed as previously described (Pitot and Pries, 1964). The mean of values of SDH activity in the nonimmune control samples (5.5×10^{-2} enzyme unit) was used as the 0% value for calculation of percent inhibition. The closed circles and open circles indicate anti-SDH Fab′ and nonimmune Fab′, respectively, with brackets representing the standard deviations of the mean.

Results

Inhibition of Serine Dehydratase Activity by Anti-SDH Fab′ Molecules. It has been reported (Jost et al., 1968) that the antigen–antibody precipitate formed upon incubation of anti-serine dehydratase IgG with liver cell extracts does not possess serine dehydratase activity. To test whether or not the binding of the anti-SDH Fab′ molecule to serine dehydratase resulted in a loss of enzyme activity, increasing amounts of anti-SDH Fab′ or nonimmune control Fab′ were added to a constant aliquot of crude SDH in the form of liver cell supernatant (Figure 1). Determination of serine dehydratase activity in the incubated samples revealed that the anti-SDH Fab′-treated, but not the nonimmune Fab′-treated, liver cell supernatants exhibited an inhibition of enzyme activity (Figure 1). Inhibition of serine dehydratase enzyme activity by the

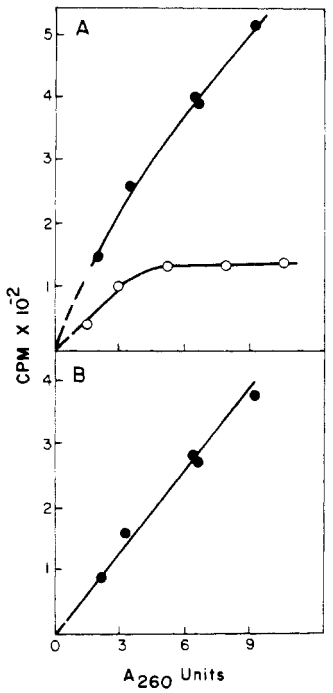

FIGURE 2: Binding of anti-SDH and nonimmune Fab′ preparations to hepatic polyribosomes. ^{125}I-labeled Fab′ preparations iodinated using Chloramine-T as a catalyst (see Materials and Methods) were incubated at 4 °C for 1 h with varying amounts of total polyribosomes. The incubated polyribosomes were washed three times by sedimentation through sucrose gradients (see text for further experimental details). The pellets from the third centrifugation were assayed for the level of ^{125}I-labeled Fab′ binding as described in the Materials and Methods. In A, the closed and open circles represent the determinations of the radioactivity and the absorbancy for anti-serine dehydratase Fab′ and nonimmune Fab′ assays, respectively. In B, the anti-serine dehydratase Fab′ values corrected for the nonimmune control values are presented.

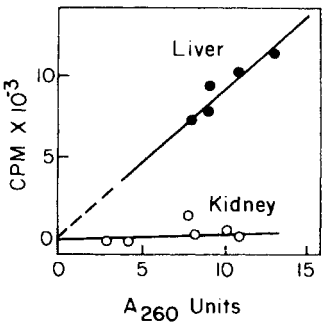

FIGURE 3: Binding of anti-serine dehydratase Fab′ to liver and kidney polyribosomes. Liver and kidney total polyribosomes were isolated from rats maintained on a 90% protein diet fed ad libitum for 6 days (see Materials and Methods). Kidney polyribosomes were incubated with a 105 000g supernatant (S_3) from the livers of these rats. The kidney polyribosomes were then sedimented through 0.44 M STKM in 0.5 M NH_4Cl. Both the liver and the washed kidney polyribosomes were incubated with anti-serine dehydratase Fab′ (2.8×10^6 cpm) (see Materials and Methods, lactoperoxidase iodination procedure) for 1 h at 4 °C. The assay for the binding of ^{125}I-labeled Fab′ preparations was accomplished as is described under Materials and Methods. The binding of anti-serine dehydratase Fab′ to liver and kidney polyribosomes is presented as closed and open circles, respectively.

iodinated or unlabeled anti-SDH Fab′ preparations was used as a conveniently measured parameter of biological activity of the antibody preparations.

