The biological significance of the intercalation specificity found for the aromatic-containing peptides is difficult to evaluate at present. It has been proposed that the ten distinctly different intercalation sites possible in DNA may serve as a specific means for recognition of DNA sequence by an intercalating molecule (Gabbay *et al.*, 1972b). In a sense, the different intercalation sites can be considered as pages in a book with the intercalating molecule acting as a selective bookmark. Thus, the "bookmark" hypothesis proposed earlier by Brown (1970) takes on added significance, now that it has been shown that the aromatic amino acid residues not only can intercalate, but do so with some degree of selectivity with respect to primary sequence of the peptide as well as base-pair specificity.

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Nature and Possible Functions of Association between Glutamate Dehydrogenase and Cardiolipin†

Catherine Godinot‡

ABSTRACT: Addition of cardiolipin to glutamate dehydrogenase inhibited the enzyme and induced aggregation of lipid and protein. The inhibition was dependent on enzyme concentration. Both apolar and polar parts of cardiolipin were implicated in the binding. Indeed, hydrolysis of cardiolipin fatty acid chains or addition of a detergent such as Lubrol WX which destroyed hydrophobic bonds prevented the inhibition. The fact that acetylation of glutamate dehydrogenase amino groups hindered the aggregation suggested that they were involved in binding the cardiolipin polar head. The amount of lipid and enzyme found in the aggregates depended upon their relative concentrations and on the presence of cofactors. Glutamate increased and NADH diminished the aggregation. Additional cardiolipin, ADP, GTP, or glutamate could release this inhibition. The presence of glutamate could also selectively prevent the release of glutamate dehydrogenase from inner membrane–matrix mitochondrial fractions, suggesting that glutamate increased the binding of the enzyme to the membrane. The possible role that this association can play in the assembly of mitochondrial membranes and in the regulation of glutamate dehydrogenase activity *in situ* is discussed.

The key to an understanding of the assembly and functions of mitochondrial membranes is the nature of the interactions between lipids and proteins and the new properties that these associations may generate. The active role played by lipids in the activity of membrane-bound enzymes of the mitochondria has been demonstrated in numerous studies (*cf.* Rothfield and Romeo, 1971). However, interferences of lipids with more soluble mitochondrial enzymes have received less attention.

Glutamate dehydrogenase (EC 1.4.1.3) (Norum *et al.*, 1966; Schnaitman and Greenawalt, 1968) and cardiolipin (Getz *et al.*, 1962) are both selectively located in the mitochondria. Earlier investigations on model systems have shown that cardiolipin is a potent inhibitor of glutamate dehydro-

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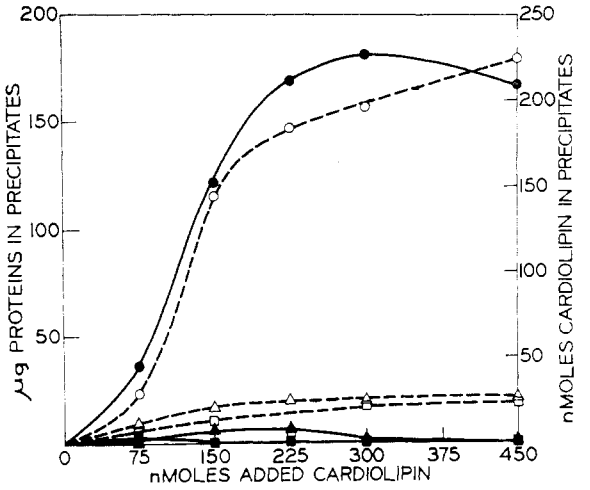

FIGURE 1: Aggregation of normal and acetylated enzyme with cardiolipin. Conditions are as described under Experimental Procedure: open symbols linked by dashed lines, cardiolipin content of the aggregates; closed symbols linked by plain lines, protein content; circles, normal enzyme; squares, active acetylated enzyme (acetylation in the presence of 10^{-4} M GTP and 10^{-4} M NADH); triangles, inactive acetylated enzyme (acetylation without protectors); 200 μg of glutamate dehydrogenase was added in each assay.

