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Dihydrolipoamide Dehydrogenase from Halophilic Archaebacteria: Purification and Properties of the Enzyme from *Halobacterium halobium*†

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ABSTRACT: Halophilic archaebacteria possess dihydrolipoamide dehydrogenase activity but apparently lack the 2-oxoacid dehydrogenase multienzyme complexes of which it is usually an integral component [Danson, M. J., Eisenthal, R., Hall, S., Kessell, S. R., & Williams, D. L. (1984) *Biochem. J. 218*, 811–818]. In this paper, the purification of dihydrolipoamide dehydrogenase from *Halobacterium halobium* is reported. The enzyme is a dimer with a polypeptide chain M_r of 58 000 (±3000). The amino acid composition of the enzyme is compared with those of the eubacterial and eukaryotic dihydrolipoamide dehydrogenases, and evidence is presented to suggest that the N-terminal amino acid of the *H. halobium* enzyme is blocked. Chemical modification with the trivalent arsenical reagent (*p*-aminophenyl)dichloroarsine indicates the involvement of a reversibly reducible disulfide bond in the enzyme's catalytic mechanism. The possible metabolic role of this dihydrolipoamide dehydrogenase in the absence of 2-oxoacid dehydrogenase complexes is discussed.

Dihydrolipoamide dehydrogenase (EC 1.6.4.3) catalyzes the NAD-dependent oxidation of dihydrolipoamide [reviewed by Williams (1976)]:

$$\text{dihydrolipoamide} + NAD^+ \rightleftarrows \text{lipoamide} + NADH + H^+$$

The enzyme fulfills this function in the pyruvate, 2-oxoglutarate, and branched-chain 2-oxoacid dehydrogenase multienzyme complexes. It is an integral component of each of these multienzyme structures, which are found in most eubacteria and all eukaryotes (Reed, 1974; Perham, 1975; Pettit et al., 1978). However, archaebacteria have been found to convert pyruvate and 2-oxoglutarate to their corresponding acyl-CoA thio esters via less complex oxidoreductases (Kerscher & Oesterhelt, 1982). In the halophiles and thermoacidophiles, ferredoxin, and not NAD^+, serves as the electron acceptor in these enzymes (Kerscher & Oesterhelt, 1981a,b; Kerscher et al., 1982), and methanogens use the deazaflavin derivative F_{420} (Zeikus et al., 1977). Lipoic acid is absent from the oxidoreductases, emphasizing a fundamental difference between these enzymes and the 2-oxoacid dehydrogenase multienzyme complexes.

Considering these data, it is unexpected that the halophilic archaebacteria possess a dihydrolipoamide dehydrogenase that, as is the case for the eubacterial and eukaryotic enzyme, is specific for NAD^+ and dihydrolipoamide (Danson et al., 1984). The basic enzymological and kinetic properties of this enzyme have been reported (Danson et al., 1984) although in the absence of the 2-oxoacid dehydrogenase complexes its function remained unclear. A more detailed molecular characterization of the archaebacterial dihydrolipoamide dehydrogenase was warranted, and in this paper we report the purification and properties of the enzyme from *Halobacterium halobium*.

Experimental Procedures

Materials

All chemicals used were of analytical grade. NAD^+, NADH, and aminopeptidase M were from C. F. Boehringer und Soehne, Mannheim, West Germany; DL-lipoamide was from British Drug Houses; DEAE-Sepharose CL-6B was from Pharmacia; hydroxylapatite–Bio-Gel HT was from Bio-Rad Laboratories, Richmond, CA; protamine sulfate (salmine) was purchased from Schwarz/Mann.

Methods

Bacterial Strain and Growth. Halobacterium halobium (C.C.M. 2090) was kindly provided by Dr. W. D. Grant (Department of Microbiology, University of Leicester, Leicester, U.K.). The organism was grown aerobically in liquid shake culture in the medium described by Payne et al. (1960).

