Lienhard, G. E., and Wang, T.-C. (1968), *J. Am. Chem. Soc. 90*, 3781.
Matsukawa, T., and Yurugi, S. (1951a), *J. Pharm. Soc. Japan 71*, 1423.
Matsukawa, T., and Yurugi, S. (1951b), *J. Pharm. Soc. Japan 71*, 1450.
Matsukawa, T., and Yurugi, S. (1952), *J. Pharm. Soc. Japan 72*, 33.
Murata, K. (1965), *in* Review of Japanese Literature on Berberi and Thiamine, Shimazono, N., and Katsura, E., Ed., Tokyo, Igaku Shoin Ltd., p 220.
Osborne, A. R., Schofield, K., and Short, L. N. (1956), *J. Chem. Soc.*, 4191.
Pearson, R. G., Sobel, H., and Songstad, J. (1968), *J. Am. Chem. Soc. 90*, 319.
Raffauf, R. F. (1950), *Helv. Chim. Acta 33*, 102.
Stein, G. A., Sampson, W. L., Cline, J. K., and Stevens, J. R. (1941), *J. Am. Chem. Soc. 63*, 2061.
Streitweiser, Jr., A. (1956), *Chem. Rev. 56*, 571.
Wittliff, J. L., and Airth, R. L. (1968), *Biochemistry 7*, 736.

Isozymes of Rat Liver Mitochondrial Malate Dehydrogenase. Evidence for the Existence of Nonidentical Subunits*

K. G. Mann† and C. S. Vestling

ABSTRACT: The isozymes of rat liver mitochondrial malate dehydrogenase have been partially resolved by carboxymethyl-Sephadex chromatography. The isolated isozymes have been studied by the techniques of reversible acid dissociation and two-dimensional tryptic fingerprinting. In addition, the purified isozyme mixture has been studied by acrylamide gel electrophoresis under dissociating conditions. The results obtained from these studies indicate that rat liver mitochondrial malate dehydrogenase is composed of two nonidentical subunits.

Rat liver mitochondrial malate dehydrogenase (hereafter referred to as mitochondrial enzyme) has a native molecular weight of 66,300, and is composed of two subunits of similar or identical molecular weight (Mann and Vestling, 1969). Either crude extracts or highly purified samples of the rat liver enzyme can be resolved into five catalytically active forms by the technique of starch gel electrophoresis (Mann and Vestling, 1968). Similar observations of multiple catalytic forms of the mitochondrial enzyme have been reported for a number of species (Grimm and Doherty, 1961; Thorne *et al.*, 1963). The isozymes of mitochondrial enzyme have been given the letter designations A, B, C, D, E, beginning with the most cationic isoenzyme (Kitto *et al.*, 1966a). In the rat liver mitochondrial isozyme series A, B, and C account for about 90% of the total enzymatic activity.

Kitto *et al.* (1966b) have reported studies conducted on the separated isozymes of chicken heart mitochondrial enzyme which suggest that the mitochondrial isozymes are conformational isomers of the same polypeptide chain(s). However, Schecter and Epstein (1968) have reported reversible denaturation studies conducted on the partially resolved chicken heart isozymes which are inconsistent with the "conformer" hypothesis of Kitto *et al.* (1966a,b). A previous communication from our laboratory (Mann and Vestling, 1968) reported reversible acid dissociation studies conducted on partially resolved samples of the rat liver mitochondrial isozymes, A, B, and C, which suggested that isozymes A and C were homodimers of the types XX and YY, while isozyme B was a hybrid dimer of type XY.

We wish to report additional data which support our previous hybrid dimer hypothesis. These data involve acid hybridization, subunit electrophoresis, and fingerprint studies conducted on the partially resolved principal isozymes, mitochondrial enzymes A, B, and C from rat liver.

Materials and Methods

Rat liver mitochondrial enzyme was purified by the method of Sophianopoulos and Vestling (1962) from frozen livers supplied by Pentex. L-(1-Tosylamido-2-phenyl)ethyl chloromethyl ketone trypsin was purchased from Worthington Biochemicals Inc.