Binding of Anti-Serine Dehydratase Fab′ Molecules to Liver Polyribosomes. Ikehara and Pitot (1973) have demonstrated the specific binding of anti-SDH Fab′ preparations to liver polyribosomes by examination of the profiles of radioactivity and absorbancy at 260 nm obtained following sedimentation of polyribosomes preincubated with radiolabeled Fab′ antibodies. Since this method is somewhat laborious and is difficult to use as a quantitative assay, it was decided to utilize an assay similar to that described in the previous publication (McLaughlin and Pitot, 1976) for the binding of anti-rat serum albumin Fab′ molecules to polyribosomes. In Figure 2 it is seen that three washings of total polyribosomes preincubated for 1 h at 4 °C with 1.4×10^6 cpm of radiolabeled anti-SDH Fab′ preparations do not result in a plateauing of the binding of anti-SDH Fab′ to polyribosomes as the amount of polyribosomes is increased. However, in the case of the nonimmune Fab′ determinations, it is seen that the pelleted radioactivity is not a function of polyribosome quantity above 5.0 A_{260} units. Subtraction of the radioactivity values for the nonimmune control Fab′ determinations from the radioactivity associated with polyribosomes incubated with anti-SDH Fab′ permits extrapolation of the linear anti-SDH Fab′ binding curve through the origin of the figure. This is what one would predict since the polyribosomes are presumed to be the limiting reactant in this assay system. The finding that the subtraction of the control nonimmune values from the anti-SDH binding values permits extrapolation of specific binding values through the origin supports the contention that the polyribosomes are the limiting reactant. Thus, the corrected anti-SDH binding values represent measurement of molecular interactions characteristic of an antigen–antibody reaction.

The cosedimentation of serine dehydratase holo- or apoenzyme with polyribosomes preincubated with anti-SDH Fab′ may result in erroneous values of the localization of polyribosomes actively synthesizing serine dehydratase. In this regard an experiment was designed to ascertain whether or not anti-SDH Fab′ molecules would react with polyribosomes isolated from cells from the kidney, an organ which does not synthesize serine dehydratase. Kidney polyribosomes were incubated at 4 °C for 15 min with a 105 000g supernatant (S_3) from the livers of rats fed a 90% protein diet for 6 days. The kidney polyribosomes were then sedimented through 0.44 M STKM in 0.5 M NH_4Cl to remove the soluble proteins including serine dehydratase. Both the liver and the washed kidney polyribosomes were incubated with antiserine dehydratase Fab′ (2.8×10^6 cpm) for 1 h at 4 °C. Figure 3 shows that kidney polyribosomes, even when exposed to soluble SDH during isolation of those ribosomes, do not bind anti-SDH Fab′ molecules. The same antibody preparation is capable of binding in a quantitative manner to liver polyribosomes isolated under circumstances similar to those used in the isolation of the kidney polyribosomes. From these data, it is concluded that soluble serine dehydratase does not cosediment with polyribosomes through the 0.44 M sucrose solutions used to wash the kidney polyribosomes. In addition, these data demonstrate that the anti-serine dehydratase Fab′ does not react with polyribosomes which are not involved in the synthesis of serine dehydratase.

Localization of Serine Dehydratase Synthesizing Polysomes in Livers of Rats Subjected to Various Nutritional Conditions. The rate of synthesis of rat liver serine dehydratase in animals pretreated with amino acids is markedly reduced within 4 h following treatment of those animals with glucose (Jost et al., 1968). In a related study, Pitot and Jost (1968) were able to demonstrate an apparent increase in the amount of serine dehydratase antigen labeled by administration of [^{14}C]valine into the portal vein 1 h after the administration of

