Proc. Nat. Acad. Sci. USA
Vol. 69, No. 4, pp. 925–929, April 1972

Limiting Availability of Binding Sites for Dehydrogenases on the Cell Membrane of *Escherichia coli*

(acyl-CoA/glycerophosphate/lactate/succinate/respiration)

HSIANG-FU KUNG* AND ULF HENNING†

Max-Planck-Institut für Biologie, Tübingen, West-Germany

Communicated by E. R. Stadtman, February 2, 1972

ABSTRACT **Experiments are reported that demonstrate that in *E. coli* the pyridine nucleotide-independent D- and L-lactate dehydrogenases and the aerobic L-α-glycerophosphate dehydrogenase are membrane bound. These enzymes differed from succinate dehydrogenase in that they could be solubilized by treatment with nonionic detergent while succinate dehydrogenase could not. The binding of these enzymes to membrane was measured in mutants constitutive for the synthesis of various dehydrogenases: in cells in which the amount of dehydrogenases synthesized was greater than in others, the enzymes described above (except succinate dehydrogenase) were found in part in the soluble fraction of the cell extracts. Experiments of oxygen uptake indicate that when a fraction of the enzymes became soluble, this soluble fraction is no longer functional in respiration. These results indicate that it is possible to prevent membrane attachment of certain dehydrogenases by the excess production of other dehydrogenases; it may be that dehydrogenases compete for identical binding sites.**

In *Escherichia coli* as in other bacteria several dehydrogenases serving aerobic electron transport and not using pyridine nucleotides as cofactors are associated with the cell envelope fraction. Although no direct evidence is available on whether such enzymes are attached to the cytoplasmic or outer membrane, functional considerations strongly suggest that they are on the cytoplasmic membrane. All such dehydrogenases must somehow be physically connected to the respiratory chain.

Three possibilities for membrane attachment are: (*a*) the enzyme is a membrane constituent and is necessary for functional membrane assembly; (*b*) the enzyme is not necessary for membrane assembly but binds to a unique binding site on the membrane; and (*c*) the enzyme is not necessary for membrane assembly but binds to a binding site that does not distinguish between different dehydrogenases. The necessity of a physical connection of these dehydrogenases to the respiratory chain makes it unlikely that the binding sites for these dehydrogenases are nonspecific.

Possibility (*a*) could be eliminated if the structural genes for the dehydrogenases were known and if deletion mutations spanning these genes were isolated. The two remaining possibilities should also be experimentally distinguishable. If binding sites were specific for individual dehydrogenases, there should be no competition between different enzymes for the same binding site. Such competition would indicate that the binding sites possessed specificity for any one of a group of dehydrogenases. If so, binding sites would become limiting with excessive production of any one dehydrogenase. Some dehydrogenases might then become soluble; we cannot predict whether the soluble fraction would remain enzymatically active. Another possibility is that only as much dehydrogenase is produced as can become attached to the membrane. In this case, if competition for binding sites occurs a lower enzyme activity might be found for some dehydrogenases under conditions of excess synthesis of others.

We undertook the present study to investigate these possibilities. Our observations on succinate, D-lactate, L-lactate, and L-α-glycerophosphate dehydrogenases support the idea of competition for binding sites.

METHODS

Strains and preparation of extracts

Strains 8 and 72 and their Hfr C progenitor, strain 1, were the generous gifts of Dr. E. C. C. Lin (1). Strain 8 carries a constitutive *glp R* allele, and strain 72 is constitutive for the *glp* system as a result of a deletion extending into the *glp R* gene. *Dec*$^-$ mutants are constitutive for the fatty acid-degrading enzymes. Mutants with different numbers are independent isolates; 1-*dec*-1, 1-*dec*-13, and 8-*dec*-22, are spontaneous *dec*$^-$ mutants derived from strains 1 and 8, respectively. All other mutants were obtained after mutagenesis with diethylsulfate. *Dec*$^-$ mutants were selected as described by Overath *et al.* (2).