CM-Sephadex was purchased from Pharmacia Fine Chemicals and purified as follows. The resin was allowed to swell in distilled water for 24 hr. The slurry was filtered and suspended in three volumes of 0.5 N HCl for 1 hr. The suspension was then filtered and washed with distilled water until free of chloride ion. The resin in the H^+ form was then suspended in 0.5 N NaOH for 1 hr, filtered, and washed with distilled water

* From the Department of Biochemistry, University of Iowa, Iowa City, Iowa 52240. *Received March 30, 1970.* Supported by a grant from the National Institutes of Health (CA 07617), and by a National Aeronautics Space Administration traineeship and a predoctoral fellowship award from the U. S. Public Health Service (A-F1-GM-34,119) to K. G. M. Inquiries concerning this report should be directed to C. S. V.

† This paper includes portions of the thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, University of Iowa, August 1967. Present address: Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55101.

until the pH was approximately 9. The resin was then suspended in 0.02 M sodium citrate, pH 6.0, and titrated to pH 6.0 with 1 M citric acid. The slurry was then washed with 0.02 M sodium citrate, pH 6.0, on a sintered-glass funnel and resuspended in the same buffer and the titration process repeated. These titration and washing procedures were repeated until the pH remained constant for 12 hr. (Poor separations were obtained with unpurified resin.)

Hydrolyzed starch for gel electrophoresis was purchased from Connaught Medical Laboratories, University of Toronto, Canada. Acrylamide, *N,N'*-bisacrylamide, and *N,N,N',N'*-tetramethylenediamine and 2-mercaptoethanol were purchased from Eastman. Urea was purchased from the Fisher Scientific Co. Phenazine methosulfate and nitroblue tetrazolium chloride were purchased from the Aldrich Chemical Co.

Chromatography. CM-Sephadex chromatography was performed in a 2.5 × 45 cm Pharmacia column. The equilibrated resin was packed to a bed height of approximately 42 cm (225 ml of resin). After packing, the column was washed for 12 hr with 0.02 M sodium citrate–0.002 M 2-mercaptoethanol, pH 6.0. An amount of purified mitochondrial enzyme (20–30 mg) in the same buffer was applied to the top of the column. The enzyme was eluted by a pH gradient established by the presence of 1 l. of 0.02 M sodium citrate–0.002 M 2-mercaptoethanol, pH 6.0, in the closed mixing flask, and 1 l. of 0.02 M sodium citrate–0.002 M 2-mercaptoethanol, pH 7.0 in the open reservoir. Fractions between 5 and 10 ml were collected by drop counting.

Enzyme Assays. Measurements of enzyme activity were performed spectrophotometrically from the malate side of the equilibrium using the assay method described by Sophianopoulos and Vestling (1962).

Electrophoresis. Starch gel electrophoresis was performed by a modification of the method described by Fine and Costello (1963). Starch gels (14%) were prepared using hydrolyzed starch dispersed in 0.005 M sodium citrate, pH 6.0. Samples were placed in 1-mm cylindrical wells in the cast gels (a technique suggested by Dr. John R. Paulsrud). Electrophoresis was routinely carried out (4°) for 18 hr at a voltage of 8.6 V/cm and a current of 30 mA in 21 × 8.3 × 1 cm Plexiglas trays. After electrophoresis was completed, the gels were sectioned longitudinally into two or three slabs and stained for catalytic activity using the NAD^+-linked nitroblue tetrazolium method of Dewey and Conklin (1960), as described by Fine and Costello (1963).

A comparison of starch gel electrophoretograms of the purified enzyme, stained for catalytic activity using the nitroblue tetrazolium chloride method, and for protein using either of the dyes, Amido Black or ponceau S, revealed only the same protein components.

Polyacrylamide disc electrophoresis was performed according to methods described by Panyim and Chalkley (1969) with the use of 7.5% acrylamide gels prepared in 0.9 M acetic acid–6.25 M urea, pH 3.2. Electrophoresis was carried out for 2 hr at a current of 3 mA/tube. Following electrophoresis, the acrylamide gels were stained for protein using Amido Black.

Hybridization. Reversible acid dissociation of isozyme mixtures was performed by a technique previously described (Mann and Vestling, 1968, 1969).

