The N-Ethylmaleimide-sensitive Cysteine Residue in the pH-dependent Subunit Interactions of Malate Dehydrogenase*

David C. Wood, C. Thomas Hodges, Sandra M. Howell, L. Gail Clary, and John H. Harrison†

From the Kenan Laboratories of Chemistry, University of North Carolina, Chapel Hill North Carolina 27514

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The specific chemical modification by N-ethylmaleimide of a cysteine residue at pH 5.0 in porcine heart mitochondrial malate dehydrogenase (l-malate:NAD+ oxidoreductase, EC 1.1.1.37) has been shown to result in an enzymatically inactive, monomeric product, which does not reassociate at pH 7.5 to yield the native dimer. In this regard, an investigation of proton release and uptake upon NADH binding to the native enzyme and to the N-ethylmaleimide-modified enzyme has implicated the above cysteine residue as being directly linked to the pH-dependent subunit dissociation of mitochondrial malate dehydrogenase. The results are consistent with the view that the modified cysteine residue is not located at the subunit interaction site, although it is probably near this site. A recent study from this laboratory has demonstrated that the monomeric enzyme obtained at pH 5.0 exists in a conformation which is enzymatically inactive and which has an enhanced intrinsic protein fluorescence. Interpretation of protein fluorescence data has suggested that the N-ethylmaleimide modification results in inactivation of the enzyme by preventing the pH-induced conformational change to the active dimer. However, NADH is able to induce reassociation of the N-ethylmaleimide-modified enzyme at pH 7.5 but not at pH 5.0. This reassociation at pH 7.5 is accompanied by a significant regain of enzymatic activity, indicating that NADH binding is able to partially overcome the negative effect of the cysteine modification on the pH-dependent subunit reassociation of mitochondrial malate dehydrogenase.

The study of structure-function relationships in enzymes often centers on the identification of active center residues and on their role in the catalytic mechanism. Such studies on oligomeric enzymes should also include the role of subunit interactions in catalysis and in maintenance of enzyme structure (1). In this regard, a specific goal is the identification of amino acid residues at the contact area between subunits and on their role in the catalytic mechanism. Such studies on essential ionic interactions or by steric hindrance of the pH-induced conformational change which apparently disrupts both the active site and the subunit interaction site (11) of this molecule. However, total regain of enzymatic activity in the native enzyme is established upon adjustment of the pH to 7.5.

The sulfhydryl group modified by MalNet* at pH 5.0 appears to be one which is essential for maintenance of the enzyme's native tertiary structure. The modification of this sulfhydryl group apparently inactivates the enzyme by preventing the correct refolding of the enzyme to the native conformation at pH 7.5. This modification may prevent the attainment of the native tertiary structure by the elimination of essential ionic interactions or by steric hindrance of the pH-induced conformational change. However, the addition of NADH to the modified enzyme at pH 7.5 has been found to induce a conformational transition to a dimeric species having significant enzymatic activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—Porcine heart mitochondrial malate dehydrogenase was purified as previously described (9), NADH, oxalacetic acid, and MalNet were purchased from Sigma Chemical Co. and used without further purification.

**Enzymatic Assays**—Enzymatic activity was determined by the decrease in absorbance upon oxidation of NADH to NAD*. The assay conditions were 50 mM Na phosphate buffer, pH 7.5, 0.20 mM NADH, 0.25 mM oxalacetic, 25 °C, except where otherwise noted. Protein concentrations were determined spectrophotometrically using the extinction coefficient ε\text{1000} = 2.53 (9).

**Sedimentation Velocity Ultracentrifugation**—Sedimentation coefficients were determined with a Spinco model E analytical ultracen-

* The abbreviation used is MalNet, N-ethylmaleimide.
trifuge (Beckman) as previously described (10). For samples of MalNet-modified enzyme containing NADH, a solution of the cofactor was prepared in 50 mM Na phosphate buffer and adjusted to the appropriate pH. An aliquot of this solution was then added to the enzyme solution to give a final NADH concentration of 1.0 mM.

**pH Stat Titration and Sample Preparation—**Enzyme samples were prepared and dialyzed against a Radiometer pH-Stat as previously described (12). Aliquots of NADH were added to yield an NADH concentration at the end of the titration of 1.0 mM.

