Journal of Solid-Phase Biochemistry, Vol. 5, No. 1, 1980

KINETIC AND STRUCTURAL PROPERTIES OF NAD-DEPENDENT BACTERIAL FORMATE DEHYDROGENASE

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Accepted October 29, 1979

A kinetic analysis of NAD-dependent formate dehydrogenase from methylotrophic bacteria, strain no. 1, has been carried out. Initial velocity, product, and dead-end inhibition studies are compatible with the rapid-equilibrium random kinetic mechanism. Chemical modification studies of formate dehydrogenase reveal essential cysteine and arginine residues. The influence of enzyme inactivation on the modification of hystidine and lysine residues was also investigated. The model of the enzyme active centre is proposed. Some practical applications of formate dehydrogenase are discussed.

INTRODUCTION

The use of organic compounds in fuel cells is limited because of the absence of effective catalysts of oxidation. Application of enzymes opens up new possibilities for creating bioelectrochemical sources of current. One of the promising fuels is methanol, which can be completely oxidized to CO_2 by a complex of microbial NAD-dependent dehydrogenases with conjugated reduction of three molecules of electron carriers. In contrast to electrochemical oxidation of methanol, enzymatic oxidation involves three distinct stages, i.e.,

$$CH_3OH \xrightarrow[NAD\ NADH]{} CH_2O \xrightarrow[NAD\ NADH]{} HCOOH \xrightarrow[NAD\ NADH]{} CO_2$$

The last stage of oxidation is catalyzed by NAD-dependent formate dehydrogenase (formate: NAD-oxidoreductase, EC 1.2.1.2.; FDH[1]), which was found in the majority of methylotrophic microorganisms (1–5). The irreversibility of this stage of oxidation allows high degrees of substrate conversion and cofactor reduction to be achieved.

[1]*Abbreviations used:* FDH, formate dehydrogenase; DTNB, 5,5'-dithiobis(2-nitrobenzoate); IAA, iodacetamide; DPC, diethylpyrocarbonate; BD, 2,3-butandione.

0146-0641/80/0300-0019$03.00/0

The aim of the present investigation is to describe the structure of the active center and the kinetic mechanism of action of bacterial FDH. The conditions for stabilization of the enzyme in solution and its utilization in a multienzyme system of bioelectrochemical oxidation of formate are discussed.

We have previously demonstrated that FDH of methylotrophic bacteria (strain no. 1) consists of two identical subunits, each of a molecular weight about 46,000, and contains two independent noninteracting active centers [6]. According to the results of low angle x-ray scattering, FDH has a molecular weight of $80{,}000 \pm 8000$ and is an ellipsoid with an axis ratio of 1 : 2 : 3 (7). FDH is stable over a wide range of temperatures (up to 50°C) and pH (5–11); see ref. [8].

MATERIALS AND METHODS

FDH was isolated from methanol-utilizing bacteria, strain no. 1, as described previously (8); the procedure included fractionation of the crude extracts by ammonium sulfate, ion-exchange chromatography on DEAE-cellulose, and gel filtration through Ultragel AcA-44. The activity assay of FDH was carried out spectrophotometrically by registering the change in the optical density at 340 nm in a Reaction Rate Analyser, Model 8600 (LKB, Sweden) or a Hitachi 200-20 recording spectrophotometer (Japan). The fluorometric measurements were carried out in a Hitachi 512 recording spectrofluorometer (Japan), and analytical ultracentrifugation in a Spinco model E ultracentrifuge (Beckman, USA). Amino acid analysis was performed on a Hitachi KLM instrument after protein samples were hydrolyzed in 6 N HCl for 24 h. The SH-groups were modified by DTNB or iodacetamide (9). Histidine residues were blocked by diethylpyrocarbonate (10), lysine residues by formaldehyde in the presence of sodium borohydride (11), and arginine residues by 2,3-butanedione (12).

The electrochemical measurements were carried out in a device with a rotating disk electrode with the help of a three-electrode scheme. The study of the two-enzyme system (FDH-NADH dehydrogenase) was carried out using a rotating disk pyrographite electrode (surface area, 0.07 cm^2) in argon, which was blown through the gas phase of the cell for 30 min prior to the experiment.