Fingerprint Studies. Fingerprints of the separated isozymes were carried out using the method of Katz *et al.* (1959). Samples were dialyzed exhaustively *vs.* 0.001 M HCl–0.1 M 2-mercaptoethanol and were heat denatured by bringing the temperature to 100°. The denaturated enzyme solution was brought to 0.1 M with respect to NH_4HCO_3, and incubated for 12 hr at 37° with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone trypsin. The hydrolysates were then lyophilized, and the dry hydrolysate was taken up in distilled water, and from 1 to 3 mg was spotted on a full sheet of Whatman No. 3MM filter paper. Descending chromatography was carried out for 24 hr in butanol–acetic acid–water (4:1:5, v/v). This was followed by high-voltage electrophoresis for 1 hr at 3200 V (~250 mA) in a Gilson Medical Electronics high-voltage electrophoresis instrument. The buffer used was pyridine–acetic acid–water (1:10:289, v/v), pH 3.7. Peptides were visualized by a ninhydrin spray.

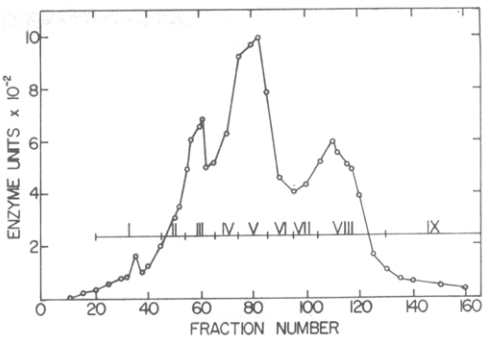

FIGURE 1: Chromatography profile for rat liver mitochondrial enzyme on CM-Sephadex. Purified mitochondrial enzyme (30 mg) was applied to column (see text), and 10-ml fractions were collected.

Results

Separation of the Mitochondrial Isozymes. The chromatography profile for an experiment in which purified rat liver mitochondrial enzyme (30 mg) was applied to a CM-Sephadex column is shown in Figure 1. Three overlapping peaks of enzymatic activity are clearly discernible. The fractions from the column (10 ml) were pooled as indicated by the spaced Roman numeral designations and concentrated by ultrafiltra-

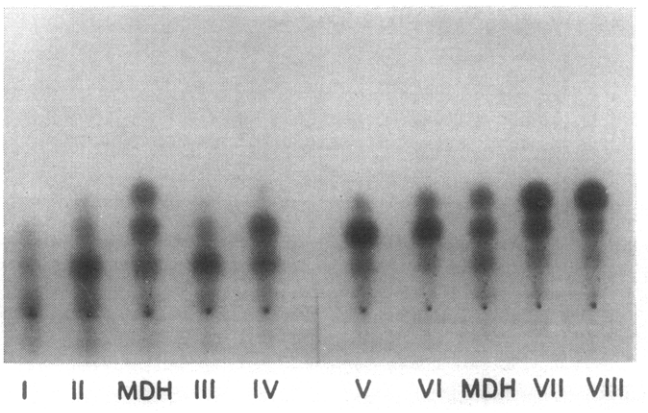

FIGURE 2: Starch gel electrophoretogram showing isozyme patterns of fractions obtained from column of Figure 1. (For details, see text.) Cathode at top.

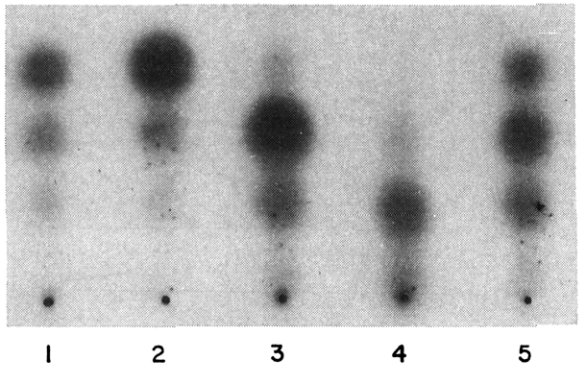

FIGURE 3: Starch gel electrophoretogram showing patterns (left to right) of (1) crude rat liver mitochondrial enzyme; (2) mitochondrial enzyme A; (3) mitochondrial enzyme B; (4) mitochondrial enzyme C; (5) purified mitochondrial enzyme.

tion. The concentrated fractions were then analyzed for their isozyme composition by electrophoresis. A photograph of the electrophoretogram which shows the relative isozyme distribution in each pool is shown in Figure 2. The samples used in this study were obtained from pools II and III (mitochondrial enzyme C), pool V (mitochondrial enzyme B), and pool VIII (mitochondrial enzyme A).