Cells were grown with vigorous aeration at 37° in a minimal medium (3) containing 0.4% glycerol, 0.4% sodium L-lactate, 0.1% potassium oleate or 0.1% potassium decanoate as carbon source. All media contained 1 μg of thiamine/ml. Cultures were harvested in late exponential phase. The cells were washed once with 0.1 M Tris·HCl (pH 8) containing 1 mM $MgCl_2$, and then 1 g (wet weight) of cells was resuspended in 1.5 ml of fresh buffer. Extracts were obtained by the freeze (−50°)-thawing method (4) with five sequential freezings and thawings.

Assays

Succinate, D-, and L-lactate dehydrogenases were measured by a modification of the procedure of Arrigoni and Singer (6, 7). L-α-glycerophosphate dehydrogenase, acyl-CoA syn-

Abbreviations: Mutant alleles conferring constitutivity to the fatty acid-degrading enzymes, *dec*$^-$; to the L-α-glycerophosphate system, *glp R*^{c}.

* Present address: Roche Institute of Molecular Biology, Nutley, N.J. 07110.

† To whom requests for reprints should be sent.

thetase (EC 6.2.1.3 substrate: oleate), acyl-CoA-dehydrogenase (EC 1.3.99.3 substrate: butyryl-CoA), β-galactosidase (EC 3.2.1.23), and pyruvate dehydrogenase complex (pyruvate dehydrogenase EC 1.2.4.1 plus dihydrolipoamide transacetylase EC 2.3.1.12 plus dihydrolipoamide dehydrogenase EC 1.6.4.3) were measured by described methods (8, 2, 9–11, respectively). Protein was determined by the biuret method (12). Oxygen uptake was measured, at 30° with 0.05% carbon source in minimal medium, with a Clark type oxygen electrode. In respiration experiments cells were first starved for 3 hr at 37° in minimal medium without any carbon source. Dry weight of cells was determined by the following unpublished procedure of Dr. K. Rehn. Formic acid was added to the cell suspensions to a final concentration of 3 N. After centrifugation, the cells were washed twice with 3 N formic acid. They were then dried for 20 hr at 120° and weighed.

Test for solubility

We had to test many extracts for solubility of the enzymes under investigation and a simple method was desired. In extracts obtained as described above, centrifugation for 30 min at 15,000 × *g* suffices to distinguish between the soluble and bound states. The validity of this procedure has been checked in several cases by sucrose density gradient centrifugation. Succinate dehydrogenase activity always and completely travelled to the bottom of the gradient while the activities of the other dehydrogenases, which stayed in the supernatant of the 15,000 × *g* centrifugation, moved as symmetrical peaks within the gradients [procedure: 4.5 ml linear gradient of 5–30% sucrose in 0.1 M Tris buffer (pH 7.2), containing 1 mM $MgCl_2$, 2.5 hr at 38,000 rpm in the SW39L Spinco rotor]. In extracts of strain 1, prepared in this way, all the succinate, D-, and L-lactate dehydrogenase activities were completely sedimented by centrifugation for 30 min at 15,000 × *g*. In contrast, more than 90% of the activity of one of the largest soluble enzymes, pyruvate dehydrogenase complex [molecular weight about 4×10^6 (5)], remained in the supernatant. 50–70% of the total protein present in the broken cell preparation (40–60 mg/ml) was found in the supernatant.

RESULTS

Succinate, D-lactate, L-lactate, and L-α-glycerophosphate dehydrogenases

Succinate dehydrogenase is bound to the cytoplasmic membrane (13), but it is not as clear whether the *E. coli* D- and L-lactate dehydrogenases (14, 15) are entirely membrane-bound enzymes and whether these two enzymes use NAD as electron acceptor. In fact, several observations (15) strongly suggest that NAD is not a cofactor for these two enzymes. The role of D-lactate dehydrogenase in amino acid and carbohydrate transport (16, 17) also strongly supports the view that the enzyme is a NAD-independent flavoprotein. At least part of the D- and L-lactate dehydrogenases in *E. coli* are not soluble (14–17). A different enzyme, a soluble D-lactate dehydrogenase linked to NAD has been studied in detail (18), but since it is not membrane bound it will not be discussed here.