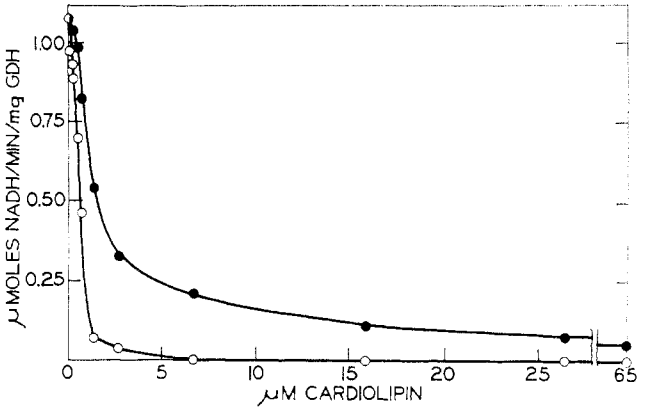

FIGURE 2: Cardiolipin inhibition of normal and acetylated active glutamate dehydrogenase. Glutamate dehydrogenase (GDH) (2 mg/ml) was acetylated at 0° by the addition of 2×10^{-3} M acetic anhydride in standard Tris buffer (0.1 M Tris-acetate (pH 7.15)) containing 1 mM phosphate (Na), 0.1 mM EDTA, 10^{-4} M GTP, and 10^{-4} M NADH (Colman and Frieden, 1966b). After 15 min, the enzyme was dialyzed against standard Tris buffer in an Amincon ultrafiltration cell equipped with a membrane PM30 until no more nucleotides could be detected in the dialysate. The enzyme kept 100% activity. GDH activity was measured as described in Table I: open circles, native enzyme; closed circles, active acetylated enzyme.

genase (Julliard and Gautheron, 1972; Dodd, 1973). When the effects of other phospholipids were compared, the extent of inhibition was determined by the charge of the lipid polar head and was diminished by increasing ionic strength. This implied an electrostatic interaction but nuclear magnetic resonance (nmr) studies suggested that apolar lipid chains should also be involved. Previous research on biogenesis of glutamate dehydrogenase in rat liver led us to postulate that integration of this enzyme in the mitochondria could be related to its association with cardiolipin (Godinot and Lardy, 1973). The present investigation concerns the nature of the binding between glutamate dehydrogenase and cardiolipin, the influence of some of the factors that govern the formation of this complex, and the biological role that this association might play.

Experimental Procedure

Glutamate dehydrogenase (Sigma Chemical Co.) was prepared and acetylated in 0.1 M Tris-acetate buffer (pH 7.15) containing 1 mM phosphate (Na) and 0.1 mM EDTA as described by Colman and Frieden (1966a). Glutamate dehydrogenase activity was determined according to Julliard and Gautheron (1972) unless otherwise indicated. Sucrose gradient centrifugation was conducted according to Eisenkraft and Veeger (1969).

Preparation of rat liver mitochondria has been described previously (Godinot and Lardy, 1973). Mitochondria were depleted of their outer membranes to prepare inner membrane–matrix mitochondrial fractions according to Sottocasa *et al.* (1967).

Chromatographically pure cardiolipin, phosphatidylcholine, or phosphatidylethanolamine (General Biochemicals or Sigma Chemical Co.) were used to prepare fresh micelles daily by sonication according to Fleischer and Fleischer (1967). All operations were conducted at 0° under nitrogen. Special care was taken to prevent oxidation of cardiolipin as described by Dodd (1973) since preliminary experiments have shown that, with oxidized samples or cardiolipin, the extent of inhibition of glutamate dehydrogenase activity was decreased. Cardiolipin concentration in the micelles was determined by measuring their phosphate content by a technique adapted from Bartlett (Dittmer and Wells, 1969). Bisglycerophosphorylglycerol was prepared by mild alkaline hydrolysis of cardiolipin according to Chang and Kennedy (1967). Its concentration was measured by phosphate determination. Its purity was monitored by paper chromatography (Hostetler *et al.*, 1971).

