AGAR GEL ELECTROPHORETIC DEMONSTRATION OF CHARGE ALTERATION IN MUTANT BACTERIAL PROTEINS*

C.R. Roe[1], K.S. You[2,++] and N.O. Kaplan[2,++]

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SUMMARY

A mutant of Pseudomonas testosteroni (designated as STDH-m) isolated by Teller and Bongiovanni (1963) is characterized by a highly specific 3-β-A/B trans-hydroxysteroid dehydrogenase. Agar gel electrophoretic comparisons of the hydroxysteroid dehydrogenases revealed an anodal change in charge in the mutant enzyme. Numerous other enzymes of the mutant when compared with wild type Ps. testosteroni enzymes were found to be similarly altered in charge. It was also found that all mutant proteins are anodal while the parent organism and a revertant (designated as M_3) from the mutant have both anodal and cathodal proteins. These protein alterations are associated with a marked alteration in the buoyant density of the mutant DNA.

Pseudomonas testosteroni is known to contain a number of steroid dehydrogenases (Hurlock and Talalay, 1957; Delin et al. (1964); Squire et al. (1964); Boyer et al. (1965)). A presumed mutant of this organism was found not to oxidize testosterone and is lacking in 17 β-hydroxysteroid dehydrogenase activity (Teller and Bongiovanni, 1963). This communication compares enzyme

1 Duke University Medical School, Division of Metabolism, Department of Pediatrics, Durham, North Carolina 27706

2 Brandeis University, Graduate Department of Biochemistry, Waltham, Massachusetts 02154

++ Present Address: Department of Chemistry, University of California, San Diego, La Jolla, California 92037

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differences in the mutant and wild type organisms as well as a revertant.

EXPERIMENTAL PROCEDURES

The Ps. testosteroni strains and STDH-m were grown in an identical medium to that described by Marcus and Talalay (1956). An overnight inoculum was transferred to a 10-fold volume of the same medium. Testosterone (0.4 gm/liter of medium) was added 4 to 6 hours after transfer for induction of steroid dehydrogenase activity. The partial revertant, M_3, has been obtained routinely after 20 hours fermentation of the mutant in an identical medium in the presence of testosterone (Roe, C.R. and You, K.S., in prep.). Testosterone was found not to influence either induction of enzymes other than steroid dehydrogenases, or bacterial growth rate. Therefore, the steroid could be omitted from cultures used for evaluation of other enzymes. The cells were harvested by centrifugation, washed in 0.05 M potassium phosphate buffer pH 7.0 (containing 20 percent glycerol) and recentrifuged. The washed cells were then converted into an acetone powder and extracted with the same buffer. After dialysis the extracts were examined by agar gel electrophoresis.

The agar gel electrophoresis method is essentially that of Nerenberg (1966). A boiled solution of 1 percent Ionagar and 1.5 percent hydrolyzed starch in 0.02 M veronal buffer pH 8.6 was poured on 3-1/4 x 4 inch glass plates. The electrode chambers contained 0.06 M veronal buffer pH 8.6. The electrophoresis was carried out for 40 to 60 minutes, depending on the mobility of a given protein at 35 ma. and 200 volts. The gels were stained for steroid dehydrogenase by using the following mixture: 42 ml. of 0.06 M sodium phosphate buffer pH 8.0, 1.2 ml. of DPN (10 mg/ml), 0.6 ml. of phenazine methosulfate (5 mg/ml), 2.4 ml. of Nitro BT (10 mg/ml, Mann Research Laboratories) and 2 ml. of steroid substrate (1 mg/ml) in methanol. 11-hydroxyandrosterone was the substrate for the detection of α-hydroxysteroid dehydrogenase activity and dehydroepiandrosterone (Mann Research Laboratories) indicated the β-hydroxysteroid dehydrogenase activity. Localization of the activity zone of malic dehydrogenase, isocitric dehydrogenase, and glutamic dehydrogenase are des-

cribed elsewhere (Fine and Costello, 1963).