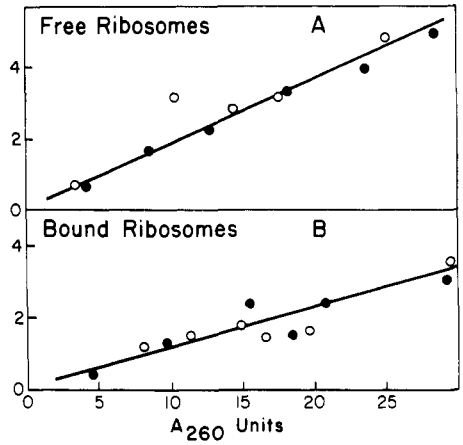

FIGURE 4: The effect of glucose administered in vivo on the binding of anti-SDH Fab′ to hepatic polyribosomes. Rats were maintained on a 0% protein diet fed ad libitum for 5 days. At the end of this period, the animals were fasted overnight. At 6 a.m., ten rats received an equimolar mixture of amino acids (200 μg per 100 g of body weight) (see Materials and Methods) by gastric intubation. Six hours after administration of the amino acids, five of the animals were given glucose in water (1 g per 100 g of body weight by gastric intubation) and the remaining five animals were given water only. Seven hours after administration of the amino acids, the animals were sacrificed. The liver free and bound polyribosomes were isolated as is described in the Materials and Methods. The determination of the binding of anti-SDH Fab′ (2.8×10^6 cpm) to varying amounts of free polyribosomes and bound ribosomes is presented in A and B, respectively. The values for the anti-SDH Fab′ binding have been corrected for nonimmune Fab′ control values. The open and closed circles represent anti-SDH Fab′ binding values for the polyribosomes from control and from glucose-treated animals, respectively.

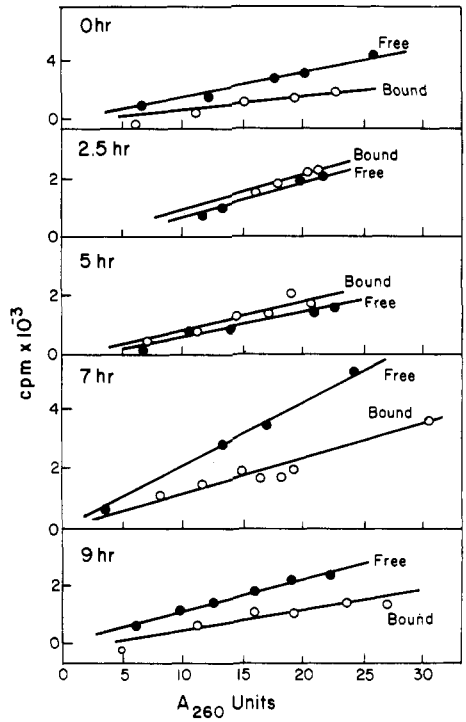

FIGURE 5: The binding of anti-SDH Fab′ to free and membrane-bound hepatic polyribosomes from rats intubated with amino acids. Rats were maintained on a 0% protein diet fed ad libitum for 5 days. Each of the rats was given an equimolar mixture of amino acids (200 mg per 100 g of body weight) (see Materials and Methods). At the times indicated thereafter as seen in the figure, rats were sacrificed. Free and membrane-bound polyribosomes were isolated from the livers as described in the Materials and Methods. The binding of anti-SDH Fab′ to isolated polyribosomes was determined as previously described (see Materials and Methods and Figure 6 legend for experimental details). The values for the binding of anti-SDH Fab′ to free (●) and membrane-bound (○) polyribosomes have been corrected for nonimmune Fab′ controls.

glucose. These data indicated that more labeled serine dehydratase antigen was associated with the rough endoplasmic reticulum than with free polysomes. The data seen in Figure 4 indicate no difference in the levels of binding of anti-SDH Fab′ molecules to the isolated free and membrane-bound polyribosomes isolated from the livers of animals given glucose 1 h earlier as compared with controls. Thus the changes demonstrated by Pitot and Jost (1968) may be related directly to the association of polyribosomes with the membranes rather than to a distinct difference in the amount of polysome-bound enzyme in these two ribosomal fractions. No experiments were carried out at later periods after glucose administration where there has been demonstrated a marked decrease in the rate of synthesis of serine dehydratase (Jost et al., 1968).