Aggregation between glutamate dehydrogenase and cardiolipin was induced by mixing the enzyme with cardiolipin micelles of determined phosphate content in 0.3 ml (final volume) of 40 mM Tris-acetate buffer (pH 7.15) containing 16 mM phosphate (Na), 0.1 mM EDTA, and effectors as indicated. After 1 hr at room temperature, the aggregates were sedimented (2500*g*, 30 min) at 0°. Protein remaining in the supernatant solution was determined by the method of Lowry *et al.* (1951) after treatment of samples with 2% cholate to eliminate phospholipid interference. Lipids were estimated by the hydroxamate method of Rapport and Alonzo (1955) after lyophilization of the samples. Blanks without glutamate dehydrogenase or cardiolipin were run for each assay to allow calculation, by difference, of the amounts of proteins and lipids precipitated in the aggregates and to eliminate any nonspecific precipitation of lipids or proteins.

Results

Aggregation of Glutamate Dehydrogenase with Cardiolipin Micelles. That the interaction between cardiolipin micelles and glutamate dehydrogenase leads to an aggregate was first demonstrated by high-speed centrifugation in sucrose gradient. When cardiolipin micelles were put on a 5–20% sucrose gradient they were found between 8 and 11% after 12 hr centrifugation at 25,000*g*. Glutamate dehydrogenase alone formed a peak at 13.5% sucrose. By mixing glutamate dehydrogenase and cardiolipin at increasing protein:lipid ratios, glutamate dehydrogenase moved toward the bottom of the tube, accompanied by the cardiolipin micelles until both were

TABLE I: Effect of Lubrol WX on Glutamate Dehydrogenase Inhibition by Cardiolipin.[a]

Lubrol WX (mg/ml)	[Cardiolipin] (μM)	Act. (%)
0.1	0	95
0	0.25	30
0.1	0.25	95

[a] Glutamate dehydrogenase activity was measured in the presence of 20 mM phosphate (K^+), 1 mM 2-mercaptoethanol, 0.5 mM EDTA, 0.6 mM NAD^+, and 10 mM glutamate (pH 8.0), 25°. The final volume was 1 ml; enzyme, 8 μg/ml; 100% activity, 1.1 μmol of NADH produced/min per mg of enzyme.

sedimented as a pellet. The size of the aggregates thus formed increased with glutamate dehydrogenase concentration. These aggregates could also be sedimented in the absence of sucrose at much lower centrifugal forces. Centrifugation at 2500*g* for 30 min sedimented the glutamate dehydrogenase–cardiolipin mixture in 16 mM phosphate (Na) (pH 7.15); another centrifugation of the supernatant fractions at 105,000*g* for 4 hr did not sediment any additional material. Therefore, in subsequent experiments, aggregation between glutamate dehydrogenase and cardiolipin micelles was studied by measuring the amount of lipid and protein that sedimented at 2500*g* for 30 min. As shown in Figure 1, when increasing amounts of cardiolipin were mixed with 210 μg of glutamate dehydrogenase, the quantity of glutamate dehydrogenase that precipitated increased up to a maximum. No significant glutamate dehydrogenase precipitation could be detected in the presence of similar concentrations of phosphatidylcholine or phosphatidylethanolamine.

Interaction of Cardiolipin with Acetylated Glutamate Dehydrogenase. Figure 1 shows also that after acetylation in the presence or absence of 10^{-4} M GTP + 10^{-4} M NADH which gave an enzyme fully active or 97% inactive, respectively, glutamate dehydrogenase could not form significant aggregates with cardiolipin. The addition of 0.5 mM ADP to the aggregation medium did not induce any more precipitation.