The L-α-glycerophosphate dehydrogenase we have chosen for this study belongs to the inducible L-α-glycerophosphate system (*glp*) (1) which includes glycerol kinase and the glycerophosphate transport system. These enzymes are essential for the aerobic growth of *E. coli* on glycerol as carbon source. NAD is also not a cofactor for this dehydrogenase (8), and this observation suggested to us that this dehydrogenase might be a membrane-bound enzyme. Since deletion mutants lacking the structural gene of the L-α-glycerophosphate dehydrogenase have been isolated (1, 19), we have studied mainly this enzyme. The existence of the mutants eliminates possibility (*a*) described above, that L-α-glycerophosphate dehydrogenase is an essential membrane constituent. The following experiments resolve some of the uncertainties described above by showing that the L-α-glycerophosphate dehydrogenase as well as the two lactate dehydrogenases are normally almost exclusively membrane-bound enzymes.

We first tried different methods for breaking cells open. Freeze-thawing in the presence of lysozyme and DNase was superior to sonification or shaking with glass beads since the latter two methods yielded extracts in which, rather poorly reproducibly, appreciable amounts (10–70%) of all dehydrogenases measured could not be sedimented by centrifugation for 30 min at 15,000 × *g*. This behavior was found, in the following way, to be at least partially due to the generation of membrane fragments of a wide size distribution. An extract obtained by sonic oscillation was subjected to sucrose density gradient centrifugation, and the gradient was analyzed for succinate dehydrogenase. About 50% of the activity had travelled to the bottom of the gradient and the other 50% was distributed as a broad smear all over the gradient. Table 1 shows the enzyme activities in the supernatants of cells opened by freeze-thawing and then subjected to centrifugation. All succinate, D-, or L-lactate dehydrogenase activities were sedimented when glycerol or L-lactate was used as carbon source for growth. About 95% of the L-α-glycerophosphate dehydrogenase was sedimentable, and this fraction decreased to 89% in strain 8 (*glp* constitutive) after growth on L-lactate. We show below that this decrease is real, and it appears to be due to a 5-fold increase of L-lactate dehydrogenase activity induced by L-lactate in the presence of constitutive levels of L-α-glycerophosphate dehydrogenase. This is, thus, preliminary evidence for competition for a common binding site leading to solubilization.

Although treatment with the nonionic detergent Brij 58 solubilized both lactate dehydrogenases and L-α-glycerophosphate dehydrogenase, the same treatment did not solubilize (or inactivate) any succinate dehydrogenase activity (Table 2). Brij solubilization of L-α-glycerophosphate dehydrogenase was confirmed by sucrose density gradient centrifugation of crude extracts in the presence and absence of Brij; the enzymatic activity moved as a symmetric peak in the presence of Brij while it was found only at the bottom of the gradient in its absence. Thus, there can be no doubt that L-α-glycerophosphate and both the lactate dehydrogenases are membrane-bound enzymes and that the membrane binding affinity of these three enzymes is different from that of succinate dehydrogenase.

Fatty acid-degrading enzymes

Unfortunately only constitutive mutants for the *glp* system have been described and they are for none of the other enzymes just discussed. Since we wanted to examine the results of excess production of many dehydrogenases at once, additional constitutive mutants were desirable. Such constitutive mutants are obtainable for the fatty acid-degrading enzymes, although this inducible system (2, 9, 20) has certain drawbacks for our purpose. This system includes an acyl-CoA dehydrogenase (and probably more than one, with different

TABLE 1. *Dehydrogenases in wild-type Escherichia coli and in strain 8 (glp R^c)*

Strain	Carbon source	Dehydrogenase: Succinate S.A.	Succinate % Soluble	L-Lactate S.A.	L-Lactate % Soluble	D-Lactate S.A.	D-Lactate % Soluble	L-α-Glycerophosphate S.A.	L-α-Glycerophosphate % Soluble
1	L-Lactate	168	0	39	0	21	0	<2	—
(wild type)	Glycerol	63	0	6	0	10	0	63	4
8	L-Lactate	135	0	64	0	11	0	67	11
(*glp R^c*)	Glycerol	146	0	13	0	23	0	170	5

In these experiments as well as in those of Tables 3 and 4 specific activities (S.A.) were measured in broken cell preparations after removal of unbroken cells by centrifugation at 2000 × *g* for 30 min. Protein concentrations in all these extracts were nearly the same so that S.A. is also a relative measure of the total amount of enzyme activity present. S.A. is expressed as nmol of substrate oxidized per min per mg of protein. The broken cell preparations were centrifuged at 15,000 × *g* for 30 min; the recovery of total activity from the pellet and supernatant was >90%. On this table as well as Tables 3 and 4, % *Soluble* was calculated based upon the activity in the supernatant relative to the broken cell preparation.