RESULTS AND DISCUSSION

The Kinetic Properties of FDH

When NAD (formate) was the variable substrate with several different constant concentrations of formate (NAD), an intersecting pattern of

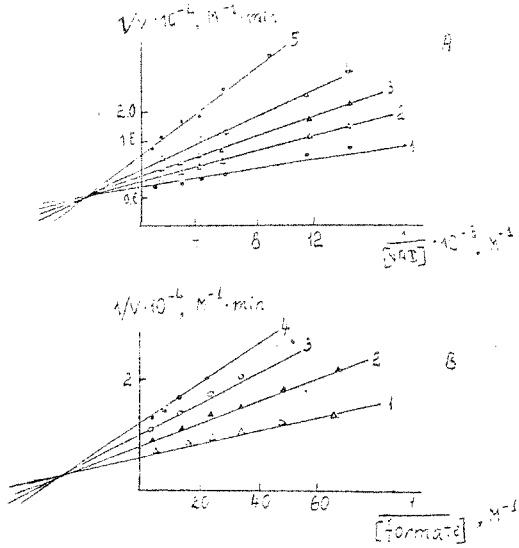

FIG. 1. Initial rate plots of formate dehydrogenase catalyzed reactions. NAD as variable substrate, with concentrations of formate (M): 1–0.15; 2–4.5×10^{-2}; 3–3.0×10^{-2}; 4–2.25×10^{-2}; 5–1.5×10^{-2}. Formate as variable substrate; with concentrations of NAD (M): 1–8.8×10^{-4}; 2–$1{\cdot}5\times10^{-4}$; 4–1.0×10^{-4}; 4–7.5×10^{-5}. 0.05 M phosphate buffer, pH 7.0, 37°C.

Lineweaver–Burk plots was obtained (Fig. 1). The values of the kinetic constants obtained in three independent series of experiments were $K_{NAD} = (6.4\pm2.1)\times10^{-5}$ M, $K_{form} = (1.9\pm0.6)\times10^{-2}$ M, and $K_{NAD,form} = (4.2\pm2.0)\times10^{-6}$M^2. The Michaelis constants determined with the saturating concentrations of the substrates were $(1.1\pm0.2)\times10^{-4}$ M and $(1.5\pm0.1)\times10^{-2}$ M for NAD and formate, respectively.

To elucidate the order of substrate binding in the course of the enzymatic reaction and to determine the position of the step limiting the total rate of the process, the effect of the products, the structural analogues of the substrates, and also the effect of isotopic substitution on the kinetic mechanism of FDH have been studied. The results of the inhibition analysis (Table 1) do not agree with the ordered kinetic schemes of action of FDH. A detailed kinetic analysis of the data shows that the most plausible is a rapid-equilibrium random mechanism of action of the enzyme. The results of the study of the isotopic effect of the kinetic parameters of the reaction catalyzed by FDH are presented in Table 2. The considerable primary isotopic effect of the maximal reaction rate should be interpreted to mean

TABLE 1. **Inhibition Analysis of NAD-Dependent Formate Dehydrogenase**[a]

Inhibitor	Varied substrate	Inhibition	K_i, M
NADH	NAD	Noncompetitive (NC)	$(2.1 \pm 0.7) \times 10^{-5}$
	Formate	Competitive (C)	
CAPAD[b]	NAD	NC	$(2.4 \pm 1.2) \times 10^{-4}$
	Formate	C	
NADP	NAD	NC	$(1.3 \pm 0.8) \times 10^{-3}$
	Formate	C	
AMP	NAD	NC	$(5.1 \pm 2.9) \times 10^{-3}$
	Formate	C	
N_3^-	NAD	Uncompetitive (UC)	$(1.5 \pm 0.6) \times 10^{-7}$
	Formate	C	
HSO_3^-	NAD	UC	$(2.0 \pm 0.5) \times 10^{-5}$
	Formate	C	
NO_3^-	NAD	UC	$(1.1 \pm 0.2) \times 10^{-3}$
	Formate	C	
HCO_3^-	NAD	NC	0.11 ± 0.02
	Formate	NC	

[a] 0.05 M phosphate buffer, pH 7.0, 37°C.
[b] CAPAD: (3-trichloroacetyl)piridine adenine dinucleotide.

that the conversion of the central ternary complex is the rate-limiting step of the enzymatic oxidation of formate.