**Enzyme Inactivation—**MalNet-modified mitochondrial malate dehydrogenase used in the sedimentation velocity and pH-Stat titration experiments was prepared as previously described (9), MalNet-modified enzyme used for NADH binding, protein fluorescence, and NADH-induced activity regain experiments was prepared by the above procedure except that the inactivation was performed at 0°C. The reaction was allowed to proceed until the inactivated sample exhibited less than 1% of the enzymatic activity of the control, whereupon the reaction was terminated by dialysis against 50 mM Na phosphate buffer at pH 5.0.

Mitochondrial malate dehydrogenase in which both the “active site” (13, 14) histidine and the MalNet-sensitive cysteine residues were modified was prepared by sequential reactions. The modification of the active center histidine with iodoacetamide was carried out first at 25°C as previously described (13, 15). The reaction was allowed to proceed until less than 1% of the control activity remained, whereupon the reaction was terminated by addition of an excess of a 10% solution of β-mercaptoethanol. This iodoacetamide-modified enzyme was subsequently dialyzed against 50 mM Na phosphate buffer at pH 5.0 and the NADH-induced modification was performed at 0°C as described above. The reaction of MalNet with the iodoacetamide-modified enzyme was allowed to proceed for 20-24 h and was then terminated by dialysis. The reaction was judged to be complete by the absence of a protein peak eluting in the region representing the elution volume of the dimer (Mr = 70,000) when a sample was applied to a previously calibrated Sephacryl S-200 gel filtration column which was equilibrated and developed at pH 7.5. The doubly modified enzyme was precipitated by dialysis against 100% saturated ammonium sulfate and stored as a 100% ammonium sulfate suspension.

**NADH Binding to Native and MalNet-modified Malate Dehydrogenase—**NADH binding was determined using Amicon Centriflo CF-25 ultrafiltration membrane cones to separate free and bound NADH. The cones were equilibrated with an NADH solution at the appropriate concentration and a 0.40-ml sample of enzyme (70-90 μM) containing 1.0 mM NADH was then added. A 100-μl volume of filtrate was collected and the NADH concentration was determined spectrophotometrically from the absorbance at 340 nm using the molar extinction coefficient of 6200 M⁻¹ cm⁻¹ (16).

For NADH binding to pH 5.0 MalNet-modified enzyme, NADH solutions were prepared in 5.0 mM Na phosphate buffer at pH 7.5 and the enzyme was dialyzed against 50 mM Na phosphate/acetate buffer at pH 5.0 when the NADH-modified enzyme was added to the enzyme solution and the filtrate was collected and immediately diluted into 50 mM Na phosphate buffer at pH 7.5 to minimize the decomposition of NADH which occurs at acidic pH values (17).

**Protein Fluorescence—**Intrinsic protein fluorescence was determined using a Hitachi Perkin-Elmer MFP-2A fluorescence spectrophotometer. Samples of native and MalNet-modified mitochondrial malate dehydrogenase were adjusted to identical protein concentrations and the protein fluorescence was determined at 16°C with excitation and emission wavelengths of 280 and 303 nm, respectively.

**RESULTS AND DISCUSSION**

**Molecular Weight of Native and MalNet-modified Mitochondrial Malate Dehydrogenase—**The pH-dependent subunit dissociation curves for native mitochondrial malate dehydrogenase and N-ethylmaleimide-modified enzyme are presented in Fig. 1. As has been previously described, the native enzyme exhibits a dissociation with an apparent pK of 5.3 under the conditions used in the analytical ultracentrifuge (10). The MalNet-modified enzyme, however, remains a monomer even at a pH value as high as 7.0 (10). Conversely, in the presence of NADH at pH values above 6.5, the MalNet-modified enzyme reassociates to a dimeric state.

Apparently, the modification of the enzyme by MalNet has perturbed a residue or residues which are required for the native pH dependence of the monom-mer-dimer equilibrium.

Previous studies have characterized the inactivation of mitochondrial malate dehydrogenase by MalNet and indicated that the exposure and subsequent modification of a specific sulfhydryl group can be correlated with the subunit dissociation (10). This sulfhydryl group may be essential for the native properties of the enzyme, but the ability of NADH to induce the formation of a dimeric enzyme at high pH but not at low pH values in the MalNet-modified enzyme suggests that the sulfhydryl group is not directly blocking the subunit interface, since dimerization is still possible under the proper conditions.