Acetylated active glutamate dehydrogenase still reacts with cardiolipin for the latter inhibits enzyme activity (Figure 2). The cardiolipin concentration necessary to obtain 50% inhibition was, however, increased from 0.5 μM with the normal enzyme to 1.5 μM with the acetylated active enzyme.

Role of Apolar Chains of Cardiolipin in Glutamate Dehydrogenase Inhibition. After removal of the cardiolipin apolar chains by mild alkaline hydrolysis, the water-soluble product (bisglycerophosphorylglycerol) when assayed up to a concentration of 1 mM did not inhibit glutamate dehydrogenase activity.

Addition of the nonionic detergent, Lubrol WX, which destroys hydrophobic interactions, but has almost no effect on glutamate dehydrogenase activity, released cardiolipin inhibition (Table I).

Influence of Enzyme Concentration on Cardiolipin Inhibition. Cardiolipin concentration necessary to produce 50% inhibition of glutamate dehydrogenase depends on enzyme concentration (Figure 3A). The relation is not quite linear, but between 10 and 100 μg of glutamate dehydrogenase/ml, taking the value of 312,000 for the molecular weight of the smallest active unit of glutamate dehydrogenase (Cohen and Mire, 1971), and about 30 μmol of cardiolipin/μmol of glutamate dehydrogenase were necessary to produce 50% inhibition.

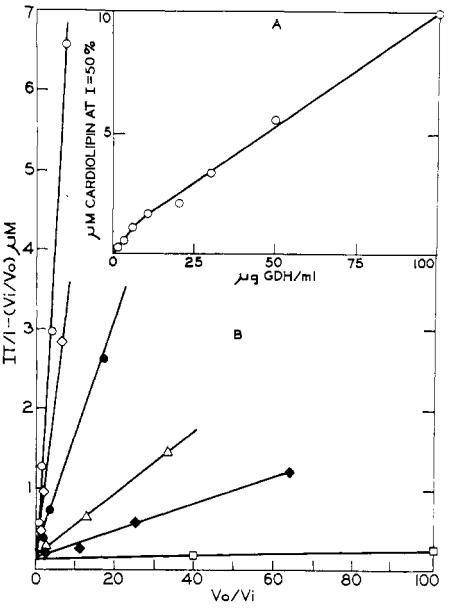

FIGURE 3: Effect of enzyme concentration on cardiolipin inhibition. Conditions as in Table I except that glutamate was 3.3 mM and enzyme concentrations were as indicated. (A) Cardiolipin concentration corresponding to 50% inhibition as a function of enzyme concentration. (B) $I_t/[1 - (v_i/v_0)]$ as a function of v_0/v_i; I_t = concentration of added cardiolipin; v_0 = enzyme activity in the absence of cardiolipin; v_i = enzyme activity in the presence of cardiolipin. Enzyme concentrations are in micrograms per milliliter: ○, 50; ◇, 30; ●, 20; △, 10; ◆, 5; □, 2.5.

This type of effect could be attributed to a mutual depletion of enzyme and inhibitor (Webb, 1963). In this type of kinetics, the mathematical treatment predicts that by plotting $I_t/[1 - (v_i/v_0)]$ as a function of v_0/v_i at different enzyme concentrations, one obtains a family of straight lines whose intercepts are a direct measure of the concentration of inhibitor binding sites (Myers, 1952; Henderson, 1972). Figure 3B shows that this plot gives a family of apparent straight lines of increasing slopes but whose intercepts are practically independent of enzyme concentration. Therefore, this model cannot be applied to determine the character of the inhibition and the absolute concentration of inhibitor binding sites. This unexpected result may be related to the complexity of glutamate dehydrogenase kinetics and/or to the fact that cardiolipin molecules may bind to each other. Both factors can modify the limits of applicability of the model.