The study of the isotopic effect has lent support to the validity of the random kinetic scheme of action of FDH. In fact, in the case of the ordered mechanism, NAD being the first substrate, the ratio of K_{NAD}/k_{cat} is equal to the reciprocal of the kinetic rate constant of the binding of the cofactor to the enzyme. It is obvious that this value should not depend on the nature of the second substrate, while as follows from the data of Table 2, the value of K_{NAD}/V equal to K_{NAD}/k_{cat} with the accuracy of the concentration of the enzyme increases 2.3-fold if deuterated formate is used as a substrate.

It should be noted that the peculiarity of FDH compared to other NAD-dependent dehydrogenases is an unusual character of inhibition by

TABLE 2. **Isotopic Effects of Kinetic Parameters of Formate Dehydrogenase Catalyzed Reactions**

K^H_{NAD}/K^D_{NAD}	K^H_{form}/K^D_{form}	$V^H V^D$	$K_{NAD}/k_{at} \cdot E_0$ (min)
1.3 ± 0.3	1.5 ± 0.2	3.0 ± 0.2	0.70 ($HCOO^-$) 1.61 ($DCOO^-$)

NADH, and also by the structural analogues of the coenzyme, which are noncompetitive inhibitors with respect to NAD and competitive inhibitors with respect to formate. The simplest way to account for the experimental data is to introduce into the scheme an abortive complex of a FDH-NAD-I type, where I is either NADH or an analogue of the coenzyme.

The method of multiple inhibition was used to demonstrate that the pairs NADH-KN_3 and NADH-KNO_3 were mutually dependent inhibitors: the binding of one of them rules out the binding of the other. As both azide and nitrate compete with formate, the results obtained point to the existence of a ternary complex of a FDH-NAD-NADH type, in which NADH seems to occupy or screen the substrate site.

Figure 2 shows the pH profiles of the kinetic parameters of the reaction catalyzed by FDH. The value of V in the pH range studied (5.2–10.5), is almost constant, which means that the rate of the hydride transfer, which determines the total rate of the enzymatic process, does not depend on the pH of the medium. The values of K_m remain constant for both substrates if the pH changes from 6 to 9. At pH below 6 and above 9, K_m increases. As there is no change in the value of the catalytic constant in the studied range, it is quite probable that this dependence is due to the ionization of the groups responsible for the binding of the substrates.

The pH dependence of K_m for NAD in the acidic range seems to be indicative of poor binding of the coenzyme on protonation of the phosphate groups, although the participation of the carboxyl groups or histidine of the

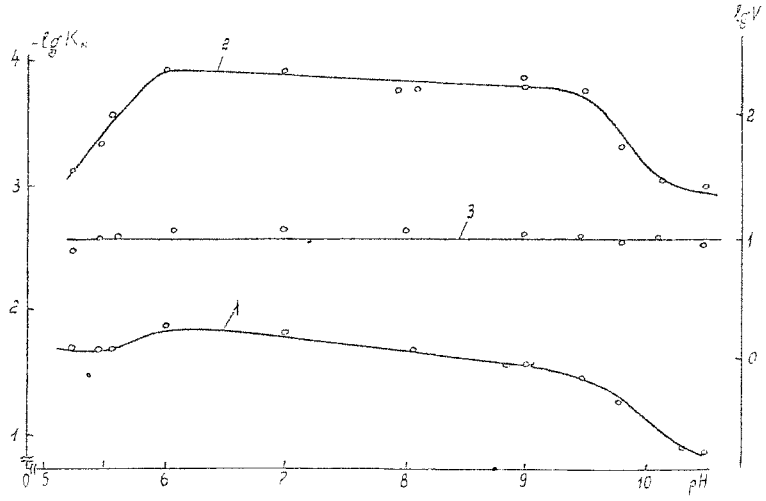

FIG. 2. Effect of pH on the kinetic parameters of formate dehydrogenase. (1) K_m for formate; (2) K_m for NAD; (3) V^*_{max}.

protein globule cannot be excluded. The K_m of formate in this range changes insignificantly. This agrees with the low pK value of the formic acid, which is 3.8. The apparent pK values calculated for the alkaline side of the pH dependence of K_m are $9{\cdot}1 \pm 0{\cdot}2$ and $9{\cdot}5 \pm 0{\cdot}2$ for formate and NAD, respectively. The same values are characteristic of ε-amino groups of lysine, of the phenol hydroxyl, and of the SH-group of cysteine. Some of the amino acid residues that have close values of pK (arginine, histidine) can also be essential for the maintenance of the enzymatic activity. The interpretation of this pH dependence is difficult because the range of the alteration of K_m is close to the values of pH at which an irreversible inactivation of the enzyme occurs (at pH below 5.0 and above 10.7).