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Table I: Purification of Dihydrolipoamide Dehydrogenase from *H. halobium*

purification step	vol (mL)	total enzyme (units)	total protein (mg)	sp act. (units/mg)	recovery (%)	overall purification (x-fold)
cell-free extract	120	103	1716	0.06	100	
protamine sulfate	125	96	1125	0.09	93	1.5
DEAE-Sepharose CL-6B	33	100	105	0.95	97	16
hydroxylapatite–Bio-Gel HT	15	57	4.7	12.1	55	202
Mono Q chromatography	1.6	42	1.2	35.0	41	583

Enzyme Assays. Dihydrolipoamide dehydrogenase was assayed at 22 °C in 50 mM potassium phosphate, pH 7.0, 3 mM NAD^+, 0.4 mM dihydrolipoamide, and 2 M NaCl. The reaction, in a final volume of 1 mL, was started with enzyme, and the increase in A_{340} was followed with time. Specific activity is expressed as μmol of NADH produced min^{-1} (mg of protein)$^{-1}$.

Synthesis of DL-*Dihydrolipoamide.* DL-Dihydrolipoamide was synthesized by the reduction of DL-lipoamide with $NaBH_4$ (Reed et al., 1958).

Synthesis of (p-Aminophenyl)dichloroarsine. (*p*-Aminophenyl)dichloroarsine ($NH_2PhAsCl_2$) was synthesized as described by Stevenson et al. (1978).

SDS[1] *Gel Electrophoresis.* Gel electrophoresis at pH 6.7 in 0.1% SDS was carried out with 7.5% polyacrylamide gels (Perham & Thomas, 1971).

Amino Acid Analysis. Samples of dihydrolipoamide dehydrogenase were reduced and S-carboxymethylated with iodoacetic acid (Perham, 1978) and then hydrolyzed in vacuo in 6 M HCl containing 0.1% phenol and 0.1% thioglycolic acid at 110 °C for 24, 48, and 72 h. Analyses were performed on a Beckman 6300 amino acid analyzer. Values for tryptophan were obtained after hydrolysis for 24 h in 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole.

Chemical Modification with $NH_2PhAsCl_2$. Modification of dihydrolipoamide dehydrogenase with $NH_2PhAsCl_2$ was carried out at 4 °C in 50 mM potassium phosphate, pH 7.0, containing 2 M NaCl and in the presence and absence of either 0.06 mM NADH or 0.1 mM dihydrolipoamide.

Purification of Dihydrolipoamide Dehydrogenase. H. halobium cells (20 g wet weight) were suspended in 100 mL of 50 mM potassium phosphate buffer, pH 7.0, containing 2 mM EDTA. The suspension was sonicated at 0 °C for four periods of 30 s at 75 W with an 11-mm probe on a sonifier cell disruptor (W-350); cell debris was removed at 10000*g* at 4 °C. An aqueous 2% (w/v) solution of protamine sulfate was added dropwise to the supernatant at 4 °C (1 mg of protamine/15 mg of protein), and the suspension was stirred for 15 min. The precipitate was removed by centrifugation at 15000*g*, and the supernatant was applied to a column (2.7 cm × 35 cm) of DEAE-Sepharose CL-6B previously equilibrated with 50 mM potassium phosphate buffer, pH 7.0 at 10 °C. The column was washed with a further 300 mL of this buffer followed by 150 mL of the buffer containing 0.5 M NaCl. Finally, a gradient of 0.5–1.5 M NaCl in 400 ml of the phosphate buffer, pH 7.0, was applied at a flow rate of 30 mL/h. Dihydrolipoamide dehydrogenase was eluted at approximately 1 M NaCl, and the pooled enzyme was applied directly to a column (2.8 cm × 6 cm) of hydroxylapatite Bio-Gel HT equilibrated at 22 °C with 50 mM potassium phosphate buffer, pH 7.0, containing 1 M NaCl. The column was washed with 100 mL of this buffer followed by 100 mL of 100 mM potassium phosphate buffer, pH 7.0, containing 1 M NaCl. The enzyme was eluted with a gradient of 0.1–0.5 M potassium phosphate buffer, pH 7.0, containing 1 M NaCl, at a flow rate of 30 mL/h. Fractions containing enzyme at a specific activity of greater than 10 units/mg were pooled and dialyzed at 4 °C against 20 mM Tris-HCl buffer, pH 7.5. The resultant enzyme solution was subjected to ion-exchange chromatography at 25 °C on the Pharmacia fast protein liquid chromatography system using the Mono Q anion exchanger in 20 mM Tris-HCl buffer, pH 7.5. Protein was eluted with a linear gradient of 0–1.0 M NaCl. Fractions containing enzymic activity were tested for purity by SDS–polyacrylamide gel electrophoresis.