chain-length specificities, see ref. 21) that does not use NAD as cofactor (9). One might suspect that this activity in *E. coli*, as in other organisms, is coupled to the respiratory chain *via* electron transfer proteins and that it is bound to the cytoplasmic membrane; but we could not confirm the suspicion in any simple way. We found that this dehydrogenase, when assayed with butyryl-CoA, did not behave as a one-component system. Part sedimented with the pellet: after centrifugation of crude extracts mixing of supernatant and pellet was necessary to restore dehydrogenase activity. Dialysis (35 hr against 0.1 M imidazole buffer, pH 7.3, containing 10 mM mercaptoethanol and 1 mM $MgCl_2$) of the crude extract caused a large decrease of activity that was not restorable by the addition of an undialyzed centrifuge supernatant. We could not obtain any information on the factor(s) that remains in the supernatant on centrifugation. However, because of the catalytic function, it remains extremely likely that the *E. coli* acyl-CoA dehydrogenase(s) is bound to the cytoplasmic membrane.

Another difficulty with the fatty acid-degrading enzymes is that the acyl-CoA dehydrogenase(s) certainly is not the only membrane-bound enzyme among them (2), and induced synthesis of these enzymes will therefore also increase the amount of other membrane-bound enzymes. Despite these uncertainties we have used the system because constitutive *dec*$^-$ mutants can easily be obtained (2, 9). Such mutants were isolated both from wild type (strain 1) and from *glp* constitutive mutants (strains 8 and 72). Double mutants constitutive for both *glp* and *dec* systems could thus be compared with wild type and with mutants constitutive for either the *glp* or *dec* system.

Dehydrogenases in constitutive mutants

The relevant behavior of the D- and L-lactate, succinate, and L-α-glycerophosphate dehydrogenases is shown in Table 3. When using glycerol as carbon source, *dec*$^-$ mutations have no measurable influence on the membrane-bound state of succinate dehydrogenase or on either of the two lactate dehydrogenases. However, the amount of L-α-glycerophosphate dehydrogenase that is soluble increases 3- to 5-fold in strains carrying a *dec*$^-$ mutation. When L-lactate is the carbon source several parameters change. L-Lactate dehydrogenase activity increases 6- to 12-fold. We have measured acyl-CoA synthetase activity as a measure of expression of the *dec*$^-$ alleles, and this activity also increases about 2- to 5-fold, certainly due to a release of catabolite repression (20). Under these conditions the influence of *dec*$^-$ mutations became pleiotropic. First, in strain 1-*dec*-1 about 10–20% of both lactate dehydrogenases are now soluble; this strain grown on lactate as carbon source will not, of course, produce measurable quantities of L-α-glycerophosphate dehydrogenase. In the double constitutive mutants (strains 8-*dec*-22 and 8-*dec*-43) an excess of L-α-glycerophosphate dehydrogenase is produced in addition to the fatty acid-degrading enzymes. Almost 40% of all L-α-glycerophosphate dehydrogenase activity present has now become soluble. Sucrose density gradient centrifugation of such an extract showed that the soluble fractions of the enzymes assayed moved as symmetric peaks while all succinate dehydrogenase activity sedimented to the bottom of the gradient.

We could not measure the degree of binding of the acyl-CoA dehydrogenase to the membrane, and the number of different acyl-CoA dehydrogenases in *E. coli* is not known. Induction or constitutivity of the fatty acid-degrading en-

TABLE 2. *Solubilization of dehydrogenases by Brij 58*

Final concentration of Brij 58 (%)	% Solubilized: Protein	Dehydrogenase: Succinate	L-Lactate	D-Lactate	L-α-Glycerophosphate
0	0	0	0	0	0
0.05	7	0	27	35	47
0.1	21	0	64	62	71
0.2	23	0	65	64	73

Strain 8 (*glp R^c*) was grown on glycerol as carbon source and the particulate fraction was prepared by centrifugation for 30 min at 15,000 × *g*. Treatment with Brij 58 was for 30 min at 0° in 0.1 M Tris·HCl (pH 8.0), containing 1 mM $MgCl_2$, and at a protein concentration of 10 mg/ml. The mixtures were then centrifuged for 30 min at 27,000 × *g*. All activities were measured in pellets and supernatants, with a total recovery in all cases of >90%.