In general, neither purification of the enzyme nor separation

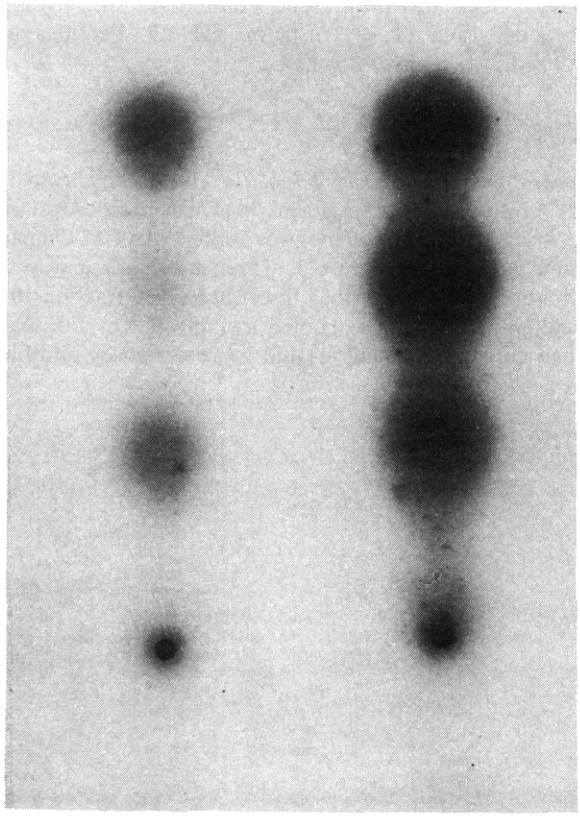

FIGURE 4: Starch gel electrophoretogram. Left-hand pattern: mixture of mitochondrial isozymes A and C. Right-hand pattern: mixture of isozymes A and C after reversible acid dissociation. (Total mitochondrial enzyme in right-hand pattern several times that in left-hand pattern.)

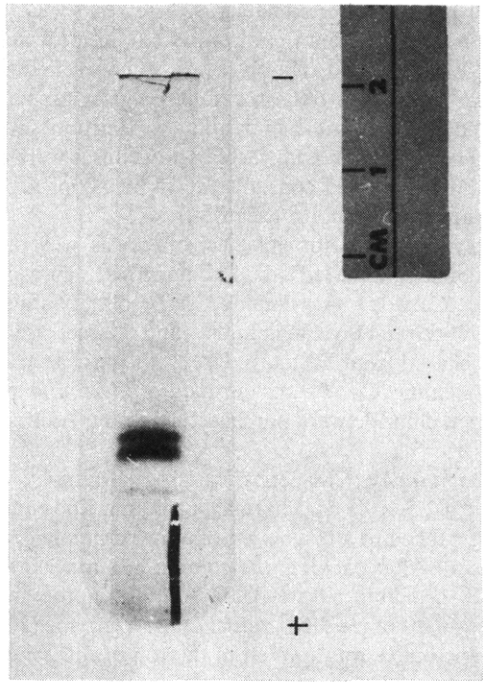

FIGURE 5: Polyacrylamide disc gel electrophoretogram of purified mitochondrial enzyme in 0.9 M acetic acid–6.25 M urea, pH 3.2.

of the isozymes altered the electrophoretic mobility of the three principal mitochondrial isozymes. This fact is evident in Figure 3, in which mitochondrial enzyme crude extract is compared with the purified enzyme, and the three partially resolved isozyme samples obtained from the column of Figure 1. It can be seen, however, that purification of the enzyme does alter the relative distribution of the mitochondrial isozymes.

Hybridization Experiments. In a previous communication (Mann and Vestling, 1968) it was reported that reversible acid dissociation of mitochondrial isozyme B led to the formation of isozymes A and C. In view of these previous experiments, it was decided to attempt to test the hybrid hypothesis in the alternate fashion that is, to prepare isozyme B from a mixture of the parent homodimers, mitochondrial enzymes A and C.