**Proton Uptake and Release upon Binding of NADH to Native and MalNet-modified Malate Dehydrogenase—**In order to determine whether the sulfhydryl residue modified by MalNet was the residue directly linked to the pH-dependent subunit equilibrium, the degree of proton release and uptake upon NADH binding to the enzyme was investigated. NADH has previously been shown to induce dimerization of native mitochondrial malate dehydrogenase at pH 5.0, i.e. conditions under which the enzyme would normally exist as a monomer (7).

A quantitative determination of the proton release or uptake upon NADH binding to mitochondrial malate dehydrogenase as a function of pH is indicated in Fig. 2. Curves are presented for the native enzyme and for MalNet-modified enzyme. At pH 5.0, the native enzyme has released a limited number of protons as dimerization occurs upon saturation with NADH. This result implies that residues exist in the protonated form at pH 5.0 where the enzyme is monomeric and that these residues become deprotonated upon an increase in the pH or upon addition of the cofactor NADH. NADH binding apparently has the effect of inducing a conformation of the enzyme in which the pK of the dissociation-linked residue shifts to a lower value, resulting in the observed proton release.

The release of protons seems to correlate well with the reassociation of the enzyme induced by NADH. The fraction of enzyme in the dissociated form increased as the pH was decreased (Fig. 1) and the magnitude of proton release observed upon NADH binding also increased (Fig. 2). In addi-
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The pH dependence of proton uptake and release upon NADH binding to MalNet-modified mitochondrial malate dehydrogenase and to enzyme doubly modified with both MalNet and iodoacetamide. Aliquots containing 50 to 60 mg of enzyme at the indicated pH were titrated with NADH on a Radiometer pH-Stat as described under "Experimental Procedures." The enzyme samples titrated were native malate dehydrogenase (○), MalNet-modified malate dehydrogenase (■), and malate dehydrogenase that was doubly modified with MalNet and iodoacetamide (▲) as described under "Experimental Procedures." The data for native and MalNet-modified enzyme were obtained from Ref. 18.

The data in Fig. 2 indicate that under the same conditions the enzyme at low pH. Binding at pH values above 6.5 is known to occur since dimerization of the modified enzyme at these pH values was induced by NADH. Thus, it was necessary to determine directly whether NADH does bind to MalNet-modified enzyme at pH 5.0.

NADH binding was quantitated by use of an ultrafiltration technique as described under "Experimental Procedures." Fig. 3 contains binding curves for native enzyme at pH 7.5 and for MalNet-modified enzyme at pH 5.0 and 7.5. It is apparent from the data that NADH binds to the modified enzyme with the same stoichiometry as with native enzyme, but with slightly decreased affinity. Also, the binding affinity appears to be approximately the same for the MalNet-modified malate dehydrogenase at pH 5.0 and 7.5.

These data suggest that the binding site for NADH may have been altered but certainly not eliminated by the modification with N-ethylmaleimide. Therefore, since NADH does bind to MalNet-modified enzyme at pH 5.0, the cysteine residue blocked by MalNet is strongly implicated as the residue directly involved in the observed proton release. Apparently, protein release cannot occur since the acidic sulfhydral residue modified by MalNet may be the protonatable group from which proton release has occurred at pH 5.0 for MalNet-modified enzyme was that NADH did not bind to the modified enzyme at low pH. Binding at pH values above 6.5 is known to occur since dimerization of the modified enzyme at these pH values was induced by NADH. Thus, it was necessary to determine directly whether NADH does bind to MalNet-modified enzyme at pH 5.0.

NADH binding to native and MalNet-modified mitochondrial Malate Dehydrogenase—In order to more directly implicate the sulfhydryl group modified by N-ethylmaleimide as the group from which protons are released upon dimerization, the binding of NADH to the modified enzyme was measured directly. Conceivably, the reason that no dimerization and no proton release was observed at pH 5.0 for MalNet-modified enzyme was that NADH did not bind to the modified enzyme at low pH. Binding at pH values above 6.5 is known to occur since dimerization of the modified enzyme at these pH values was induced by NADH. Thus, it was necessary to determine directly whether NADH does bind to MalNet-modified enzyme at pH 5.0.