Chemical Modification of the Functional Groups of FDH

To elucidate the function of the SH-groups in the molecule of FDH, the process of inactivation of the enzyme under the action of irreversible inhibitors such as DTNB and iodacetamide (IAA) has been studied. The results of the study of the effect of modification of the SH-groups on the activity of FDH are shown in Fig. 3. The data on the inactivation of FDH by IAA agrees with the results of titration of the SH-groups of the enzyme with DTNB. In both cases total inactivation of the enzyme occurs when one SH-group per subunit is modified.

Inactivation of FDH under the action of excess IAA over the 6.9–10.7 pH range obeys the pseudo-first-order reaction kinetics. As the pH of the solution is raised from 7 to 9, there is an increase in the rate constant of inactivation, and at the pH values higher than 9.8, the value of the rate constant does not markedly change. The value of pK for the SH-group modified under the action of IAA is 9.6 ± 0.15 (25°C).

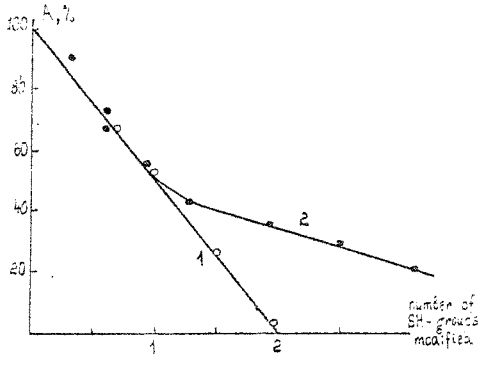

FIG. 3. Inactivation of formate dehydrogenase in the presence of (1) DTNB, (2) iodacetamide.

No increase in NADH fluorescence occurs on interaction with inactivated FDH. Coenzymes do not quench the fluorescence of the modified protein either. These results show that the coenzyme does not bind to the enzyme preparations, the SH-groups of which are blocked by IAA.

NAD, NADH, and NAD in the presence of azide effectively protect FDH from being inactivated. Their stabilizing effect is enhanced in the following order, NAD < NADH < (NAD + azide), and correlates with the binding constants of the binary and ternary complexes formed. In the presence of NAD + azide, the enzymatic activity is almost totally retained throughout the modification, and one SH-group per subunit is protected from the action of IAA.

The close pK value of the ionogenic group that is responsible for the binding of NAD (9.5, 37°C) and the pK of the SH-group (9.6, 25°C) give grounds for believing that the essential thiol residue of FDH can be directly involved in the binding of the coenzyme. At the same time the possibility of inactivation of FDH upon blocking of SH-groups due to steric hindrances or conformational alterations of the protein globule cannot be excluded.

On the basis of the experiments on inactivation of FDH by DTNB and IAA, it was established that each of the subunits of FDH has one essential SH-group. These thiol residues seem to be located either in or close to the coenzyme binding site and play an important role in the binding of the cofactor. The SH-group is not likely to participate in the catalytic conversion of the substrate. Inactivation of FDH upon blocking of the SH-groups seems to be due to the fact that the binding of NAD in the active centre of the modified enzyme is impossible.

Modification with diethylpyrocarbonate (DPC) leads to the loss of the enzymatic activity and is accompanied by the appearance of a new absorption maximum in the region of 238–245 in the differential absorption spectrum, which is characteristic of carbotoxilated histidine. The enzymatic activity of FDH after inactivation of DPC is restored under the action of hydroxylamine, which shows the specific character of the blocking of the histidine residues by the modifying agent.

Figure 4 shows plots of $\ln(V_t/V_0 \times 100)$ versus the time of incubation at three different concentrations of DPC. Both the fast and the slow stages of inactivation of FDH under the action of the modifying agent obey first-order reaction kinetics with respect to the concentration of the inhibitor. The same type of plots were obtained when the kinetics of modification of histidine residues was studied. The values of the inactivation rates of the enzyme and the rates of modification of the histidine residues are close.

