SHORT COMMUNICATIONS

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Relationship of L-alanine and L-glutamate dehydrogenases of *Bacillus thuringienses*

The molecular relationship between alanine dehydrogenase (L-alanine:NAD^+ oxidoreductase, EC 1.4.1.1) and glutamate dehydrogenase (L-glutamate:NAD^+ oxidoreductase (deaminating), EC 1.4.1.2) from mammalian sources is well-documented. Conformational changes and/or subunit associations brought about by small molecule allosteric effectors, such as malate[1] and GTP and ADP[2], can drastically alter the alanine dehydrogenase/glutamate dehydrogenase activity. In contrast, glutamate dehydrogenases isolated from some bacteria have been reported to be insensitive to allosteric effectors[3]. *Thiobacillus novellus* contains both an NAD^+- and an $NADP^+$-specific glutamate dehydrogenase; AMP produced sigmoid kinetics with the former enzyme but did not affect the latter[4]. Among the bacteria, Bacillus species are generally thought to have little or no glutamate dehydrogenase activity[5,6]. Glutamate dehydrogenase has been demonstrated in *Bacillus subtilis* and was shown not to respond to regulation by purine nucleotides[3]. Alanine dehydrogenase activity incorporated into the spores of *Bacillus cereus* strain T (ref. 5) and *B. subtilis*[7] has been implicated as a key to the germination mechanism. The alanine dehydrogenase from both *B. cereus*[5] and *B. subtilis*[8] has been highly purified. No evidence was obtained for the interconversion of the *B. cereus* alanine dehydrogenase into glutamate dehydrogenase[5].

We wish to communicate that *Bacillus thuringiensis* extracts have alanine dehydrogenase(NAD^+) and glutamate dehydrogenase(NAD^+) activities which show differential response to the nucleotides GTP and ADP and have a molecular weight ratio of approx. 3:4. *B. thuringiensis* var. *thuringiensis* Berliner was grown on a citrate–salts medium[9] supplemented with trace metals, 0.1% glucose and 0.033% casein amino acids. Cells were harvested by centrifugation and disrupted by sonication. Enzyme activity was assayed in the supernatant fluid remaining after centrifugation. The spectrophotometric assay conditions were as follows: 1.0 ml of 0.05 M phosphate buffer (pH 9.0), 0.5 ml of NAD (5.4 mg/ml), 0.5 ml of potassium L-glutamate or L-alanine (0.5 M, pH 9.0), 0.7 ml of water, and 0.3 ml of enzyme preparation. The rate of reaction was equal to the rate of increase in absorbance at 340 nm as measured with a Gilford Model 2000 Spectrophotometer. Protein was determined by a microbiuret procedure[10]. Electrophoresis on 7% polyacrylamide gels was carried out according to the procedure of DAVIS[11]. The gels were stained for dehydrogenase activities[12] using NAD^+, phenazine methosulfate, nitro-blue tetrazolium and 0.05 M (pH 8.5) L-glutamate or L-alanine as substrate. Two bands identical for both alanine dehydrogenase and glutamate dehydrogenase were revealed on the gels with migration rates of 0.28 and 0.41 relative to the bromophenol blue front. No bands were obtained when $NADP^+$ replaced the NAD^+. The front-running band contained more than 90% of

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the alanine dehydrogenase activity and less than 5% of the glutamate dehydrogenase activity, as estimated from a scan of the stained gels obtained with a Joyce–Loebl Chromoscan. The most prominent glutamate dehydrogenase band appeared considerably less active than the largest alanine dehydrogenase band. These bands were individually excised from unstained gel halves, and each was subjected to a second electrophoresis. Each band maintained its separate identity, indicating that these conditions did not favor subunit reassociation. The pattern neither changed throughout growth and sporulation on the medium described, when L-glutamate or L-alanine replaced glucose as the carbon source, nor when they replaced NH_4^+ as the nitrogen source.