A succinic dehydrogenase band was stained using the following mixture: 14.8 ml of 0.03 M potassium phosphate buffer pH 7.6, 0.7 ml of 0.5 M succinate (disodium salt), 0.5 ml of Nitro BT (10 mg/ml) and 1 ml of phenazine methosulfate (5 mg/ml). Amido black was used as 0.01 percent solution in 5 percent acetic acid for protein staining. Destaining of the gel was accomplished either electrophoretically, or by washing with slowly running tap water. The latter method gives the best result within a few hours.**

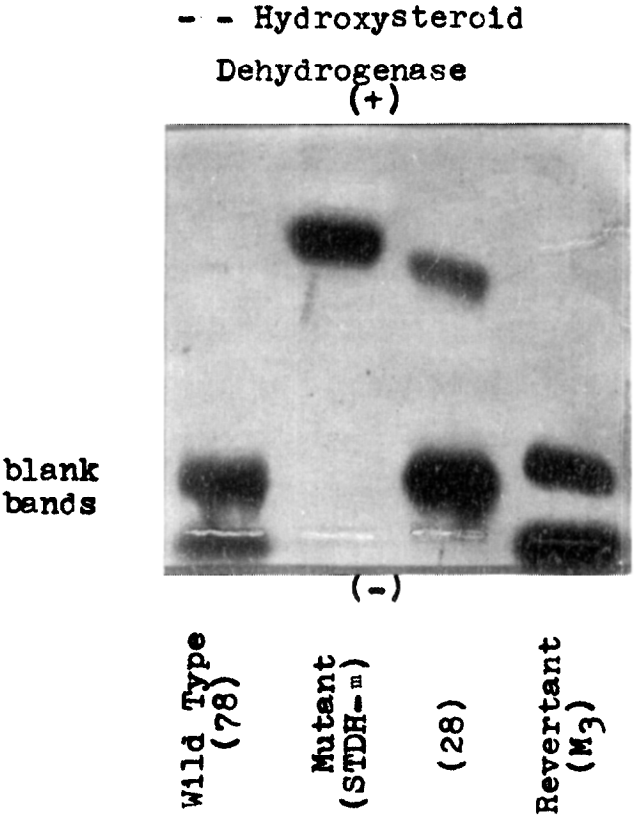

Fig. 1: Electrophoretic mobility comparison of 3-α-hydroxysteroid dehydrogenase activity from (left to right) Ps. testosteroni strain #78, STDH-m, strain #28, and M_3. The anode is at the top of all agar gel slides.

Blank bands (which were observed in the absence of Steroid) are DPN dependent. Their role is not understood.

** This report, to the best of our knowledge, is the first successful demonstration of the staining of succinic dehydrogenase. Mammalian succinic dehydrogenase (for example, bovine heart enzyme of Singer, Kearney and Bernath (1956) can be stained equally well by this method.

RESULTS

Extracts of the parent organism of Ps. testosteroni (ATCC #11996, Stanier strain #78) were compared with Stanier strain #28 (ATCC #17410), STDH-m and a partial revertant (M_3) obtained from the mutant during prolonged fermentation. The guanine-cytosine content of these organisms, determined by buoyant density in CsCl, is 60 to 61 moles percent (1.720 gm/cm^3) for Stanier strains and the partial revertant (Mandel, 1966: Roe and You, in prep.) while STDH-m DNA is 70 moles percent (1.728 gm/cm^3) (Roe and You,in prep.). Figure 1 demons-