Influence of Effectors on Aggregation of Cardiolipin with Glutamate Dehydrogenase. Several nucleotides are known to affect glutamate dehydrogenase activity and the association between its monomers (Frieden, 1963a). Figure 4A shows that these nucleotides also modified the precipitation of glutamate dehydrogenase by cardiolipin. Addition of ADP increased the amount of precipitated GDH[1] while GTP, ITP, ATP, or NAD^+ decreased it slightly. The effects of these nucleotides on the amount of precipitated cardiolipin (Figure $4A_2$) were insignificant except at the highest concentration of cardiolipin (2.4 mM). In the presence of 10^{-4} M NADH (Figure 4B), the precipitation of glutamate dehydrogenase was hardly detectable at low cardiolipin concentrations. It increased slowly when the cardiolipin concentration reached the level of maximum precipitation in the absence of effector (Figure $4A_1$ and B_1). In the presence of 10^{-4} M NADH, cardiolipin precipitation was decreased about 50% (Figure $4B_2$). As a result, the

[1] Abbreviation used is: GDH, glutamate dehydrogenase.

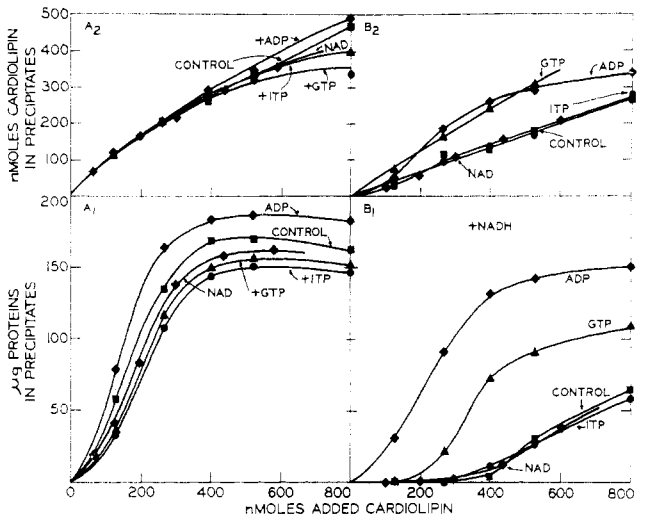

FIGURE 4: (A) Effects of ADP, GTP, and ITP on the aggregation of cardiolipin and glutamate dehydrogenase. (B) Effects of ADP, GTP, and ITP on the inhibition by NADH of the aggregation of cardiolipin and glutamate dehydrogenase. Conditions are as described under Experimental Procedure. All nucleotide concentrations were 10^{-4} M; 220 μg of glutamate dehydrogenase was present in each assay.

amount of glutamate dehydrogenase bound per mole of cardiolipin was drastically decreased by NADH addition especially at low concentration of added cardiolipin. This inhibition by NADH was released by adding 10^{-4} M GTP or better 10^{-4} M ADP, but ITP or NAD^+ failed to release it. The glutamate dehydrogenase and cardiolipin precipitated by fixed amounts of added cardiolipin varied quantitatively from one sonicate to another but the relative effects of the different nucleotides were in very good agreement from one experiment to another.

In the absence of nucleotides, addition of glutamate increased the amount of precipitated GDH. This effect was demonstrable only when the added cardiolipin was below the concentration corresponding to maximum precipitation; it was amplified in the presence of 10^{-4} M NADH, and glutamate released the NADH inhibition (Figure 5).

Influence of Effectors on the Release of Glutamate Dehydrogenase from Mitochondrial Inner Membrane–Matrix Fractions. To determine whether the binding of glutamate dehydrogenase to inner mitochondrial membranes, which contain the cellular cardiolipin, can be modified by the effectors working in the model system, mitochondria were depleted of their outer membranes, incubated in the presence of effectors at concentrations used in the previous model system, and sedimented. Glutamate dehydrogenase activity and protein contents were measured in supernatant fractions. Table II shows that glutamate inhibits the release of glutamate dehydrogenase from this submitochondrial fraction. The presence of NADH partly prevents glutamate inhibition. ADP (10^{-4}–10^{-3} M) and GTP (10^{-4} M) added alone or in the presence of NADH (10^{-4} M) and cardiolipin micelles (300 nmol) failed to show any significant effect. In all cases, the amount of soluble proteins remained approximately constant and equal to about 25% of the total proteins in the assay which shows that glutamate specifically prevents glutamate dehydrogenase release. The difference in the soluble protein content that this diminution of glutamate dehydrogenase involves must be too low to be detected.