[1] Abbreviations: SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid disodium salt; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride.

Table II: Amino Acid Composition of *H. halobium* Dihydrolipoamide Dehydrogenase[a]

amino acid	residues/subunit (M_r 58 000)	amino acid	residues/subunit (M_r 58 000)
CM-Cys	3.2 (±0.2)	Met	10.6 (±0.1)
Asp	58.6 (±0.6)	Ile	23.8 (±0.2)
Thr	35.5 (±0.8)	Leu	45.3 (±0.2)
Ser	27.4 (±0.8)	Tyr	10.1 (±0.2)
Glu	57.7 (±0.3)	Phe	17.8 (±0.1)
Pro	20.1 (±0.8)	His	14.4 (±0.5)
Gly	75.3 (±0.7)	Trp	1.6 (±0.1)
Ala	75.2 (±0.4)	Lys	10.9 (±0.1)
Val	66.4 (±0.2)	Arg	14.3 (±0.1)

[a] Values for threonine and serine were obtained by extrapolation to zero time of hydrolysis. Values for valine and isoleucine are from 48- and 72-h values only. Tryptophan was determined by hydrolysis with 4 M methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole. Values of other amino acids were determined from duplicate samples taken after 24-, 48-, and 72-h hydrolyses in 6 M HCl at 110 °C. CM-Cys refers to *S*-(carboxymethyl)cysteine.

RESULTS

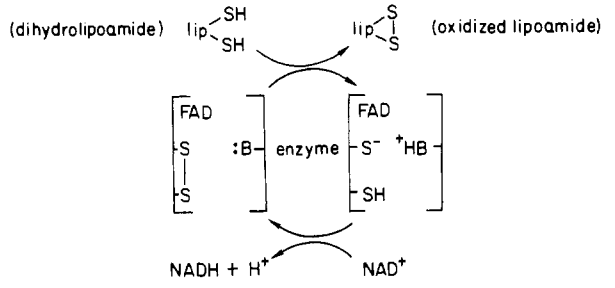
Purification of Dihydrolipoamide Dehydrogenase. The results of a typical purification of the enzyme are given in Table I. The two steps involving ion-exchange columns, namely, chromatography on DEAE-Sepharose and on the Mono Q matrix, require the enzyme to be in low concentrations of NaCl for considerable periods. Similar to other halophilic enzymes, the dihydrolipoamide dehydrogenase was completely inactive when assayed in the absence of NaCl. However, we have found that the readdition of 2 M NaCl to this inactive enzyme, even after 24 h at 4 °C, completely restored enzymic activity within minutes. This finding was of crucial importance in the purification of the dihydrolipoamide dehydrogenase. The spectrum of the pure dihydrolipoamide dehydrogenase showed peaks at 370, 455, and 485 nm, characteristic of the presence of flavin in the enzyme (Williams, 1976).

Determination of Purity and Subunit Molecular Weight. Electrophoresis of the purified dihydrolipoamide dehydrogenase on SDS–polyacrylamide gels gave a single protein band, confirming the homogeneity of the preparation. Comparison of the electrophoretic mobility with those of standard proteins run simultaneously gave a polypeptide chain M_r of 58 000 (SEM ±3000). Taken with the previously determined molecular weight of the native protein of 112 000 (Danson et al., 1984), these data give the number of subunits per enzyme molecule as 1.9 (SEM ±0.1).