Samples from glucose-grown cells harvested at the end of exponential growth had maximal activity for both dehydrogenases and were used for further study. A preparation with glutamate dehydrogenase specific activity of 206 nmoles/h per mg protein and alanine dehydrogenase specific activity of 427 showed values of 70 and 350 in the presence of 1.034 mM GTP, and values of 58 and 407 in the presence of 1.034 mM ADP, respectively. The alanine dehydrogenase, which had initially twice the activity of the GDH, was only inhibited 18% by GTP and 5% by ADP, whereas the GDH was inhibited 66% and 72%, respectively. Further characterization of the alanine dehydrogenase and glutamate dehydrogenase was made by use of the gel electrophoresis procedure of HEDRICK AND SMITH[13] which allows one to distinguish between a size-isomer family of proteins and a charge-isomer family. When the log of the protein mobility relative to the dye front is plotted *versus* polyacrylamide gel concentration, size isomeric proteins give a family of nonparallel lines extrapolating to a common point in the vicinity of 0% gel concentration; charge isomeric proteins give a parallel family of lines. Proteins differing in both charge and size give nonparallel lines which intersect at a point other than 0% gel concentration. The slopes of such plots were shown to be related to molecular weight in such a way that the molecular weight could be determined with an accuracy of $\pm 4\%$. We adapted the more favorable running pH gel system of DAVIS[11] to the procedure of HEDRICK AND SMITH[13] in the following way: modified Solution C contained 40 g acrylamide (recrystallized from chloroform) and 1.333 g *N,N'*-methylenebisacrylamide (recrystallized from acetone)

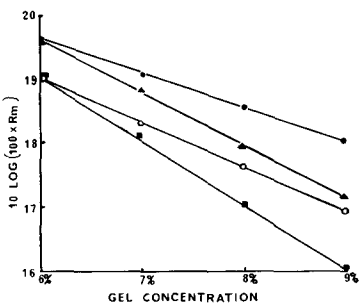

Fig. 1. Electrophoretic migration as a function of gel concentration. The circles represent the standard proteins, ovalbumin (mol. wt. 45 000; closed circles) and bovine albumin (mol. wt. 67 000). The triangles are for alanine dehydrogenase; the squares are for glutamate dehydrogenase. Projections of the latter two lines intersect near 0% gel concentration. Slopes obtained were multiplied by 10 to correspond to plots of 100 log (Rm × 100) (ref. 13; Rm = relative mobility).

in a total volume of 100 ml water. The modified Solution C in the gel formulation yielded a 10% polyacrylamide gel; substitution of equal volumes of water for portions of Solution C yielded suitable gels down to 2% polyacrylamide. The Tris–glycine reservoir buffer was only diluted 1:1 instead of 1:10. Ovalbumin (2 × crystallized, Mann Research Laboratories) and crystalline bovine serum albumin (Mann Research Laboratories) with molecular weights for the monomers being 45 000 and 67 000, respectively, were used as our standards; they were detected on the gels by amido-schwartz staining. The alanine dehydrogenase and glutamate dehydrogenase were detected by the tetrazolium stain[12] indicated previously, the heavier-staining front-running band being considered alanine dehydrogenase in each case. The results are shown in Fig. 1. The slopes obtained from these data were −5.6 for ovalbumin and −7.2 for bovine serum albumin. Slopes of −8.2 and −10.0 were obtained for alanine dehydrogenase and glutamate dehydrogenase, respectively, corresponding to molecular weights of $8.2 \cdot 10^4$ and $10.7 \cdot 10^4$. These values for size isomers could be explained by a model in which a trimer (alanine dehydrogenase) is interconvertible with a tetramer (glutamate dehydrogenase). The inactive monomeric subunit would have a molecular weight of about $2.7 \cdot 10^4$.

The alanine dehydrogenase/glutamate dehydrogenase activities of *B. thuringiensis* appear to differ from those of other bacilli[3,5,7] in size and response to nucleotide regulation. It possibly represents a much simpler system than the corresponding mammalian system and, therefore, should be suitable for an in-depth study of the association and regulation phenomena.

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