TABLE 3. *Dehydrogenases in wild type and mutants, with glycerol or L-lactate as carbon source for growth*

Strain (relevant genotype)	Dehydrogenase								Acyl-CoA synthetase
	Succinate		L-Lactate		D-Lactate		L-α-Glycerophosphate		
	Relative S.A.	% Soluble	Relative S.A.	% Soluble	Relative S.A.	% Soluble	Relative S.A.	% Soluble	S.A.
				Glycerol					
Wild type	1	0	1	0	1	0	1	4	<0.005
8 (*glp R*^{c})	1.4	0	2	0	2.3	0	2.7	5	<0.005
1-*dec*-13 (*dec*$^-$)	2.8	0	2.6	0	2.1	0	2.7	15	0.1
1-*dec*-1 (*dec*$^-$)	2.8	0	1.3	0	2.1	0	1.7	13	0.06
8-*dec*-22 (*glp R*^{c}, *dec*$^-$)	2.5	0	2	0	2.8	0	2.2	20	0.16
				L-Lactate					
Wild type	1.6	0	6.5	0	2	0	<0.03	—	<0.005
8 (*glp R*^{c})	1.3	0	10.5	0	1	0	6.7	11	<0.005
1-dec-1 (*dec*$^-$)	1.7	0	11	23	4	11	<0.03	—	0.56
8-*dec*-22 (*glp R*^{c}, *dec*$^-$)	1.7	0	12	18	3.4	11	5.6	38	0.42
8-*dec*-43 (*glp R*^{c}, *dec*$^-$)	2	0	11	16	2.2	22	2.3	34	0.28

Strain designations are as described in *Methods* and experimental details as described in the legend to Table 1. Relative specific activities (S.A.) were calculated by setting the S.A. of wild type = 1 (for absolute values see Table 1). S.A. of acyl-CoA synthetase are given as μmol acyl-CoA formed per hr per mg of protein.

zymes may also cause an increase of other membrane-bound enzymes of this pathway. Furthermore, it is possible that membrane-bound dehydrogenases other than those examined here change their rates of syntheses under different growth conditions. Therefore, it is not possible to determine the stoichiometric relationship between solubility and the total amounts of the dehydrogenases involved.

Dehydrogenase activities *in vivo* and *in vitro*

If part of the dehydrogenases were no longer associated with the cytoplasmic membrane *in vivo*, this fraction might no longer function as an electron donor for respiration and the ratio of *in vitro* specific enzyme activity to *in vivo* oxygen uptake might change. The data of Table 4 show that the solubilization of dehydrogenases found *in vitro* is, in fact, accompanied by a reduction of the rate of respiration of whole cells with the corresponding substrate. With L-lactate as substrate for the *dec*$^-$ mutants, the rates of oxygen uptake decrease about as much as L-lactate dehydrogenase becomes soluble. With L-α-glycerophosphate as substrate this decrease is considerably larger than the degree of apparent solubilization. Since, at least for *E. coli*, no information exists on the structure-function relationship of membrane-bound dehydrogenases and their coupling to the respiratory chain, one does not know what to expect. All possibilities are easily conceivable, i.e., more, equal, or less loss of O_2 uptake than loss of membrane-bound dehydrogenase.

As expected when succinate is the substrate, the different strains have the same relative amounts of specific dehydrogenase activity and oxygen uptake.

In these experiments we have also used a different *glp R*^{c} mutant, strain 72, to eliminate the possibility that the solubilization effect observed is specific only for strain 8. It is clear that the general effect is the same although the quantitative amount of solubilization for the different enzymes is somewhat different from that found with strain 8. Another difference, concerning L-α-glycerophosphate and L-lactate dehydrogenases, is obvious where 13 and 20%, respectively, is already soluble in the *dec*$^+$ strain. We have never seen this with strain 8. It could be that it is due to the higher levels of the two enzymes in the extract used (see Table 1). We encountered difficulties in measuring oxygen uptake using D-lactate as substrate with strain 72 (several rates were obviously erratic), and this system is therefore not included in Table 4.