Amino Acid Composition and N-Terminal Sequence. The amino acid composition of *H. halobium* dihydrolipoamide dehydrogenase is given in Table II. With the method of Cornish-Bowden (1977), these data have been compared with the amino acid compositions of dihydrolipoamide dehydrogenase from *Escherichia coli* (Stephens et al., 1983) and from pig heart (Williams, 1976) to give an estimate of differences in sequence. No sequence homology was detected between the *H. halobium* enzyme and those from *E. coli* and pig heart. However, the analysis estimated a homology of approximately 70% between *E. coli* and pig heart dihydrolipoamide dehydrogenases. This value is in reasonably good agreement with that of up to 57% identity on the basis of partial sequence comparisons of the two enzymes (Stephens et al., 1983).

Several attempts were made to obtain an N-terminal sequence of the *H. halobium* enzyme on an Applied Biosystems 470A gas-phase sequencer with 3–4 nmol of both S-carboxymethylated and unmodified enzyme. No sequence was obtained, strongly suggesting that the N-terminal amino acid is blocked. This conclusion was supported by incubation of the enzyme with the exopeptidase aminopeptidase M in 20 mM Tris-HCl, pH 7.5, at 25 °C. No loss of dihydrolipoamide dehydrogenase activity was observed after 24 h, and no change in the polypeptide chain molecular weight was found by SDS–polyacrylamide gel electrophoresis. Under identical conditions, aminopeptidase M proteolyzed rabbit muscle glyceraldehyde-3-phosphate dehydrogenase, which is known to have an unblocked N-terminus.

Chemical Modification of Dihydrolipoamide Dehydrogenase with $NH_2PhAsCl_2$. The catalytic mechanism of dihydrolipoamide dehydrogenase from eubacterial and eukaryotic species involves the alternate oxidation and reduction of an intrachain disulfide bond:



In this mechanism, B is a base on the enzyme.

We have now confirmed the presence of the dithiol form of *H. halobium* dihydrolipoamide dehydrogenase by chemical modification of the enzyme with $NH_2PhAsCl_2$. This trivalent arsenical reagent reacts specifically with vicinal thiol groups, leaving single thiols unmodified (Whittaker, 1947; Stevenson et al., 1978). The data reported in this paper are with unpurified enzyme, but all the findings were fully confirmed with pure protein.

In accordance with the above mechanism, *H. halobium* dihydrolipoamide dehydrogenase was completely inactivated by $NH_2PhAsCl_2$ in the presence of NADH (Figure 1), whereas the reagent alone resulted in <10% loss of enzymic activity over the same time period (Figure 2). In the presence of dihydrolipoamide, the enzyme is reduced, and approximately 50% inactivation by the arsenical was observed (Figure 2). Under the latter conditions, $NH_2PhAsCl_2$ will react rapidly with excess dihydrolipoamide. The reduced enzyme can then be either modified by the arsenical or reoxidized by the oxidized lipoamide initially generated; hence, incomplete inactivation will be observed. Consistent with this proposal, addition of NADH to the 50% inactivated enzyme results in complete loss of the remaining enzymic activity. Similarly, addition of excess dihydrolipoamide, followed by more $NH_2PhAsCl_2$, brings about another 50% loss of activity (Figure 2).

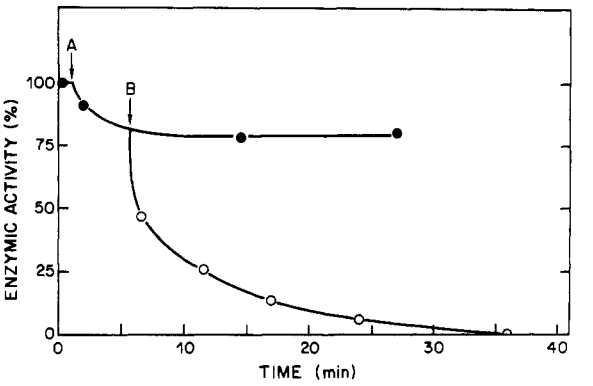


FIGURE 1: Chemical modification of dihydrolipoamide dehydrogenase with $NH_2PhAsCl_2$. Cell-free extracts of *H. halobium* were incubated at 4 °C in 50 mM potassium phosphate buffer, pH 7.0, containing 2 M NaCl, in the presence of (a) 0.06 mM NADH (●) and (b) 0.06 mM NADH and 0.35 mM $NH_2PhAsCl_2$ (○). The times of addition of NADH (A) and of $NH_2PhAsCl_2$ (B) are indicated by arrows. Dihydrolipoamide dehydrogenase was assayed spectrophotometrically at 340 nm as described under Experimental Procedures.