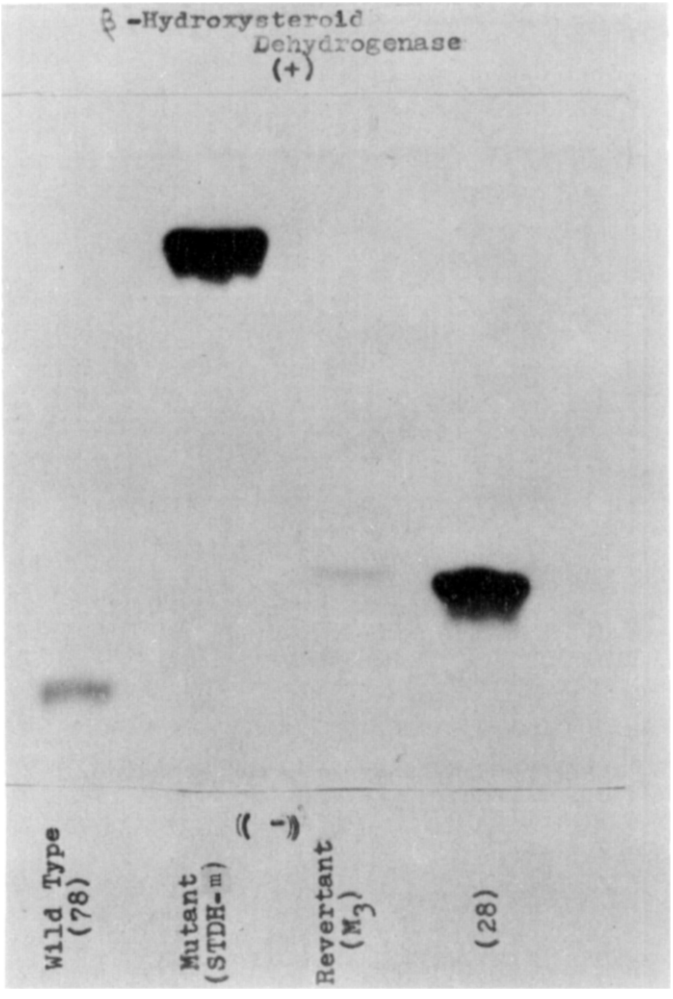

Fig. 2: Analysis of 3-β-hydroxysteroid dehydrogenase activity (left to right) strain #78, STDH-m, M_3, and strain #28.

trates the extreme difference in net charge of the α-hydroxysteroid dehydrogenase of the mutant, the Stanier strains, and M_3. Figure 2 illustrates the variation in charge of the β-hydroxysteroid dehydrogenases of these organisms. This comparison demonstrates the acidic nature of the mutant enzymes.

The substrate specificity of the mutant α and β enzymes has been altered (Teller and Bongiovanni, 1963: Roe and Kaplan, in prep.). Examination of electrophoretic mobilities of other enzymes were carried out because it was expected that the drastic alteration of DNA in the mutant, might carry a gross

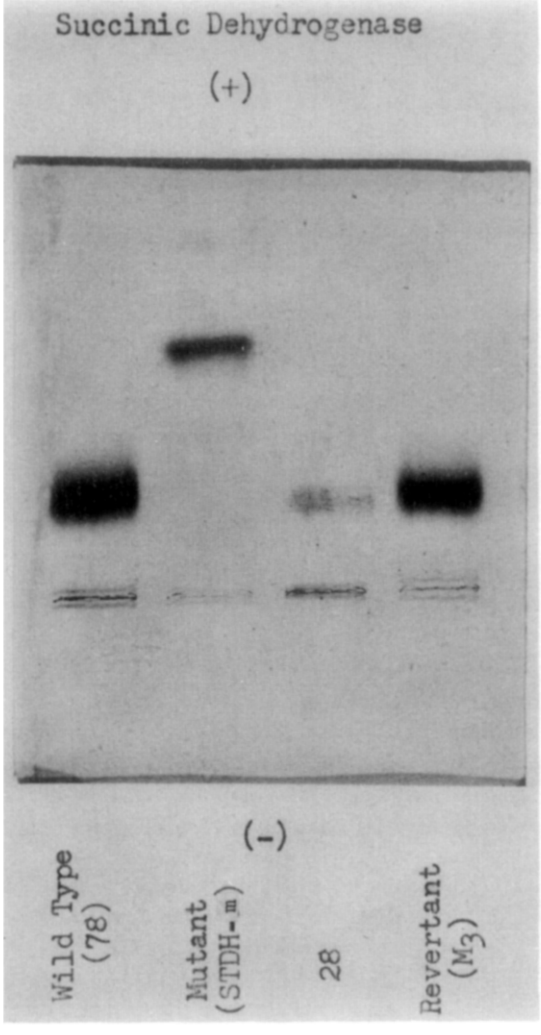

Fig. 3: Activity bands of succinic dehydrogenase (left to right). Same order as Fig. 1.

alteration in many other proteins other than steroid dehydrogenases. Several other enzymes were compared: succinic dehydrogenase (Fig. 3), malic dehydrogenase (Fig. 4), TPN dependent isocitric dehydrogenase (Fig. 5), and TPN dependent glutamic dehydrogenase (Fig. 6, STDH-m lacks this enzymatic activity). The mutant enzyme was always more acidic than the corresponding activities in the Stanier and M_3 strains. The enzymes of the Stanier strains and M_3 have marked similarities between them

Rather than compare additional enzymes, it was more desirable to scan the entire bulk protein which is present in the organisms. Dialyzed soluble extracts from the acetone powder of the three organisms were compared for total protein alteration. Figure 7 demonstrates the finding that all mutant