Söling et al. (1969) have described the sensitivity and resistance of serine dehydratase synthesis to actinomycin D treatment by immunochemical determinations of ^{3}H-labeled amino acid incorporation into serine dehydratase in liver supernatants from rats given a single dose of casein hydrolysate followed by a second dose of casein hydrolysate with and without actinomycin D. They found that, between 2–3 and 6–8 h after initiation of induction of the enzyme by amino acids, the synthesis of SDH was resistant to the administration in vivo of actinomycin D. Before and after this 6-h period of time, the synthesis of serine dehydratase was sensitive to actinomycin D administration. In an attempt to understand better the mechanism of this phenomenon of changing resistance and sensitivity of synthesis of serine dehydratase to actinomycin D administration, the free and membrane-bound polyribosomes were isolated from rats maintained on a protein-free diet fed ad libitum for 5 days followed by administration of a single dose of amino acids (Figure 5). The level of binding of anti-SDH Fab′ to the free and membrane-bound polyribosomes isolated from rats sacrificed at various times following the administration of the amino acids is presented in Figure 5. The relative degree of binding of anti-SDH Fab′ molecules to the free in comparison with the membrane-bound polyribosomes varies with respect to the time that the animals were sacrificed following administration of 200 mg of amino acids per 100 g of body weight. The membrane-bound polyribosomes isolated from the livers of rats sacrificed at 2.5 and 5 h following administration of the amino acids bind anti-SDH Fab′ molecules to approximately the same degree as do the free polyribosomes isolated from the same livers. Conversely, at all other time points examined the free polyribosomes, when compared with the membrane-bound polyribosomes isolated from the livers of the same rats, preferentially bind the radiolabeled antibody molecule. In subsequent experiments (data not presented), it was found that between 0–2 and 6–8 h after initiation of the induction process somewhat variable results were obtained regarding the relative binding of anti-SDH Fab′ molecules to free vs. membrane-bound polyribosomes. However, anti-SDH Fab′ binding to membrane-bound polyribosomes was consistently equal to or greater than the binding to free polyribosomes isolated from the livers of rats sacrificed between 2 and 5 h following administration of amino acids. This is the approximate period during the induction of serine dehydratase when enzyme induction is resistant to the administration of actinomycin D in vivo (Söling et al., 1969).

Reynolds et al. (1971) examined the changes in serine dehydratase activity in livers of rats shifted from a 12 to a 60% protein diet. The activity reached a maximum level on day 4

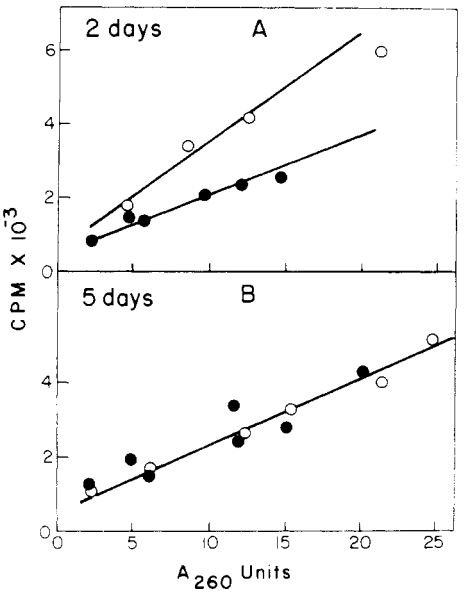

FIGURE 6: The binding of anti-SDH Fab′ to the free and membrane-bound polyribosomes from animals maintained on a 90% protein diet fed ad libitum. Rats maintained on a chow diet (see Materials and Methods) fed ad libitum for 1 month were given a 90% protein diet fed ad libitum for 2 (A) or 5 days (B). The rats were sacrificed and the free and membrane-bound polyribosomes isolated from the livers (see Materials and Methods). Varying amounts of the polyribosomes were incubated with ^{125}I-labeled Fab′ preparations (2.2 × 10^6 cpm) and the values for the determination of anti-SDH Fab′ binding have been corrected for nonimmune Fab′ controls (see Materials and Methods). The binding values for free and membrane-bound polyribosomes are represented by closed and open circles, respectively.

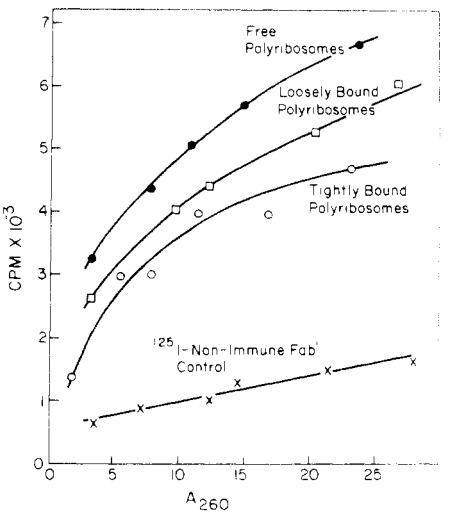

FIGURE 7: The binding of anti-serine dehydratase Fab′ preparations to loosely and tightly membrane-bound polyribosomes and to free polyribosomes of rat liver. Loosely and tightly membrane-bound polyribosomes and free polyribosomes were isolated (see Materials and Methods) from rats maintained on a 90% protein diet fed ad libitum for 5 days. The polyribosomes were incubated with ^{125}I-labeled Fab′ preparations and the binding of the ^{125}I-labeled Fab′ preparations was determined as is described in the Materials and Methods. The values for the binding of anti-serine dehydratase Fab′ to free (●), loosely membrane-bound (□), and tightly membrane-bound (○) polyribosomes are presented. The binding of nonimmune Fab′ to free polyribosomes (X) is also presented.