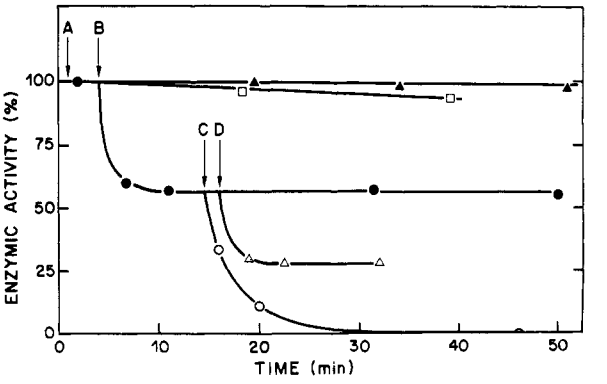


FIGURE 2: Chemical modification of dihydrolipoamide dehydrogenase with $NH_2PhAsCl_2$. Cell-free extracts of *H. halobium* were incubated at 4 °C in 50 mM potassium phosphate buffer, pH 7.0, containing 2 M NaCl, in the presence of 0.1 mM dihydrolipoamide and 0.35 mM $NH_2PhAsCl_2$ (●), 0.1 mM dihydrolipoamide (▲), and 0.35 mM $NH_2PhAsCl_2$ (□). The times of addition of dihydrolipoamide (A) and of $NH_2PhAsCl_2$ (B) are indicated by arrows. To two separate samples of the enzyme partially inactivated by $NH_2PhAsCl_2$ in the presence of dihydrolipoamide (●) were added 0.06 mM NADH (○) at t = 14.5 min (C) and 0.35 mM dihydrolipoamide followed by 0.3 mM $NH_2PhAsCl_2$ (△) at t = 17 min (D). Dihydrolipoamide dehydrogenase was assayed spectrophotometrically at 340 nm as described under Experimental Procedures.

Reagents with vicinal thiol groups can reverse the reaction between trivalent arsenicals and lipoic acid, but monothiols are much less effective (Whittaker, 1947; Stocken & Thompson, 1949; Stevenson et al., 1978). Additionally, the cyclic lipoyl dithioarsenite complex will undergo slow hydrolysis in aqueous solution. The results from attempts to reverse the inactivation of *H. halobium* dihydrolipoamide dehydrogenase caused by $NH_2PhAsCl_2$ in the presence of dihydrolipoamide are given in Table III. Partial reactivation by various dithiol reagents is observed, and much greater concentrations of the monothiol 2-mercaptoethanol are required to give the same effect. However, almost complete reactivation was achieved by removal of excess $NH_2PhAsCl_2$ by dialysis against oxidized lipoamide. The enzyme was found to be much less stable in its reduced form than in its oxidized

Table III: Reactivation of Dihydrolipoamide Dehydrogenase Inactivated by $NH_2PhAsCl_2$ in the Presence of Dihydrolipoamide

enzyme	enzymic activity (%)
native enzyme	100
enzyme inactivated by $NH_2PhAsCl_2$[a]	50
inactivated enzyme treated with[b]	
1.3 mM oxidized lipoamide (16 h)	75
2.0 mM 2,3-dithiopropanol (10 min)	76
2.4 mM 3,4-dimercaptotoluene (10 min)	67
2.4 mM dithiothreitol (10 min)	56
23.0 mM 2-mercaptoethanol (10 min)	66
dialysis vs. 0.5 mM oxidized lipoamide (16 h)	96

[a] Dihydrolipoamide dehydrogenase was incubated at 4 °C in 50 mM potassium phosphate buffer, pH 7.0, containing 2 M NaCl, in the presence of 0.1 mM dihydrolipoamide and 0.35 mM $NH_2PhAsCl_2$ (see Figure 2 for the time course of inactivation). [b] The enzyme, inactivated to 50% of its original activity, was incubated at 4 °C with the thiol-containing reagents listed. The times of incubation are those giving maximum reactivation.

form. Treatment with reagents that leave the enzyme in its reduced dithiol form is unlikely to be as successful as that with oxidized substrate, which, after hydrolysis of the dithioarsenite, yields the disulfide form of the enzyme.