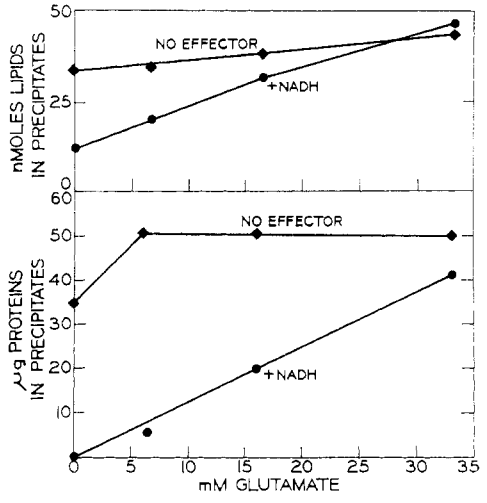

FIGURE 5: Effect of glutamate on the aggregation of cardiolipin and glutamate dehydrogenase association. Conditions are as described under Experimental Procedure; 250 μg of glutamate dehydrogenase and 75 nmol of cardiolipin micelles were present in each assay.

TABLE II: Influence of Glutamate and NADH on the Release of Glutamate Dehydrogenase from Inner Membrane–Matrix Mitochondrial Fractions.[a]

[Glutamate] (mM)	Solubilized Glutamate Dehydrogenase Act.[b] No addition	N[c]	+10^{-4} M NADH	N
0	5.4 ± 0.07	8	5.3 ± 0.17	5
2	4.4 ± 0.12	6	4.7 ± 0.08	5
4	2.9 ± 0.09	3	5.0 ± 0.10	3
6	2.3 ± 0.14	3	5.3 ± 0.19	3
10	2.0 ± 0.19	5	4.9 ± 0.26	5
20	2.5 ± 0.07	5	4.9 ± 0.19	5

[a] Inner membrane–matrix mitochondrial fractions were suspended in 0.25 M sucrose incubated at 30° for 10 min in the presence of effectors as indicated and centrifuged at 60,000*g* for 15 min (Rendon and Waksman, 1973). Supernatant fractions were analyzed for protein contents by the procedure of Lowry *et al.* (1951) and for glutamate dehydrogenase activity on 2-μl aliquots as described previously (Godinot and Lardy, 1973). The final volume of incubation was 0.5 ml and contained 10 mg of submitochondrial proteins. The given values are followed by the standard error of the mean. [b] GDH activity is expressed as μmol of NADH oxidized/min per mg of protein present in supernatant fractions. [c] *N* = number of experiments.

Discussion

Cardiolipin inhibition is dependent on enzyme concentration. This explains the large differences observed in previously reported values for the cardiolipin concentration required to obtain 50% inhibition (Julliard and Gautheron, 1972; Dodd, 1973).

The nature of binding of glutamate dehydrogenase to cardiolipin is dependent on hydrophobic and electrostatic interactions. Previous work had shown that glutamate dehydrogenase inhibition by cardiolipin was lowered by increasing ionic strength (Dodd, 1973) suggesting an ionic interaction, but nmr studies implicated the apolar part of the

molecule in hydrophobic interactions. The lack of glutamate dehydrogenase inhibition by bisglycerophosphorylglycerol and the release of inhibition by Lubrol WX suggest that the apolar residues of cardiolipin interacting with glutamate dehydrogenase by hydrophobic bonds are indispensable to observe the catalytic inhibition of the enzyme.