In summary, the results support the view that the soluble fraction of the enzymes is no longer functional in respiration.

DISCUSSION

Our data show that D-lactate, L-lactate, and L-α-glycerophosphate dehydrogenases, but not succinate dehydrogenase, may be obtained free of the *E. coli* cytoplasmic membrane under certain conditions. The difference is strengthened by the fact that only succinate dehydrogenase cannot be solubilized by the detergent Brij 58.

Can the partial solubilization of the enzymes be interpreted as competition for binding sites? The term, binding site, has to be used in a general sense. Such a site could be physically located on the membrane itself or on a multienzyme complex that functions as the respiratory chain. Perhaps the sites are topologically different for different dehydrogenases but a common factor is required for binding; this factor itself would be the limiting agent. Regardless of the sort of site involved, availability of sites for the three enzymes studied and their synthesis are not coupled, i.e., limitation of sites does not inhibit enzyme synthesis by feedback mechanisms. There is no proof that the effects we have observed are due to competition; it is possible to construct arguments against competition. For example, we could assume that cells grown on L-lactate produce a membrane different from that when grown on glycerol and that *dec*$^-$ mutants synthesize an altered "*dec*-membrane." However, such *ad hoc* assumptions are not very attractive, and there is a good correlation between the solubility of one or several dehydrogenases and the corresponding specific activities. Competition certainly re-

TABLE 4. *Dehydrogenase activity and respiration, with succinate, L-lactate, and L-α-glycerophosphate as substrates*

Succinate				
Strain (relevant genotype)	Relative S.A., succinate dehydrogenase	% Soluble	O_2 uptake	
			Found	% Of expected
72 (*glp* R^c)	1	0	1	
72-*dec*-35 (*glp* R^c, *dec*$^-$)	0.4	0	0.45	100
72-*dec*-20 (*glp* R^c, *dec*$^-$)	0.7	0	0.72	100

L-Lactate				
Relative S.A., L-lactate dehydrogenase	% Bound	O_2 uptake		
		Found	Expected for bound enzyme only	Expected for all enzyme
1	80	1		
0.68	50	0.34	0.34/0.80 = 0.43	0.68/1 = 0.68
0.68	50	0.46	0.34/0.80 = 0.43	0.68/1 = 0.68

L-α-Glycerophosphate				
Relative S.A., L-α-glycerophosphate dehydrogenase	% Bound	O_2 uptake		
		Found	Expected for bound enzyme only	Expected for all enzyme
1	87	1		
1.6	76	0.5	1.22/0.87 = 1.4	1.6/1 = 1.6
1.3	74	0.65	0.96/0.87 = 1.1	1.3/1 = 1.3

Strain designations are as described in *Methods*. The carbon source for growth was 0.4% L-lactate. Specific activities (S.A.) were measured in broken-cell preparations and O_2 uptake with whole cells. Relative S.A. were calculated by setting the values of strain 72 = 1, the absolute values (nmol/min per mg) are 495 (succinate), 108 (L-lactate), and 154 (L-α-glycerophosphate). Relative O_2 uptake was calculated correspondingly, the absolute values (mm Hg/min per mg cell dry weight) are 36 (succinate), 55 (L-lactate), and 38 (L-α-glycerophosphate). The expected O_2 uptake values were calculated under the assumptions that only the bound fraction or that all enzyme measured would participate in respiration.

mains the simplest explanation although it is not clear whether the dehydrogenases are the only competing species. We pointed out above that constitutive synthesis of the fatty acid-degrading enzymes involves excess synthesis of membrane-bound enzymes other than acyl-CoA dehydrogenase(s).

It would be of interest to see whether other groups of enzymes that have similar functions, e.g., binding proteins or hydrolytic enzymes of the periplasmatic space, would also exhibit the same apparent competitive behavior among themselves.