With enzyme inactivated by $NH_2PhAsCl_2$ in the presence of NADH, incubation with 2,3-dithiopropanol, dithiothreitol, or 2-mercaptoethanol caused no reactivation. After dialysis against 0.5 mM oxidized lipoamide, less than 10% of the original enzymic activity was regained. These observations were unexpected considering that lipoamide dehydrogenase from *E. coli*, inhibited with $NH_2PhAsCl_2$, was reactivated with 2,3-dithiopropanol (Adamson & Stevenson, 1981). In the presence of NADH and $NH_2PhAsCl_2$, the dithioarsenite bonds so formed within the *H. halobium* enzyme appear to be unavailable to dithiol reagents, perhaps due to changes arising in the protein conformation.

Discussion

In eubacterial and eukaryotic organisms, dihydrolipoamide dehydrogenase functions in the 2-oxoacid dehydrogenase complexes. However, we have reported that halophilic archaebacteria also possess this enzymic activity (Danson et al., 1984). This was an unexpected find in that, in all archaebacteria tested so far, the oxidative decarboxylations of pyruvate and 2-oxoglutarate are catalyzed by ferredoxin oxidoreductases (Kerscher & Oesterhelt, 1981a,b, 1982; Kerscher et al., 1982) and, in contrast with the situation in aerobic eubacteria and eukaryotes, the 2-oxoacid dehydrogenase complexes have never been detected (Aitkin & Brown, 1969; Kerscher & Oesterhelt, 1982; Danson et al., 1984).

To probe the metabolic and evolutionary significance of this halophilic dihydrolipoamide dehydrogenase in the absence of the 2-oxoacid dehydrogenases, we report in this paper the purification and characterization of the enzyme from *H. halobium*. The enzyme is shown to be a dimeric flavoprotein of polypeptide chain M_r 58 000 (±3000). Chemical modification with $NH_2PhAsCl_2$ in the presence and absence of dihydrolipoamide or NADH indicates the involvement in catalysis of a reversibly reducible disulfide bond. Taken with the catalytic and kinetic properties reported previously (Danson et al., 1984), it is clear that the halophilic dihydrolipoamide dehydrogenase is remarkably similar to its counterpart in nonarchaebacterial species (Williams, 1976).

To explore these similarities further, we had hoped to compare the N-terminal sequence of this protein with that of the *E. coli* and pig heart enzymes as it is within the first 50 amino acids that the active site disulfide is found (Stephens et al., 1983). However, this is not possible at this stage as the N-terminal amino acid of the *H. halobium* enzyme appears to be blocked. A comparison of amino acid compositions as a measure of sequence similarities (Cornish-Bowden, 1977) gave no detectable homology between the *H. halobium* enzyme and those from *E. coli* and pig heart. This is possibly a consequence of adaptation of the halophilic dihydrolipoamide dehydrogenase to conditions of high-salt concentrations. A comparison of halophilic and nonhalophilic proteins [reviewed by Lanyi (1979)] revealed the trend that the halophilic proteins contain a significantly higher frequency of acidic residues (especially glutamate) and the borderline hydrophobic residues (e.g., alanine and glycine) and a lower frequency of basic amino acids (especially lysine) than their nonhalophilic counterparts. This is entirely consistent with the pattern observed for *H. halobium* dihydrolipoamide dehydrogenase: compared with the *E. coli* and pig heart enzymes, the halophilic protein has higher mole fractions of glutamate, threonine, glycine, alanine, valine, and leucine but lower amounts of isoleucine, lysine, and arginine.