The stoichiometry of NADH binding for native enzyme, pH 7.5 (○); MalNet-modified enzyme, pH 7.5 (■); and MalNet-modified enzyme, pH 5.0 (▲) was determined using an ultrafiltration method as described under "Experimental Procedures."
dryl proton has been eliminated by the modification with N-ethylmaleimide. Although the above cysteine residue appears likely to be the residue from which proton release occurs upon dimerization, the possibility cannot be ruled out that the sulfhydryl modification has perturbed a nearby residue which is the actual source of protons. The possibility must also be considered that the cysteine modification may sterically prevent a conformational change in the enzyme's native structure, thus preventing proton release from a residue other than cysteine from occurring.

Effect of MalNet Modification on pH-dependent Conformational Change—Recent studies from this laboratory have characterized the monomeric species of mitochondrial malate dehydrogenase obtained by decreasing the pH to 5.0 (11). The kinetics of subunit reassociation has also been determined by means of pH-jump experiments. From these studies, a model was proposed which suggested that the enzyme monomer at pH 5.0 is enzymatically inactive and also exhibits an increased intrinsic protein fluorescence relative to the active, dimeric enzyme. When the pH is raised from 5.0 to 7.5, the enzyme undergoes a conformational change from an inactive, monomeric conformation to an enzymatically active, dimeric conformation. The rate-limiting step in this conformational transition is consistent with the cis/trans isomerization about an X-Pro bond (19).

From these results it is apparent that, at pH 5.0, the N-ethylmaleimide modification occurs with an enzymatically inactive, monomeric conformation of the enzyme in which a reactive sulfhydryl group is exposed. This reaction appeared to result in a loss of enzymatic activity with time (9) because the assay conditions (pH 7.5, 0.20 mM NADH, 25 °C) strongly favor reassociation. The pH 5.0 mitochondrial malate dehydrogenase becomes partially reassociated in the assay cuvette under the above conditions. As the enzyme is allowed to react with N-ethylmaleimide, it is no longer able to rapidly reassociate and the result is apparently normal enzyme inactivation kinetics.

Since MalNet undergoes reaction with an enzymatically inactive conformation of the enzyme, an apparent inactivation of the enzyme would occur if modification of the sulfhydryl group prevented the normal pH-induced conformational change. This possibility was tested by use of the direct correlation previously made between enzymatic specific activity and intrinsic protein fluorescence (11). As indicated in Table I, the intrinsic protein fluorescence of native enzyme was observed to decrease by approximately 60% as the pH was raised from 5.0 to 7.5. This decrease is concomitant both with dimerization and the regain of full enzymatic specific activity. However, the MalNet-modified enzyme displayed essentially no change in protein fluorescence at the two pH values (Table I).

The lack of change in protein fluorescence for MalNet-modified enzyme between pH 5.0 and 7.5 implies that even at pH 7.5 the MalNet-modified enzyme is possibly locked into the enzymatically inactive conformation normally found at low pH. Apparently, the enzyme may not refold to the native conformation at high pH since the deprotonation of the essential sulfhydryl group has been prevented by the modification. The MalNet derivative of the sulfhydryl group may also introduce steric hindrance of the pH-induced conformational transition.

The data further suggest, based on the previously proposed model, that the MalNet-modified enzyme at pH 7.5 has the trans conformation about the proposed X-Pro bond (11) rather than the native cis conformation. In addition, it is apparent from the data in Table I that the intrinsic protein fluorescence of MalNet-modified enzyme was quenched relative to the native enzyme at either pH, presumably as a result of the sulfhydryl group modification. Since mitochondrial malate dehydrogenase contains no tryptophan (20), this observation implies that an exposed tyrosine residue may lie in close proximity to or may be directly affected by the sulfhydryl group modified by N-ethylmaleimide.

pH-Stat Titration of Malate Dehydrogenase Modified with Iodoacetamide and N-Ethylmaleimide—The curve of proton uptake for MalNet-modified enzyme given in Fig. 2 has indicated that the modification has altered normal proton uptake to the active site histidine. Furthermore, at these same conditions of pH and in the presence of NADH, the MalNet-modified enzyme underwent dimerization to a partially active conformation. Since dimerization in the native enzyme is accompanied by proton release, it was thought possible that the NADH-induced dimerization of MalNet-modified enzyme might also be accompanied by proton release from a residue, other than the MalNet-sensitive cysteine, which was masked by simultaneous uptake to the “active center” histidine. Therefore, this histidine was modified by iodoacetamide in the MalNet-modified enzyme and pH-Stat titration performed in order to test this possibility. Previous work has shown that the iodoacetamide modification of the active center histidine has no effect on the pH-dependent subunit dissociation (Fig. 1) and that this modification also eliminates any proton uptake upon NADH binding (13, 15). Therefore, the “active site” histidine residue may be modified without affecting the pH-dependent subunit interactions.