following the shift in the diet. Therefore we determined the relative degree of binding of anti-SDH Fab′ (2.2 × 10^6 cpm) molecules to the free and bound polyribosomes isolated from the livers of animals sacrificed 2 or 5 days after a shift from a chow diet of relatively low protein content to one having a 90% protein content. In Figure 6A preferential binding of the anti-SDH Fab′ molecules to the membrane-bound polyribosomes as compared with the Fab′ binding to free polyribosomes isolated from the livers of animals maintained on a 90% protein diet fed for 2 days is seen. Maintenance of the rats on a 90% protein diet fed for 5 days causes a further change in the pattern of binding of the anti-SDH Fab′ preparation to the free and membrane-bound polyribosomes. As is seen in Figure 6B the level of binding appears essentially equal for the two classes of polyribosomes isolated from the livers of rats maintained on a 90% protein diet for 5 days.

Serine Dehydratase Nascent Chains on "Loosely" and "Tightly" Bound Polyribosomes. Membrane-bound polyribosomes have been reported to be composed of two subclasses which Rosbash and Penman (1971a,b) named "loose" and "tight" polyribosomes. The loose class of membrane-bound polyribosomes was dissociated from the endoplasmic reticulum by treatment of the membranes with ribonuclease or EDTA or puromycin. The tight class was dissociated from the endoplasmic reticulum by solubilization of the membrane components with detergent. Tanaka and Ogata (1972) reported that the tight class of membrane-bound polyribosomes was the exclusive site of synthesis of rat serum albumin. In the previous paper, we reported (McLaughlin and Pitot, 1976) that rat serum albumin is synthesized preferentially by the tight class of membrane-bound polyribosomes but that the loose class of membrane-bound polyribosomes and free polyribosomes are also involved in synthesis of rat serum albumin. The demonstration of the synthesis of serine dehydratase by membrane-bound polyribosomes isolated from the liver (Ikehara and Pitot, 1973) has provoked the question of whether or not a distinction could be made regarding the synthesis of serine dehydratase by the tightly or loosely membrane-bound polyribosomes. In Figure 7 the binding of ^{125}I-labeled Fab′ preparations (2.2 × 10^6 cpm) to the loosely and tightly membrane-bound polyribosomes, as well as the free polyribosomes, following a 1-h incubation at 4 °C is presented. These polyribosomes were isolated from the livers of animals maintained on a 90% protein diet fed for 5 days. There is appreciable binding of the anti-SDH Fab′ preparations compared with that with the nonimmune Fab′ controls to each of the three polyribosome classes. The free polyribosomes exhibit the maximum level of binding, the loosely membrane-bound polyribosomes, an intermediate level of binding, and the tightly membrane-bound polyribosomes show the lowest level of binding to the anti-SDH Fab′ molecules. These differences in the relative levels of binding of the anti-SDH Fab′ molecules to the three classes of polyribosomes exist over a wide range of polyribosome concentrations. It should be noted that a direct comparison of the data presented in Figure 7 with that in Figure 6B is not possible as the data are presented since in Figure 7 the bound polysomes were separated into two fractions while no such distinction is made in Figure 6B.

Localization of SDH Nascent Chains in Hepatoma Polyribosomes. In light of the data presented regarding the distribution of ^{125}I-labeled anti-SDH Fab′ binding sites on free and membrane-bound hepatic polyribosomes isolated from rats subjected to various environmental stimuli (Figures 5 and 6), it became of interest to examine the binding of this antibody preparation to the polyribosomes isolated from several Morris hepatomas, namely the 5123C, 7800, and 7777. In Figure 8 data are presented for the binding of an anti-SDH Fab′ preparation (2.8 × 10^6 cpm) to the polyribosomes isolated from the

hepatomas of rats maintained on a chow diet. In comparing the level of binding of the radiolabeled antibody to the membrane-bound polyribosomes with Fab′ binding to the free polyribosomes, it can be seen that the membrane-bound polyribosomes of hepatomas 7800 and 5123C exhibit a greater Fab′ binding capacity than do the free polyribosomes from the same tumors. There was no binding of the anti-SDH Fab′ molecule to the free or the membrane-bound polyribosomes isolated from the Morris hepatoma 7777. The enzyme activity in the supernatant of cells from these tumors was found to be 86 units per g of 5123C tissue and 5.8 units per g of 7800 tissue. There was no measurable SDH activity in the supernatant of the 7777 hepatoma.