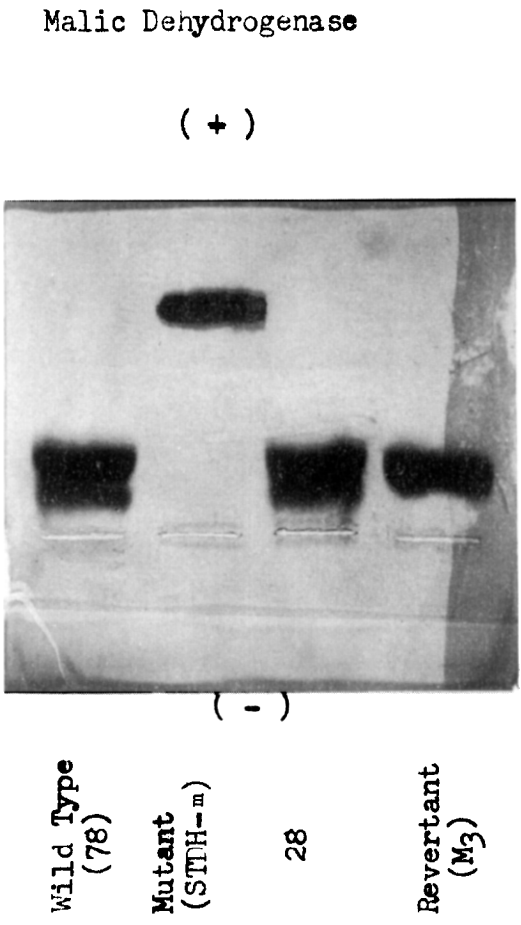

Fig. 4: Activity bands of Malic dehydrogenase (left to right). Same order as Fig. 1.

proteins are displaced to the anode and are acidic under the circumstances that electrophoresis is performed (pH 8.6, 0.06 M veronal buffer, 2-4°C). All of the known classified strains of Ps. testosteroni (Stanier, Palleroni, and Doudoroff, 1966), and the revertant M_3, were examined for total protein mobility on the agar gels. They exhibited very similar mobility patterns.

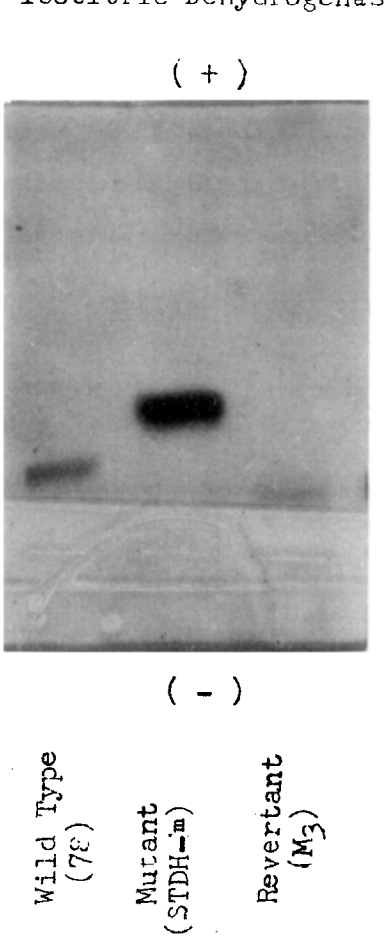

Fig. 5: Activity bands of TPN dependent isocitric dehydrogenase (left to right) strain #78, STDH-m, and M_3.

DISCUSSION

Agar gel electrophoresis depends almost exclusively on a charge separation having little if any molecular sieving effect (Wieme, 1965). This ad-