The results of the pH-Stat titration of the doubly modified enzyme, as displayed in Fig. 2, indicate that no proton release is associated with the NADH-induced dimerization of this modified enzyme. Apparently, the NADH-induced dimerization of MalNet-modified mitochondrial malate dehydrogenase at pH values above 6.5 does not result from the deprotonation of a residue or residues which are directly induced by the cofactor. Since NADH-induced dimerization of MalNet-modified enzyme occurs at pH values above 6.5 but not at lower pH values, one or more residues apparently must become deprotonated in order for dimerization to occur. The effect of such a deprotonation may be to alter the conformation of the enzyme, or to alter the interaction of NADH with the enzyme, allowing NADH binding to induce dimerization. NADH apparently does bind to the MalNet-modified enzyme at pH 5.0 and at pH 7.5, but the nature of the interaction between cofactor and enzyme must differ at the two pH values since dimerization is only induced at pH values above neutrality.

Reactivation of MalNet-modified Malate Dehydrogenase by NADH at pH 7.5—The molecular weight versus pH data in Fig. 1 indicate that the MalNet-modified enzyme does not dimerize at pH 7.5 unless NADH is also present. Apparently, NADH is able to induce a conformation of the enzyme capable of overcoming the negative effect of MalNet modification upon the pH-dependent subunit reassociation. Since in the case of the native enzyme, dimerization was directly correlated
with an increase in specific activity, the possibility of enzymatic activity regain in the MalNet-modified enzyme was investigated.

The data in Fig. 4 indicate the regain of enzymatic specific activity upon addition of NADH to MalNet-modified mitochondrial malate dehydrogenase at pH 7.5 and at pH 5.0. At pH 5.0, a negligible increase in specific activity was observed, but at pH 7.5 a first order increase in specific activity occurred. A semilogarithmic plot of the data in Fig. 4 is given in Fig. 5. The kinetics of activity regain for MalNet-modified malate dehydrogenase was similar to that observed for the pH or NADH-induced subunit reassociation of native enzyme. The conditions of enzyme concentration and NADH concentration used were such that full dimerization of the MalNet-modified enzyme was obtained (Fig. 1). The maximal activity regain was obtained at an enzyme concentration of 2 mg/ml and remained constant to a concentration as high as 5 mg/ml. Also suggesting that full dimerization of the modified enzyme was obtained. The kinetics of the regain of enzymatic activity implies that the conformational change induced by NADH in the native enzyme can be induced in the modified enzyme as well.

The resulting dimeric, modified enzyme has significant enzymatic activity, although the full specific activity of the native enzyme is not recovered. In order to more fully characterize the partially reactivated MalNet-modified mitochondrial malate dehydrogenase, the kinetic parameters for the cofactor NADH were determined. A sample of MalNet-modified enzyme was allowed to reassociate upon addition of 1.0 mM NADH as indicated in Fig. 4, and the $K_n$ and $V_{max}$ values for NADH were then determined by means of a Lineweaver-Burk plot. A $K_n$ value of 37 $\mu$M and a $V_{max}$ of 25,000 min$^{-1}$ were obtained. The corresponding values obtained for the $K_n$ and $V_{max}$ of the native enzyme are 33 $\mu$M and 80,000-100,000 min$^{-1}$, respectively. Therefore, the regain of enzymatic activity in the dimeric, MalNet-modified mitochondrial malate dehydrogenase appears to be only partial due to a decrease in the $V_{max}$ parameter, not in the $K_n$ for NADH. This decrease also correlates well with the decrease observed in the magnitude of proton uptake upon NADH binding to the MalNet-modified enzyme (Fig. 2).

The intrinsic protein fluorescence would also be expected to decrease as the MalNet-modified enzyme regains enzymatic activity. However, this parameter cannot be observed in the presence of NADH due to fluorescence energy transfer from tyrosine to NADH.