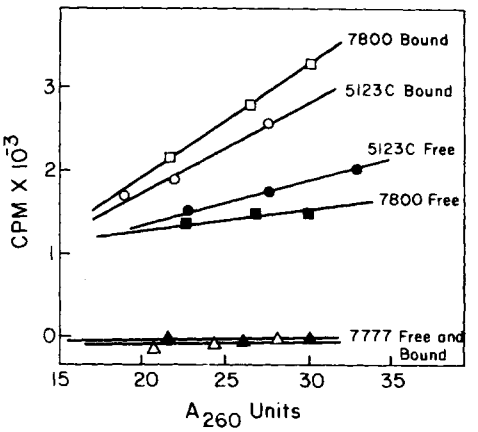

FIGURE 8: The binding of anti-SDH Fab′ to free and membrane-bound polyribosomes of several hepatomas. Animals bearing transplantable Morris hepatomas (see Materials and Methods) were maintained on chow diets fed ad libitum. Free and membrane-bound polyribosomes were isolated as is described in the Materials and Methods. The binding values for anti-SDH Fab′ preparations were determined. The closed squares, triangles, and circles represent the values for the free polyribosomes from the hepatomas 7800, 7777, and 5123C, respectively. The open symbols represent the values for the membrane-bound polyribosomes from those hepatomas. The appropriate nonimmune control values have been subtracted from the anti-SDH Fab′ binding values.

Discussion

Discrete localization of the synthesis of a particular protein by either the free or membrane-bound polyribosomes has been reported for a number of proteins. The subject has been reviewed by Shires et al. (1974). Data presented in this paper provide evidence that the synthesis of the enzyme, serine dehydratase, is not limited to either free or membrane-bound polysomes. Thus, the enzymes, serine dehydratase, NADPH-cytochrome *c* reductase (Ragnotti et al., 1969), and catalase (Sakamoto and Higashi, 1973), as well as the proteins, albumin (Ikehara and Pitot, 1973), ferritin (Redman, 1969), and immunoglobulin (Lisowska-Bernstein et al., 1970) are clear exceptions to the earlier generality that membrane-bound polyribosomes are involved only in the synthesis of proteins destined for an extracellular function, whereas the free polyribosomes are synthesizing proteins required in intracellular metabolism. In this paper we utilized the binding of the anti-serine dehydratase Fab′ preparations to hepatic free and membrane-bound polyribosomes to demonstrate that the preferential site of synthesis of serine dehydratase appears to shift between the free and membrane-bound polyribosomes.

As was discussed in the preceding paper (McLaughlin and Pitot, 1976), a clear differentiation of specific vs. nonspecific binding of antibody molecules to polyribosomes must be made when one utilizes an assay involving reactions of ^{125}I-labeled antibody with polyribosomes. Holme et al. (1971a,b) and Ikehara and Pitot (1973) demonstrated that the Fab′ portion of the IgG molecule, as prepared according to the method of Porter (1959), exhibited a significantly lesser degree of nonspecificity when reacted with polyribosomes synthesizing a specific antigen. This consideration becomes of major importance in investigations in which the protein being studied is synthesized as a small percentage of total protein synthesis. An important control for the determination of the level of nonspecific interaction is the reaction of a nonimmune Fab′ preparation with appropriate quantities of polyribosomes. Anti-serine dehydratase Fab′ does not react with polyribosomes isolated from kidney tissue (Figure 3) following exposure of those polyribosomes to soluble serine dehydratase. Washing hepatic polyribosomes with 0.5 M NH_4Cl prior to incubation with antibody preparations (see Materials and Methods) was also utilized to reduce the potential for contamination of polyribosomes with completed enzyme.