However, ionic interaction must be responsible for the primary binding of glutamate dehydrogenase to cardiolipin. This was deduced from earlier reports showing the specificity of cardiolipin as compared to other phospholipids to inhibit glutamate dehydrogenase activity. Auxiliary evidence for this ionic interaction came from experiments with acetylated glutamate dehydrogenase. Acetylation of glutamate dehydrogenase modifies some of the amino groups of this protein (Colman and Frieden, 1966b). Acetylated glutamate dehydrogenase does not aggregate with cardiolipin. This may indicate that these amino groups participate in aggregation by attracting the negatively charged groups of the cardiolipin polar head. Such a type of interaction has been proposed for cytochrome *c* and acidic phospholipid micelles (Green and Fleischer, 1963) but unlike cytochrome *c*, a basic protein whose net charge is opposite to cardiolipin, glutamate dehydrogenase shows a large excess of negative charge at physiological pH (Appella and Tomkins, 1966) with an isoelectric point between pH 4 and 5 (Olson and Anfinsen, 1952). The positively charged residues may be displayed in a position favorable for reaction with cardiolipin, presumably at the surface of the enzyme.

The overall mechanism could be of the type described by Jencks (1969) who found that charged dyes which can undergo some sort of hydrophobic interaction bind stoichiometrically to oppositely charged groups on proteins although small ions show little or no binding under the same conditions. The ion pair formation may be attributed to hydrophobic interaction between nonpolar groups and direct electrostatic interaction between the charges in a medium of low dielectric constant provided by the shielding hydrocarbon groups.

The influence of nucleotides on the aggregation of cardiolipin with glutamate dehydrogenase cannot be explained only by their effect on the state of association of the enzyme itself. Indeed, in the presence of NADH, ADP increases glutamate dehydrogenase polymerization while GTP maintains it in its monomeric form (Frieden, 1959). Figure $4A_1$ shows that ADP increases slightly and GTP decreases slightly glutamate dehydrogenase aggregation which corresponds to the effects of the nucleotides on the molecular weight of the enzyme described by Frieden (1959). But in the presence of NADH (Figure $4B_1$), both ADP and GTP release NADH inhibition. GTP contains a free amino group in position 2 which is not involved in the fixation on the inhibitory site. Acetylation experiments suggested that association of glutamate dehydrogenase with cardiolipin is dependent on the presence of free amino groups at the surface of the enzyme (this paper) and that NADH masks the more reactive amino groups (Colman and Frieden, 1966a,b). Binding of GTP brings a free amino group which would allow the electrostatic interaction of cardiolipin with glutamate dehydrogenase. This would explain the release of the NADH-induced inhibition of association of glutamate dehydrogenase with cardiolipin. This is further supported by the fact that ITP, which has the same inhibitory properties as GTP (Frieden, 1963a) but which does not contain this free amino group in the 2 position, could not release the NADH-induced inhibition of association of glutamate dehydrogenase with cardiolipin.

This assocation may play different physiological roles. As pointed out previously (Godinot and Lardy, 1973), it may be responsible for the final location of glutamate dehydrogenase in mitochondria. It was shown that conditions enhancing glutmate affinity for glutamate dehydrogenase decreased the binding of this enzyme to microsomes. On the contrary, the presence of glutamate increases the amount of glutamate dehydrogenase associated with cardiolipin (Figure 5) and prevents the release of glutamate dehydrogenase from inner mitochondria·membrane–matrix fractions (Table II). Therefore, the presence of glutamate and NAD^+ in the cytosol may detach the enzyme from microsomal membranes, site of its synthesis, and favor the binding to mitochondrial membranes. Since hydrophobic interactions are involved in the association of glutamate dehydrogenase with cardiolipin, the enzyme may reach the interior apolar part of the membrane according to the model of Singer and Nicolson (1972). It possesses an appropriate size (Josephs *et al.*, 1972) to span the entire thickness of the membrane. On the matrix side of inner membrane, the more reduced state of pyridine nucleotides that prevails in mitochondria (Krebs and Veech, 1969) may induce the dissociation of the enzyme from the membrane and release the enzyme into the matrix.