A mixture containing approximately equal amounts of mitochondrial isozymes A and C was subjected to reversible dissociation at pH 2.0 and reassociated by neutralization with 0.5 M sodium citrate–0.1 M 2-mercaptoethanol, pH 7.0. In this experiment, 64% of the initial enzymatic activity was recovered. The products obtained from reversible dissociation of the mitochondrial A–C mixture, together with the appropriate control are shown in Figure 4. The relative amount of mitochondrial B is seen to have increased considerably. Several times as much total enzyme was applied in the case of the right-hand pattern. This result is consistent with our previous interpretation of the mitochondrial isozyme system. This hypothesis suggested that isozymes A and C were homodimers, while B was hybrid dimer. Dissociation of a mixture of the two homodimers A (XX) and C (YY) gives rise on reassociation to the hybrid dimer B (XY) in addition to the original homodimers. Since previous studies have shown that isozymes A and C give better recoveries of enzymatic activity following reversible acid dissociation than a mixture of mitochondrial isozymes (Mann and Vestling, 1968), it is difficult to

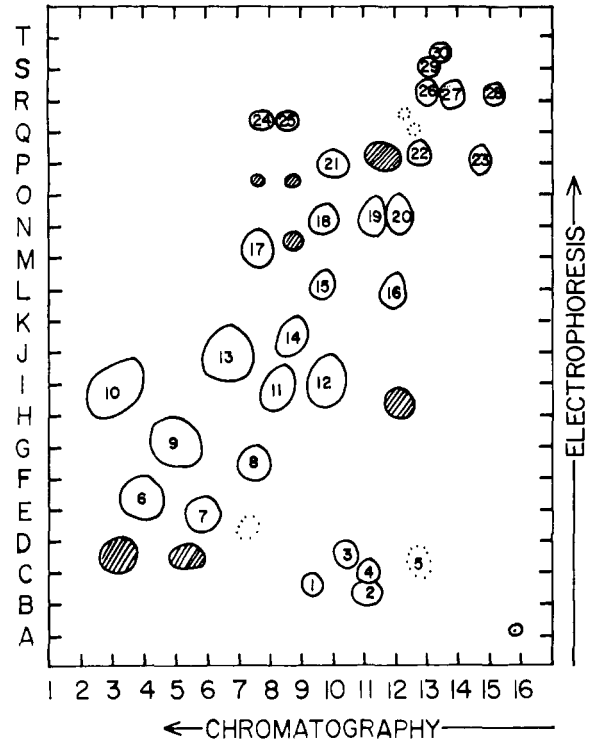

FIGURE 6: Drawing of a two-dimensional L-(1-toslyamido-2 phenyl)-ethyl chloromethyl ketone trypsin fingerprint of mitochondrial enzyme.

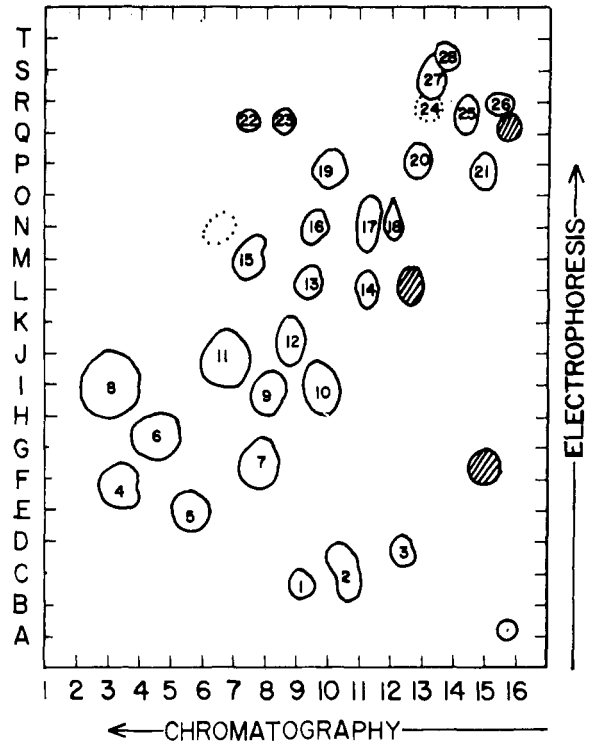

FIGURE 7: Drawing of a two-dimensional L-(1-toslyamido-2 phenyl)-ethyl chloromethyl ketone trypsin fingerprint of mitochondrial enzyme C.

interpret the observed data in terms of a selective inactivation of mitochondrial isozymes A and C.