Studies from this laboratory (Söling et al., 1969) have demonstrated that the synthesis of serine dehydratase following administration of amino acids is initially sensitive to actinomycin D, becoming resistant to its effects between 1 and 2 h after the initiation of induction. Eight hours after administration of the inducing agent (a mixture of amino acids), SDH synthesis again becomes sensitive to the effects of the antibiotic. Thus for a period of about 6 h beginning 1 to 2 h after the initiation of the induction of SDH by amino acid administration, the synthesis of the enzyme was found to occur at its maximal rate in control animals as well as animals given actinomycin D at levels known to inhibit most DNA-directed RNA synthesis in vivo (1–2 mg/kg). The data were explained on the basis that the mRNA template for serine dehydratase is transcribed upon induction of the enzyme and then is stabilized within 3 h after the initiation of induction. The stable mRNA template is capable of being translated without renewal for approximately 6 h before degradation of the SDH mRNA. We have proposed (Pitot, 1964; Pitot et al., 1969) that stabilization of mRNA templates might occur through the formation of a membrane–mRNA complex. Experimental evidence relevant to this hypothesis has been reviewed recently by Shires et al. (1974). The demonstration that serine dehydratase is preferentially synthesized by the membrane-bound polyribosomes (Figure 5) during the period when the mRNA template appears stable by the actinomycin D studies supports the hypothesis that the endoplasmic reticulum plays a role in stabilization of this template. Further evidence that the synthesis of serine dehydratase occurs preferentially on membrane-bound polyribosomes early in the induction processes is demonstrated by the data presented in Figure 6 which show that, relatively early during the induction of serine dehydratase by feeding a 90% protein diet, the synthesis of the enzyme is preferentially on membrane-bound polyribosomes.

Pitot et al. (1965) defined the period of time during which the synthesis of serine dehydratase remained totally independent of transcription as the mRNA template lifetime. This was estimated to be 6–8 h in the liver. The estimated template lifetime for this enzyme in the Morris hepatoma 5123C was found to be greater than 2 weeks (Pitot et al., 1965). If stabilization of mRNA templates occurs as a consequence of formation of polyribosome–membrane complexes which become functional translating units, one would predict that the membrane-bound polyribosomes isolated from the 5123C tumor would be the preferential sites of synthesis of serine dehydratase. In fact this is what was found (Figure 8). Thus, the data show that a hepatoma characterized by having a prolonged

template lifetime for the enzyme, serine dehydratase, synthesizes this protein preferentially on polyribosomes associated with the endoplasmic reticulum.

One of the proposed roles of the endoplasmic reticulum in the normal liver cell entails transmembrane transport of nascent chains of proteins to be excreted (Siekevitz and Palade, 1960; Sabatini and Blobel, 1970; Redman and Cherian, 1972). The membrane-bound polyribosomes synthesize a variety of proteins which are secreted by the cell (reviewed by Shires et al., 1974). Rosbash and Penman (1971a,b) reported the existence of two subclasses of membrane-bound polyribosomes which he named "loose" and "tight" polyribosomes. Data reported by Tanaka and Ogata (1972) suggested that the tight polyribosomes were the exclusive site of synthesis of rat serum albumin. In the previous paper (McLaughlin and Pitot, 1976), data were presented corroborating these findings in general but modifying them to the extent that all three classes of polyribosomes (free, loosely membrane-bound, and tightly membrane-bound) were found to synthesize rat serum albumin. Data are presented in this paper showing that the enzyme serine dehydratase is synthesized by all three classes of polyribosomes (Figure 7). It should be noted that no appreciable difference in binding of nonimmune Fab′ molecules to free polyribosomes when compared with membrane-bound polyribosomes has been observed (McLaughlin, unpublished observations). The somewhat unexpected finding that the tightly membrane-bound polyribosomes contribute to the synthetic machinery making the protein serine dehydratase which is not exported from the cell implies that the endoplasmic reticulum plays an additional role in translation besides serving to segregate newly synthesized proteins destined for extracellular use from those intended for intracellular use. Since the membrane-bound polyribosomes of the hepatocyte represent an estimated 75% of the total cellular ribosomes (Blobel and Potter, 1967), as much as 83% of the in vivo synthesis of serine dehydratase occurs on membrane-bound polyribosomes isolated from livers of rats maintained on a 90% protein diet.

We would thus propose that the distribution of actively translating specific polyribosomes between the free and membrane-bound polyribosome classes is a function of the physiological and pathophysiological state of the cell. The factors which regulate this modulation of the localization of templates we do not understand but the endoplasmic reticulum appears to play an important role in this phenomenon.

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