The linear dependence between the number of the modified residues of histidine in the molecule of the enzyme and the activity of FDH is retained up to 20–30% of the residual activity. Extrapolation shows that total

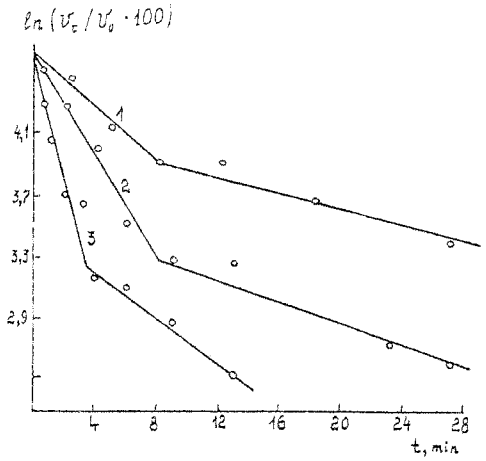

FIG. 4. Inactivation of formate dehydrogenase in the presence of diethylpyrocarbonate. Concentrations of modifying agent (mM): (1) 0.25, (2) 0.5, (3) 1.0.

inactivation of the enzyme occurs when ~10 residues of histidine per subunit of the enzyme are modified.

K_m both for NAD and formate does not alter up to the 50% modification of the histidine residues that are accessible to the action of DPC. This process corresponds to the first faster stage of inactivation of FDH. Further modification of the histidine residues involves changes in the structure of the enzyme that significantly effect the conformation of the active center and results in a three-fold increase in K_m for both substrates. In kinetic terms this process corresponds to the second slower stage of the inactivation of the enzyme. Both NAD and NADH retain the ability for binding with modified FDH, causing the quenching of the protein fluorescence.

NAD and NADH protect the enzyme against inactivation, although the same number of the histidine residues is modified as in the absence of the cofactors. The respective rate constants of modification of the histidine residues in the presence of the coenzymes are practically the same as in their absence, and formate does not affect the process of inactivation of the enzyme.

Figure 5 shows the kinetic curves for interaction of the SH-group of the native and modified FDH with DTNB. Inactivation of the enzyme involves the decrease in the concentration of the highly reactive thiol groups, which are essential for maintenance of the enzymatic activity. The blocking of the histidine residues changes the character of interaction of DTNB with the

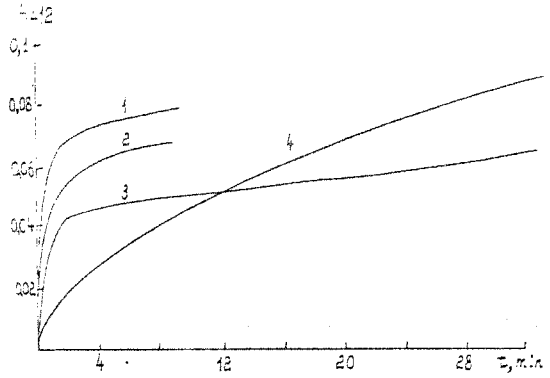

FIG. 5. Titration of the SH-groups of native and partially-inactivated by diethylpyrocarbonate formate dehydrogenase with the help of DTNB. Residual enzyme activity (%): (1) 100, (2) 62, (3) 50, (4) 13.

essential SH-groups of the enzyme. At the initial stages of modification, inactivation of the enzyme correlates with the decrease in the concentration of the reactive thiol groups. With high degrees of modification of the histidine residues, a conformational change leads to the enhancement of the reactivity of SH-groups that heretofore have been masked. The evidence allows us to suggest that one of the histidine residues that is accessible to the action of DPC can be located in close proximity of the cysteine residue, which is essential for the maintenance of the enzymatic activity.

Modification of FDH with the help of DPC does not detect the essential histidine residue, which possesses an anomalously high reactivity and is protected from the action of the inhibitor by the substrate or the coenzyme. The histidine residues that are accessible to the action of DPC do not participate in the binding of the substrates or in catalysis, which is inferred from the constant character of the kinetic parameters of the enzyme over the 6–9 pH range. It is probable that the blocked residues of histidine are located outside the active site of the enzyme or at least outside the substrate binding site. It seems that the modification of the enzyme causes the change in the conformation of the active site; however, the overall conformation of the protein globule is retained, which is indicated by the identical fluorescence and CD spectra for the native and modified FDH.