Electrophoresis under Dissociating Conditions. Polyacrylamide disc electrophoresis was performed on the purified mitochondrial isozyme mixture in gels prepared in 6.25 M urea–0.9 M acetic acid, pH 3.2. Figure 5 is a photograph of the resulting electrophoresis pattern obtained under these dissociating conditions. In this experiment, 50 μg of purified mitochondrial enzyme was applied to the polyacrylamide column. It can be seen that the dissociated enzyme is resolved into two bands of approximately equal intensity. In addition, several faint bands are discernible, which probably indicate a small amount of contaminating protein.

This result is consistent with the hypothesis that the mitochondrial isozyme system is the result of combination of two types of polypeptide chains (X and Y) to form dimer molecules of the types XX, XY and YY.

Fingerprint Studies. Two-dimensional fingerprinting studies have been carried out on the purified mitochondrial isozyme mixture, and mitochondrial isozymes A and C. Amino acid analysis of the purified mitochondrial isozyme mixture (Burck, 1962) indicates that mitochondrial enzyme contains 49 arginine residues and 18 lysine residues per unit of mol wt 66,000. On this basis, one would predict 67 sites for trypsin cleavage. If the two isozyme subunits were identical, 34–35 peptides should be produced. Peptide maps of the purified mitochondrial enzyme mixture revealed 39–43 peptides, while similar maps of mitochondrial enzymes A and C gave 36–40, and 30–32 peptides, respectively. Drawings of the tryptic fingerprints for mitochondrial enzymes A and C are shown in Figures 6 and 7. It is apparent that the fingerprints for both isozymes are very similar. Peptides which are not identical are shaded. Prominent features of these maps are the tetrad centered about coordinate J,8, and the doublets at coordinates N,12 and 0.8. Table I gives the coordinates of the peptides which are considered to be identical.

All of the shaded (nonidentical) peptides were clearly visible in the fingerprints of mitochondrial enzymes A and C, and all appeared in the fingerprint of the mitochondrial isozyme mixture. With the exception of the shaded peptides, the maps for isozymes A and C are homologous. This indicates that although the two mitochondrial enzyme subunits are nonidentical, they have a good deal of peptide sequence in common.

Discussion

The results reported here support the mitochondrial enzyme hybrid dimer hypothesis. The hybridization and reversible dissociation studies reported here and in a previous communication indicate that mitochondrial enzyme B is a hybrid dimer composed of one X type and one Y type subunit, while mitochondrial enzymes A and C are homodimers composed of X type and Y type subunits, respectively. This conclusion is supported by the results obtained on electrophoresis under dissociating conditions which indicated two components of unlike charge, and fingerprint studies which indicate the existence of dissimilar peptide sequences in mitrochondrial enzymes A and C.

The results from the fingerprint studies, however, do indicate that X and Y chains have a great many sequences in common. This observation is in agreement with the results reported by Devenyi *et al.* (1966). These authors reported

TABLE I: Identical Peptides of Mitochondrial Enzymes A and C.

Peptide		Coordinates	
A	C	A	C
1	1	B9	B9
2		B11	
3	2	C10	C10
4		C11	
5	3	C13	C12
6	4	E4	F3
7	5	E6	E6
8	6	F7	F8
9	7	G5	G5
10	8	I3	I3
11	9	I8	I8
12	10	I10	I10
13	11	J7	J7
14	12	K9	J9
15	13	L10	L9
16	14	L12	L11
17	15	M8	M7
18	16	N10	N10
19	17	N11	N11
20	18	N12	N12
21	19	P10	P10
22	20	P13	P13
23	21	P15	P15
24	22	Q8	Q7
25	23	Q9	Q8
26	24	R13	R13
27	25	R14	R14
28	26	R15	R15
29	27	S13	S13
30	28	S14	S14

that commercial samples of pig heart mitochondrial enzyme, purified by the method of Thorne (1960) gave 36–38 peptides on tryptic hydrolysis. Devenyi and coworkers also suggested that two C-terminal peptides were resolved from the tryptic hydrolysates. Thorne *et al.* (1963) have shown that the mitochondrial enzyme prepared by the techniques used by Devenyi *et al.* is a mixture of isozymes. Thus the results reported by Devenyi *et al.* are in good agreement with our data.