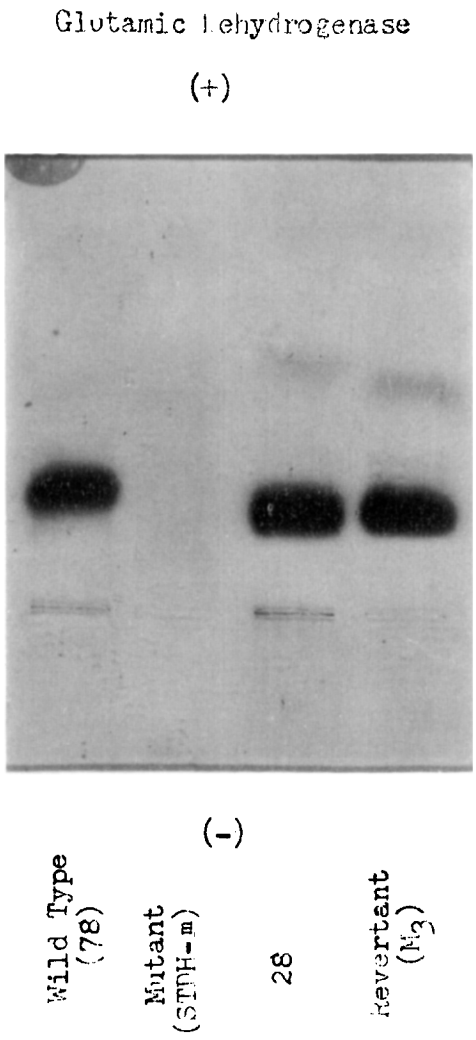

Fig. 6: Activity bands of TPN dependent glutamic dehydrogenase (left to right). Same order as Fig. 1 (STDH-m lacks this enzymatic activity).

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vantage renders the technique useful in evaluating protein migration. Gottlieb and Hepden (1966) found that there was more similarity in the mobility patterns (using acrylamide gel) between strains within one species, than between strains of different species in the system of Streptomyces. The sieving effect of acrylamide gels, however, prevent a conclusive evaluation of the Streptomyces strains. The usefulness of agar gel electrophoresis for the taxonomic evaluation of microorganisms, is demonstrated by the marked

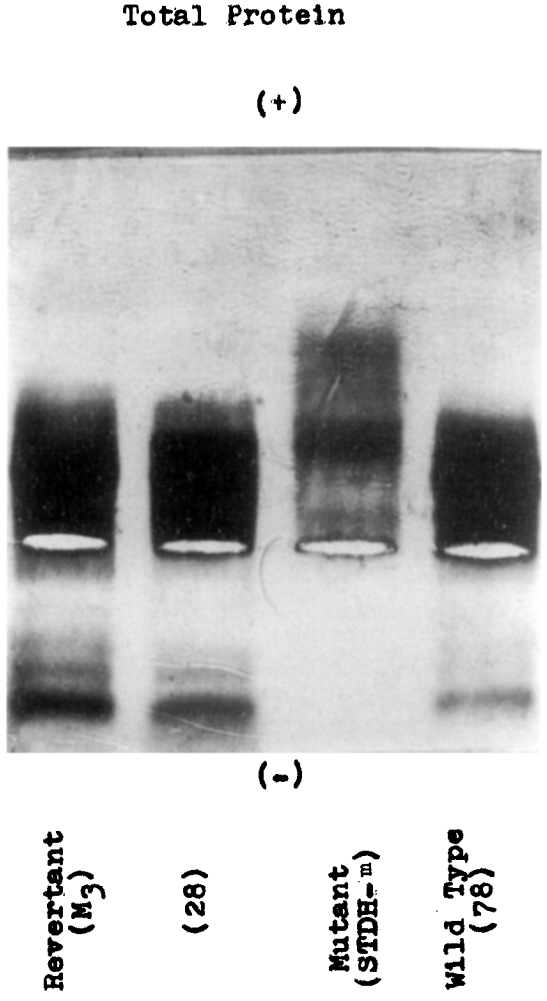

Fig. 7: Amido black stained agar gel of total soluble protein demonstrating the acidic nature of STDH-m at pH 8.6. From left to right: M_3, strain #28, STDH-m, and strain #78.

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similarities of protein banding patterns in the Ps. testosteroni system. All classified strains of Ps. testosteroni have similar banding patterns as typified by the Stanier strain #78, whereas there was a large difference between other species of Pseudomonas and Ps. testosteroni (for example, Ps. aeruginosa has most cathodal proteins right below the origin, whereas Ps. testosteroni has them far below the origin).

There are no known reports of an organism whose proteins are totally

acidic upon mutation with deletion of some enzymatic activities (17-β-hydroxy-steroid dehydrogenase [Teller and Bongiovanni, 1963] and glutamic dehydrogenase). The acidic shift in proteins appears to be a specific mutation related to altered DNA density (Roe and You, in prep.). No attempt has been made to classify the mutant, however, the exceptionally high G-C content, determined by the DNA buoyant density, would indicate that the organism is not a member of the classified genus of Pseudomonas (according to the criteria of Stanier, Palleroni and Doudoroff [1966]). Gross alteration in chemical composition of DNA in the mutant strongly indicates a great change in the covalent structure of a given protein, which is visibly revealed on the gel plates. Studies in comparative enzymology and association of protein alteration with DNA guanine-cytosine content variation among these organisms are continuing.

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