The effective binding of various inorganic anions and also the specifications of the structure of formate allow us to suggest the presence of a cationic group in the active site of FDH. Such a center might be an ε-amino group of the lysine residue or guanidine group of the arginine residue. To modify the lysine residues, we used the reaction of formaldehyde in the

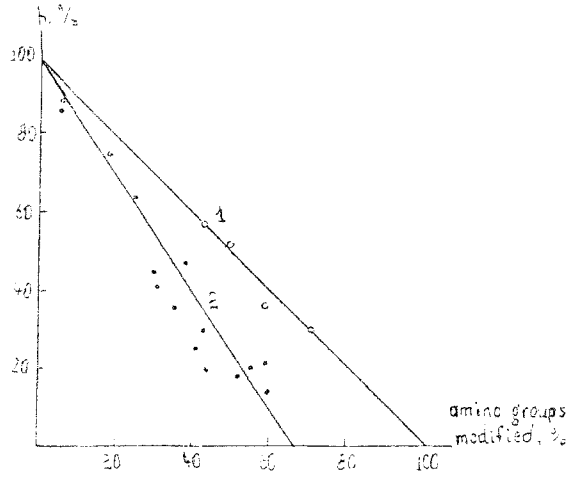

FIG. 6. Correlation between residual enzyme activity and percent of NH_2-groups modified. (1) Determination of the surface amino groups; (2) determination of the total number of amino groups.

presence of sodium borohydride, and the arginine residues were blocked by 2,3-butanedione (BD).

Figure 6 shows the results of enzyme inactivation under the action of H_2CO. The titration of the surface NH_2-groups of FDH with the help of ε-phthalic aldehyde (curve 1) and the determination of the total number of amino groups of the enzyme with fluorescamine under denaturing conditions (curve 2) allowed us to reveal a linear dependence between the residual activity and the degree of modification. Extrapolation of these dependences to the abscissa shows that complete inactivation of FDH occurs when all the surface amino groups are modified, that is, only 70% of their total number, which corresponds to the blocking of 12–13 lysine residues per subunit.

Addition of NADH or formate to the incubation mixture does not produce any effect on the process of inactivation of the enzyme. The K_m value for NAD and formate in FDH preparations inactivated by 90% does not change compared to the respective values for the native enzyme.

The inactivation of FDH under the action of an excess of BD obeys pseudo-first-order kinetics. The dependence of the inactivation rate on the concentration of the modifying reagent is hyperbolic, which testifies to the formation of a noncovalent complex between FDH and BD that precedes modification. The concentration of BD that corresponds to one-half of the maximal inactivation rate is 2×10^{-2} M (pH 8.2, 25° C).

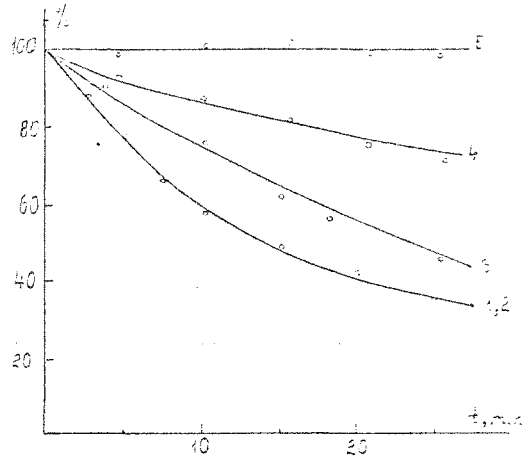

FIG. 7. Inactivation of formate dehydrogenase under the action of 2,3-butandione in the presence of different compounds. (1) control; (2) formate, 0.3 M; (3) NADH, 16.5 μM; (4) NAD, 0.65 mM; (5) NAD + NaN_3, 0.78 ± 0.04 mM.

Figure 7 shows the kinetic curves for inactivation of FDH under the action of BD in the presence of different compounds. The coenzymes alone as well as NAD in the presence of azide effectively protect FDH from inactivation. The inactivation rate decreases as the concentrations of the coenzymes increase. A kinetic analysis shows that in this case there is a complete protection of the enzyme from the action of BD. Formate produces no effect on the rate of inactivation of the enzyme.