Given our evidence that halobacteria possess a true dihydrolipoamide dehydrogenase, the question of its in vivo function remains to be answered. A further step toward such an answer will come from a search for lipoic acid, the presumed substrate of the enzyme, in the halophilic archaebacteria. If present, its function in the absence of the 2-oxoacid dehydrogenase complexes needs to be explained. It has been suggested that lipoic acid may be involved in the binding protein dependent sugar transport systems in *E. coli* (Richarme, 1985) since a lipoic acid deficient mutant of this organism was impaired in the uptake of ribose, galactose, and maltose. In addition, the observation that trivalent arsenical reagents inactivate hexose transport by adipocytes is consistent with the involvement of a molecule with vicinal thiol groups in these uptake systems (Frost & Lane, 1985).

Another possible function of lipoic acid and dihydrolipoamide dehydrogenase is in the glycine enzyme cleavage system, also called glycine synthase [reviewed by Kikuchi & Hiraga (1982)]. This system, present in various eukaryotes and eubacteria, is composed of four different proteins, one of which (the H protein) containing lipoic acid and another (the L protein) being a dihydrolipoamide dehydrogenase. The presence of such an enzyme complex has not yet been investigated in the archaebacteria.

Clearly, the biological roles of lipoic acid and dihydrolipoamide dehydrogenase are under active review. It is probable that further studies on them in the halophilic archaebacteria, where they are not found in their established function in the 2-oxoacid dehydrogenase complexes, will add to the current interest.

Registry No. *p*-$NH_2C_6H_4AsCl_2$, 79368-82-4; dihydrolipoamide dehydrogenase, 9001-18-7.

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Binding of Coagulation Factor XI to Washed Human Platelets†

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ABSTRACT: The binding of human coagulation factor XI to washed human platelets was studied in the presence of zinc ions, calcium ions, and high molecular weight kininogen. Significant factor XI binding occurred at physiological levels of these metal ions when high molecular weight kininogen was present. Binding required platelet stimulation and was specific, reversible, and saturable. Scatchard analysis of the binding yielded approximately 1500 binding sites per platelet with an apparent dissociation constant of approximately 10 nM. Since the concentration of factor XI in plasma is about 25 nM, this suggests that in plasma factor XI binding sites on stimulated platelets might be saturated. Calcium ions and high molecular weight kininogen acted synergistically to enhance the ability of low concentrations of zinc ions to promote factor XI binding. The similarity between the concentrations of metal ions optimal for factor XI binding and those optimal for high molecular weight kininogen binding, as well as the ability of high molecular weight kininogen to modulate these metal ion effects, implies that factor XI and high molecular weight kininogen may form a complex on the platelet surface as they do in solution and on artificial negatively charged surfaces.

The process of contact activation of intrinsic coagulation has been the subject of intensive investigation in recent years. A large body of data has been developed concerning the events leading to the activation of factor XI, involving the proteins factor XII, prekallikrein, and high molecular weight kininogen (HM_rK)[1] [reviewed in Cochrane & Griffin (1982) and Griffin & Cochrane (1979)]. It has been shown that these proteins are assembled on negatively charged surfaces and form a contact factor complex capable of generating factor XIa activity on the surface. The studies from which this model was derived employed a variety of artificial or nonphysiological negatively charged surfaces such as kaolin, celite, glass, ellagic acid, sulfatides, or dextran sulfate. Although such studies have been useful in elucidating the molecular interactions involved in contact activation, questions about the physiological sites of the initiation of intrinsic coagulation remain open. The finding that crude preparations of collagen support contact activation of plasma (Niewiarowski et al., 1964, 1965; Wilner et al., 1968; Cochrane et al., 1972a,b) suggests the possibility that exposed subendothelium may be one such site. However, it was proposed by Walsh (1972a) that the activated platelet surface may also serve as locus for these reactions. Clotting assays showed that activated platelets could substitute for artificial negatively charged surfaces in promoting factor XI activation by the contact system (Walsh, 1972a–d). More recently, in experiments using highly purified contact factors and well-washed platelets, activated platelets in the presence of high molecular weight kininogen were shown to promote the proteolytic activation of factor XI by factor XIIa, as well

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[1] Abbreviations: HM_rK, high molecular weight kininogen; M_r, molecular weight; BSA, bovine serum albumin; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; $K_d$app, apparent dissociation constant; Con A, concanavalin A; QAE, diethyl(2-hydroxypropyl)aminoethyl.

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