The dimeric MalNet-modified enzyme apparently has a conformation containing the cis X-Pro bond as judged by the characteristically slow regain of enzymatic activity which was observed. The Arrhenius activation energy for the reassociation of the modified enzyme was determined from the rate of regain of enzymatic activity as previously described (11) and a value for $E_A$ of 19.1 kcal/mol was obtained. This value is consistent with the proposal that a proline cis/trans isomerization is the rate-limiting step in the reassociation of the MalNet-modified enzyme. Thus, the conformation about the proposed proline residue appears to be an absolute requirement for the native, enzymatically active tertiary structure of this enzyme. However, if the sulfhydryl group modified by N-ethylmaleimide is blocked, the result is a partial loss of enzymatic activity and a shift in the pH dependence of the subunit reassociation. Therefore, the modification of the enzyme with N-ethylmaleimide appears to result in inactivation due to interference with a conformational change, not by reaction with a residue directly involved in catalysis or cofactor binding. Another indication of the interference of the MalNet modification with the enzyme structure is the decrease in magnitude and shift in the maximum of proton uptake upon NADH binding to the modified enzyme (Fig. 2). The environment of the "active site" histidine residue responsible for the observed proton uptake has apparently been perturbed by the effect of the sulfhydryl modification on the regain of the native conformation when the pH of the sample is changed from pH 5.0 to 7.5.

The studies of Bradshaw and co-workers on the primary structures and evolutionary relationships of mitochondrial malate dehydrogenase and L-3-hydroxyacyl-CoA dehydrogenase (21) have suggested structural features which may account for some of the observed pH-dependent subunit dissociation properties of mitochondrial malate dehydrogenase. The NAD$^+$-linked dehydrogenases have been shown to contain both a cofactor binding domain and a catalytic domain which contains essential active site residues (22). These domains have also been identified in mitochondrial malate dehydrogenase by comparison of its primary structure with those of L-3-hydroxyacyl-CoA dehydrogenase, lactate dehydrogenase, and cytoplasmic malate dehydrogenase (21, 23).

A model has been proposed (11) for the pH-dependent subunit dissociation of mitochondrial malate dehydrogenase in which a change in the conformation about one or more X-Pro imino bonds from cis to trans results in subunit dissociation, a loss of enzymatic activity, and an increase in intrinsic protein fluorescence. The isomerization about proline or a subsequent conformational change could also result in the

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**Fig. 4.** Regain of enzymatic activity upon addition of NADH to MalNet-modified malate dehydrogenase at pH 5.0 and 7.5. The increase in specific activity was observed at 14 °C at an enzyme concentration of 2.3 mg/ml and an NADH concentration of 1.0 mM. Samples of MalNet-modified enzyme were dialyzed against 50 mM Na phosphate buffer of the appropriate pH and the enzymatic activity was observed upon addition of NADH at pH 7.5 (●) and pH 5.0 (●).

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**Fig. 5.** First order kinetics of NADH-induced activity regain in MalNet-modified mitochondrial malate dehydrogenase at pH 7.5. The data shown are taken from Fig. 4. SA$_f$ is the final specific activity and SA$_0$ is the initial specific activity.
separation of the catalytic and cofactor binding domains. If these domains were separated in the pH 5.0 conformation of the enzyme, the result would necessarily be a loss of enzymatic activity. In support of the above model is the observation that the cofactor binding domain has apparently remained largely intact in the monomeric, pH 5.0 conformation since NADH does bind to the native monomer and to MalNet-modified enzyme at pH 5.0. The modification of a cysteine residue by N-ethylmaleimide at pH 5.0 may inactivate the enzyme by sterically preventing the catalytic and cofactor binding domains from becoming adjacent at pH 7.5. However, the regain of enzymatic activity observed in Fig. 4 does suggest that NADH may induce a conformation of the enzyme at pH 7.5 which at least partially overcomes any such steric constraint.

Although much insight into the tertiary structure of mitochondrial malate dehydrogenase has been obtained from the characterization of the pH-dependent dissociation, very little is yet known about the actual subunit contact areas of this enzyme. The cysteine residue modified by MalNet is apparently exposed upon subunit dissociation, but this residue does not necessarily reside at the subunit interface. Rather, the MalNet-sensitive sulfhydryl group could be buried in the tertiary structure of the enzyme. A more complete understanding of the role of subunit interactions in this enzyme will require the identification of residues at the subunit contact area and the determination of their relationship to the enzymatic active site. An investigation of the residues at the subunit interface of mitochondrial malate dehydrogenase is presently underway in this laboratory.

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