Modification of 14 arginine residues out of 42 present in the FDH molecule with the help of BD results in complete enzyme inactivation. In the presence of NAD + azide, the enzyme activity is not only totally retained, but one arginine residue per FDH subunit is protected from modification. Thus, modification of the lysine residues of FDH by formaldehyde in the presence of borohydride does not reveal the amino group, which is protected from the action of the modifier by the substrates. Inactivation of the enzyme can be due to both the blocking of the essential residues and the secondary changes occurring in the protein globule as a result of modification.

The blocking of the arginine residues involves a preliminary coordination of BD and the enzyme molecule. The effective protection against inactivation in the presence of cofactors allows us to suggest that at least one arginine residue is present in the active center of FDH at or near the coenzyme binding site, and that it is essential for enzymatic activity.

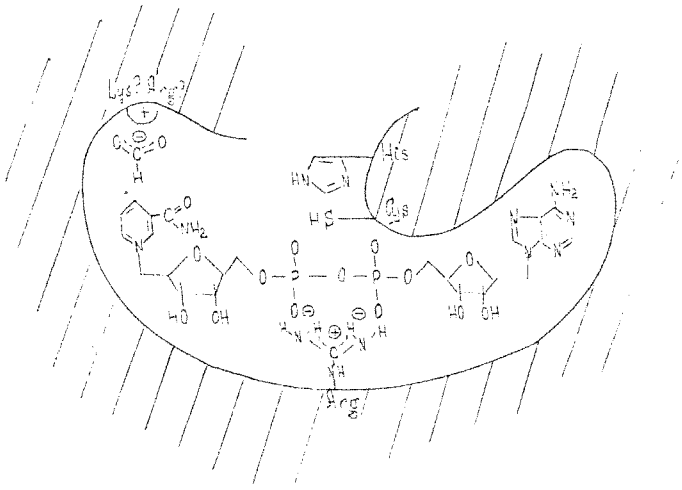

FIG. 8. Proposed schematic model of the active center of formate dehydrogenase.

The results of chemical modification studies of FDH can be schematically summarized as in Fig. 8: (*a*) NAD binds to the enzyme in an "open" conformation as in other dehydrogenases, its pyrophosphate group interacting with the arginine residue; (*b*) the essential thiol residue is located in a coenzyme binding domain of the active site of FDH, and at least one of the histidine residues is located in close proximity to this SH-group; and (*c*) the structure of the substrate binding site involves basic residues, probably lysine or arginine.

Stability of FDH and the Ways of Increasing It

Inactivation under aerobic conditions is characteristic for the majority of FDHs described in the literature. As FDH from methylotrophic bacteria is a typical SH-enzyme, it is clear that its inactivation is associated primarily with oxidation of the active thiol residues essential for the maintenance of the activity. Figure 9 shows inactivation profiles for FDH in the presence of various substances. The use of SH-compounds or EDTA considerably increases the stability of the enzyme preparations. The stabilizing role of EDTA seems to be that of formation of complexes with metal ions that catalyze the oxidation of the thiol groups of the enzyme. In the presence of 0.01 M ETDA, FDH retains total activity for as long as 6 months.

The stability of FDH increases in the presence of substrates and the products of the enzymatic reaction. The stabilizing effect of coenzymes

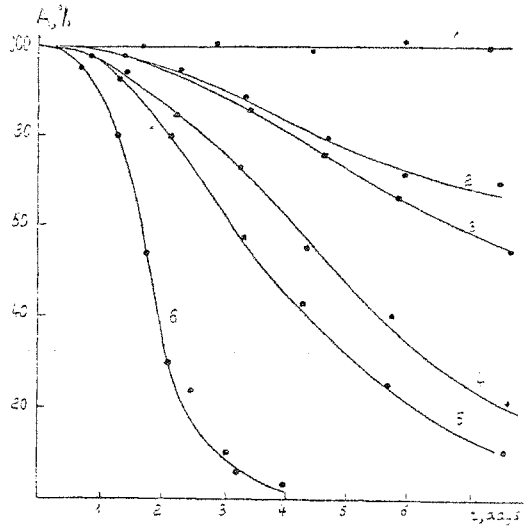

FIG. 9. Inactivation profiles for formate dehydrogenase in the presence of various compounds upon storage in 0.05 M phosphate buffer, pH 7.0, 25°C. (1) 0.01 M EDTA, (2) 3 mM NADH and 0.4 M sodium formate, (3) 0.4 M sodium formate, (4) 3 mM NAD, (5) 3 mM NADH, (6) control.

seems to be due to the decrease in the accessibility of the essential SH-groups of the active center. The results that we have obtained are very important for the practical application of FDH, as they prompt one to the possibility of considerable stabilization of the enzyme in reactors in the absence of external stabilizing agents.