The possibility that the results obtained by electrophoresis under dissociating conditions are due to approximately 50% contamination by another protein appears to be remote. Rat liver mitochondrial enzyme purified by the method of Sophianopoulos and Vestling is apparently homogeneous on sedimentation, diffusion, free-boundary electrophoresis, (Sophianopoulos and Vestling, 1962; Burck, 1962), and in high-speed sedimentation equilibrium experiments (Mann and Vestling, 1969). Thus purified rat liver mitochondrial enzyme preparations are homogeneous with respect to malate dehydrogenase activity (Mann, 1967). It appears that the possibility of a large amount of contamination by extraneous protein is excluded. The best interpretation of the electrophoresis pattern observed under dissociating conditions, therefore, is that there are subunits of different charge in the protein.

In a recent report (Kitto *et al.*, 1970) the matter of the "conformer" hypothesis for chicken heart mitochondrial isozymes was considered again, partly in view of the report by Schechter and Epstein (1968). Kitto *et al.* reported that two of the chicken heart mitochondrial isozymes (A and E) show electrophoretic interconversion (E → A) following reversible acid or guanidine hydrochloride denaturation, but that the renatured isoyzmes differ markedly with respect to thermal stability. Schechter and Epstein were unable to detect changes in electrophoretic mobility when partially resolved chicken heart mitochondrial isozymes were subjected to reversible denaturation by either guanidine hydrochloride or acid treatment. Our results with rat liver mitochondrial isozymes do not show apparent electrophoretic interconversion of isozymes A and C following reversible acid dissociation but do show interconversion when isozyme B or a mixture of isozymes A and C are so treated.

The nature of the differences between the mitochondrial enzyme subunits can only be discussed speculatively. The fingerprint data suggest that two genes are responsible for the formation of mitochondrial enzyme polypeptide chains. The chains of which mitochondrial enzyme A is composed appear to have a higher content of the basic amino acids. The possibility that some lysines in mitochondrial enzyme C might be acetylated, and thus blocked to trypsin cleavage is excluded by the studies of Stegink (1967), who has shown that rat liver mitochondrial enzyme contains no covalently bound acetyl groups.

Acknowledgments

The authors would like to thank Mr. Sakol Panyim for his assistance with the disc electrophoresis experiments, Dr. Thomas Lindell for his assistance in performing the fingerprinting experiments, and Mr. Anthony G. Sobin for technical assistance.

References

Burck, P. J. (1962), Ph.D. Thesis, University of Illinois, Urbana, Illinois.

Carpenter, F. H. (1967), *Methods Enzymol. 11*, 237.

Devenyi, T., Rogers, S. J., and Wolfe, R. G. (1966), *Nature 210*, 489.

Dewey, M. M., and Conklin, J. L. (1960), *Proc. Soc. Exp. Biol. Med. 105*, 492.

Fine, J., and Costello, L. A. (1963), *Methods Enzymol. 6*, 958.

Grimm, F. C., and Doherty, D. G. (1961), *J. Biol. Chem. 236*, 1980.

Katz, A. M., Dreyer, W. J., and Anfinsen, C. B. (1959), *J. Biol. Chem. 234*, 2897.

Kitto, G. B., Stolzenbach, F. E., and Kaplan, N. O. (1970), *Biochem. Biophys. Res. Commun. 38*, 31.

Kitto, G. B., Wassarman, P. M., and Kaplan, N. O. (1966b), *Proc. Natl. Acad. Sci. U. S. 56*, 578.

Kitto, G. B., Wassarman, P. M., Michjeda, J., and Kaplan, N. O. (1966a), *Biochem. Biophys. Res. Commun. 22*, 75.

Mann, K. G. (1967), Ph.D. Thesis, University of Iowa, Iowa City, Iowa.