Irrespective of the detailed mechanisms leading to the partial solubilizations observed with some dehydrogenases, there is certainly some sort of limitation for attachment to the cytoplasmic membrane. A similar limitation has been reported to exist for transport systems of *E. coli* (22) and yeast (23). It would be of interest to know what the maximum membrane-bindable *amounts* of the enzymes investigated in this study are. Such knowledge would give some information on the percentage of membrane space that the enzymes can occupy.

NOTE ADDED AFTER COMPLETION OF THE MANUSCRIPT

Dr. E. R. Stadtman drew our attention to the fact that lysozyme can precipitate glutamate dehydrogenase and glutamine synthetase from *E. coli* (Miller, R., and Stadtman, E. R., unpublished). Thus at least part of the binding we had measured could have been an artifact and at least part of the apparent competition for binding sites we had observed could have been competition for lysozyme. We therefore have prepared an extract by sonication of cells from strain 72 grown on L-lactate. Of the total D-lactate dehydrogenase, L-lactate dehydrogenase, and L-α-glycerophosphate dehydrogenase activities, 69, 50, and 62%, respectively, were soluble in this extract. Addition of lysozyme (50 μg/ml) and DNase (2 μg/ml) to the high speed centrifugation supernatant did not cause, upon a repeated centrifugation at 15,000 × *g* for 30 min, significant losses of any of the three dehydrogenase activities.

We thank Dr. M. Achtman for much help in the preparation of the manuscript as well as Dr. P. Overath for valuable critical comments.

1. Cozzarelli, N. R., Freedberg, W. B. & Lin, E. C. C. (1968) *J. Mol. Biol.* **31**, 371–387.
2. Overath, P., Pauli, G. & Schairer, H. U. (1969) *Eur. J. Biochem.* **7**, 559–574.
3. Vogel, H. J. & Bonner, D. M. (1956) *J. Biol. Chem.* **218**, 97–106.
4. Showe, M. K. & DeMoss, J. A. (1968) *J. Bacteriol.* **95**, 1305–1313.
5. Reed, L. J. & Oliver, R. M. (1968) *Brookhaven Symp. Biol.* **21**, 397–412.
6. Courtright, J. B. & Henning, U. (1970) *J. Bacteriol.* **102**, 722–728.
7. Arrigoni, O. & Singer, T. P. (1962) *Nature* **193**, 1256–1258.
8. Lin, E. C. C., Koch, J. P., Chused, T. M. & Jorgensen, S. E. (1962) *Proc. Nat. Acad. Sci. USA* **48**, 2145–2150.
9. Weeks, G., Shapiro, M., Burns, R. O. & Wakil, S. J. (1969) *J. Bacteriol.* **97**, 782–836.
10. Boos, W., Schaedel, P. & Wallenfels, K. (1967) *Eur. J. Biochem.* **1**, 382–394.
11. Reed, L. J., Leach, F. R. & Koike, M. (1958) *J. Biol. Chem.* **232**, 123–142.
12. Beisenherz, G., Boltze, H. J., Bücher, Th., Czok, R., Garbade, K. H., Meyer-Arendt, E. & Pfleiderer, G. (1953) *Z. Naturforschg. 8*b 555–577.
13. Sedar, A. W. & Burde, R. M. (1965) *J. Cell Biol.* **24**, 285–295.
14. Haugaard, N. (1959) *Biochim. Biophys. Acta* **31**, 66–72.
15. Kline, E. S. & Mahler, H. R. (1965) *Ann. N.Y. Acad. Sci.* **119**, 905–919.
16. Barnes, E. M. & Kaback, H. R. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 1190–1198.
17. Kaback, H. R. & Milner, L. S. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 1008–1015.
18. Tarmy, E. M. & Kaplan, N. O. (1968) *J. Biol. Chem.* **243**, 2587–2596.
19. Schwartz, M. (1966) *J. Bacteriol.* **92**, 1083–1089.
20. Overath, P., Raufuss, E. M., Stoffel, W. & Ecker, W. (1967) *Biochem. Biophys. Res. Commun.* **29**, 28–33.
21. Klein, K., Steinberg, R., Fiethen, B. & Overath, P. (1971) *Eur. J. Biochem.* **19**, 442–450.
22. Fox, C. F. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 850–855.
23. Hennaut, C., Hilger, F. & Grenson, M. (1970) *Biochem. Biophys. Res. Commun.* **39**, 666–671.