Since glutamate dehydrogenase is inactive in the presence of cardiolipin, its reversible binding may contribute to the regulation of the enzyme activity *in vivo*, explaining the preferential glutamate oxidation *via* transamination rather than oxidative deamination observed in some experimental conditions (Borst, 1962). Inhibition of aggregation of glutamate dehydrogenase with cardiolipin is much stronger by NADH than by NAD^+. This would imply that strong reducing conditions would permit the enzyme to serve in the reductive amination of α-ketoglutarate which corresponds to observations made by Williamson *et al.* (1967).

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Lactate Dehydrogenase Isozymes. Turnover in Rat Heart, Skeletal Muscle, and Liver†

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ABSTRACT: Turnover values for 12 lactate dehydrogenase isozymes from three rat tissues were estimated by ^{14}C-labeled amino acid incorporation during the adult, steady state condition. The continuous dietary administration method, accompanied by specific immunoprecipitation, was used; the data were analyzed by the zero-order synthesis, first-order degradation model. The values for heart lactate dehydrogenases 5, 4, 3, 2, and 1 were 3.2, 1.7, 11.8, 39.6, and 53.2 pmol/g per day, respectively. For skeletal muscle lactate dehydrogenases 5, 4, 3, 2, and 1, the rates of synthesis and degradation were 13.5, 0.6, 0.8, 1.3, and 2.4 pmol/g per day, respectively. According to this method of analysis, rat liver isozyme 5 turns over at a rate of 79.2 pmol/g per day, whereas the value for rat liver isozyme 4 is 0.8 pmol/g per day. No correlation between the turnover and the molecular weight of the lactate dehydrogenase isozymes was observed in skeletal or cardiac muscle. In liver there was a correlation between the relative, but not the absolute, rate of isozyme-5 and -4 degradation.

Proteins within animal cells are in a state of dynamic flux, being continuously synthesized and broken down, a phenomenon designated turnover.[1] In recent years intensive investigations of the turnover of various proteins have made it clear that theories dealing with the regulation of intracellular protein levels must take into account both synthesis and degradation (Schimke and Doyle, 1970).

Of the many enzymes that occur in animal tissues in multiple molecular forms (isozymes), rates of biosynthesis and degradation, which may control the tissue distribution of these isozymes and thereby provide an insight into their functional significance (Fritz *et al.*, 1969), have been measured only for the lactate dehydrogenases (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) (Fritz *et al.*, 1969, 1970b). We previously measured rates of synthesis and degradation of lactate dehydro-

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[1] If turnover is defined as the rate of replacement of a protein in a particular tissue (Reiner, 1953), then at steady state turnover is equal to the rate of synthesis or the rate of degradation. For the model in which synthesis is zero order and degradation is first order: turnover = $k_d[P]_{ss}$. Since half-life = $(\ln 2)/k_d$, turnover = $([P]_{ss} \ln 2)/\text{half-life}$. The symbols have the following meanings: k_d is the rate constant for degradation; k_s is the rate constant for synthesis; and $[P]_{ss}$ is the steady state concentration of the protein under investigation. Thus, it is misleading to use the terms "turnover" and "half-life" synonymously. Half-life estimates the rate of removal of a protein relative to the amount present and $k_d \times 100$ can be considered to be the per cent of the protein replaced in a given time. On the other hand, k_s is a measure of the absolute amount of the protein made (or degraded) per unit of tissue per unit of time. Proteins with short half-lives do not necessarily have a fast turnover. If the steady state protein concentration is small, the turnover will be slow. For example, rat skeletal muscle lactate dehydrogenase 5 has a half-life of 138 days, as calculated from the first-order rate constant for degradation (k_d), whereas rat skeletal muscle lactate dehydrogenase 1 has a half-life of 13 days. However, the turnover of these isozymes is 13.5 and 2.4 pmol/g per day, respectively (Table I).