Mann, K. G., and Vestling, C. S. (1968), *Biochim. Biophys. Acta 159*, 567.
Mann, K. G., and Vestling, C. S. (1969), *Biochemistry 8*, 1105.
Panyim, S., and Chalkley, R. (1969), *Arch. Biochem. Biophys. 130*, 337.
Schechter, A. N., and Epstein, C. J. (1968), *Science 159*, 997.
Sophianopoulos, A. J., and Vestling, C. S. (1962), *Biochem. Prepn. 9*, 102.
Stegink, L. D. (1967), *Anal. Biochem. 20*, 502.
Thorne, C. J. R. (1960), *Biochim. Biophys. Acta 42*, 175.
Thorne, C. J. R., Grossman, L. I., and Kaplan, N. O. (1963), *Biochim. Biophys. Acta 73*, 193.

Syncatalytic Modification of a Functional Tyrosyl Residue in Aspartate Aminotransferase*

Philipp Christen† and James F. Riordan

ABSTRACT: Chemical modification of enzymes in the presence of substrate, a procedure generally intended to *prevent* reaction of functional amino acid residues, has now been employed to *promote* the reactivity of certain residues of aspartate aminotransferase during the transamination reaction. In the presence of the substrate pair, glutamate and α-ketoglutarate, tetranitromethane abolishes the activity of this enzyme within 1 hr, concomitant with the nitration of one tyrosyl residue. However, in the absence of substrate, neither the pyridoxal 5′-phosphate nor the pyridoxamine 5′-phosphate form of the enzyme is inactivated. In the presence of competitive inhibitors, or of either substrate alone, only slight inactivation occurs. The inactive nitroenzyme is found to be in the pyridoxamine 5′-phosphate form, suggesting that nitration probably occurs during or after the transition of the enzyme–substrate complex from the aldimine to the ketimine intermediate. The quasi-substrate, α-methylaspartate, which does not form a ketimine, does not induce rapid inactivation, thus supporting this view. The susceptibility of the functional tyrosyl residue toward nitration is greatly enhanced, during the catalytic process, relative to that of model compounds seemingly reflecting a short-lived change in its environment. This *syncatalytic*, *i.e.*, synchronous with the catalytic process, activation would appear to be related to the role of this tyrosyl residue in the mechanism of transamination.

Current theories of enzyme action emphasize the importance of transient conformational changes to the overall catalytic process (Koshland and Neet, 1968; Eigen and Hammes, 1963; Hammes, 1968; Ivanov and Karpeisky, 1969). Such changes are thought to facilitate intramolecular interactions between functional groups of the enzyme, to properly align them with groups of the substrate, to induce bond strain, and to underlie allosteric regulation. These theories have been supported by considerable indirect and direct physicochemical evidence, though the latter in an as yet limited number of systems.

We have now found chemical indications that conformational changes of aspartate aminotransferase occur during the transamination reaction. A functional tyrosyl residue, unreactive in the absence of substrates, becomes unusually susceptible to chemical modification when *transamination is actually proceeding*. This change in chemical properties synchronous with catalysis, *i.e.*, syncatalytic,[1] has been detected by reaction with tetranitromethane. The increased reactivity of the essential tyrosyl residue appears only during a particular segment of the catalytic pathway, *i.e.*, during or after the transition from the aldimine to the ketimine, and likely reflects its role in the mechanism of action of the enzyme. A preliminary report of these investigations has been presented (Christen and Riordan, 1969).

Materials

Aspartate aminotransferase (EC 2.6.1.1., cytoplasmic enzyme from pig heart) prepared according to Jenkins *et al.* (1959) was obtained from Boehringer Mannheim Corp. (batch 6308221) as a suspension in 3 M ammonium sulfate–0.05 M maleate–0.0025 M α-ketoglutarate. The specific activity was 440 μ/mg (μmoles of oxalacetate per min per mg) when assayed as described below. Malate dehydrogenase and α-ketoglutaric acid were purchased from Worthington Biochemical Corp.; NADH, L-aspartic acid, pyridoxal 5′-phos-

* From the Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and the Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts. *Received March 4, 1970.* This work was aided by Grant-in-Aid GM-15003 from the National Institutes of Health of the Department of Health, Education, and Welfare.

† Recipient of a grant of the Stiftung für Biologisch-Medizinische Stipendien der Schweizerischen Akademie der Medizinischen Wissenschaften, the University of Zürich, and the American-Swiss Foundation for Scientific Exchange. Present address: Biochemistry Institute, University of Zürich, Switzerland.

[1] The term "syncatalytic" is employed to refer to those events which occur *while catalysis is actually proceeding*, *i.e.*, synchronous with the catalytic process.