We have made an attempt to stabilize FDH by immobilizing it on a water-soluble copolymer of polyvinyl-4-vinyl pyridine with acrolein quaternized by dimethyl sulfate or ethyl bromide. The water-soluble complexes of FDH with the support possess a 300 to 350-fold higher stability than the native preparations of FDH (37°C). This stabilization effect seems to be due to an excessive surface positive charge around the molecule of the enzyme; the charge prevents the heavy metal ions from penetrating into the active centre of the enzyme and causing oxidation of the essential SH-groups.

Application of FDH

Preparations of soluble or immobilized FDH can be used as catalysts for oxidation of formate for analytical purposes, for regeneration of NADH, and also for making formate-oxidizing anodes for biochemical fuel cells. It

should be noted that FDH has a number of advantages in the case of regeneration of NADH compared to other NAD-dependent dehydrogenases that are used for this purposes. This is due to the fact that the oxidation of formate to CO_2 is an almost irreversible reaction, which allows high degrees of conversion of NAD to NADH to be achieved without accumulation of byproducts. The reverse reaction of CO_2 reduction to formate is also of great interest.

In this laboratory the possibility of creating the kinetic methods for determination of formate with the help of soluble FDH and FDH immobilized on aminated sylochrome was studied. The method allows one to determine up to 2×10^{-5} M formate with an error not exceeding 5%, which is comparable with the most sensitive methods of microestimation of formate.

Owing to the fact that the effective electrochemical oxidation of NADH is difficult, we have studied the process of oxidation of formate with the help of a two-enzyme system, that is, FDH and NADH dehydrogenase that catalyzes the oxidation of NADH coupled with reduction of methyl viologen. On the whole the process of oxidation of formate is the following:

$$\begin{matrix}\text{HCOOH} \\ \text{CO}_2\end{matrix} \rightleftharpoons \begin{matrix}\text{NADH} \\ \text{NAD}\end{matrix} \rightleftharpoons \begin{matrix}2\text{MV}^{+} \\ 2\text{MV}^{++}\end{matrix} \xrightarrow{-2e} \text{electrode}$$

Figure 10 shows polarization curves of oxidation of formate on a rotating disk pyrographite electrode. The extremal values of the diffusion current, 12 mA/cm^2, were achieved with oxidation potentials higher than 0.18 V. It should be noted that in our conditions the values of activities for enzymes (60 μmol per min for FDH and 6 μmol per min for NADH-dehydrogenase) were much higher than the rate of oxidation of the reduced form of methyl viologen on the electrode. Owing to this, and also to a high

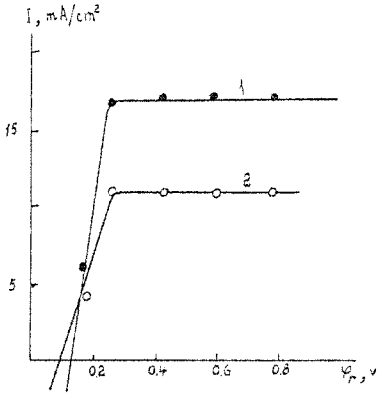

FIG. 10. Polarization curves for formate oxidation on the rotating disk pyrographite electrode in the presence of formate dehydrogenase (60 μmol/min), NADH dehydrogenase (6 μmol/min), NAD (1 mM), methyl viologen (0.5 M), sodium formate (2 M), EDTA, (10 mM), in 1 M triethanol amine-HCl buffer, pH 8.0.

concentration of methyl viologen, the total concentration of the reduced form hardly ever changed during the time when the current was measured.

Oxidation of the reduced methyl viologen occurs in the diffusion regime; this means that the value of the oxidation current is limited by the rate of delivery of the reduced methyl viologen to the surface of the electrode. The parameters of oxidation of formate did not change throughout the 14 days of the experiment. Comparison of the results obtained in the study of this system with data from the literature on direct oxidation of formate on platinum electrodes (including those modified by atoms of other elements) shows that this system is very feasible as far as the potentials for formate oxidation are concerned.

Thus, our results point to the possibility of utilization of these and similar enzyme systems for creating more economical anodes for bioelectrochemical fuel cells.

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