Aspartate Aminotransferase of Lactobacillus murinus*

G. Rollan, M.C. Manca de Nadra, A. Pesce de Ruiz Holgado and G. Oliver

Centre de Referencia para Lactobacilos (CERELA), Chacabuco 145, 4000 Tucumán, Argentina, and Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Argentina

Received August 31, 1987

ABSTRACT. Aspartate aminotransferase from Lactobacillus murinus is thermostable, its activity being not changed for two months at temperatures between 4 and −70 °C. Maximum activity was observed at 40 °C and pH 7.3 in phosphate buffer (30 mmol/L). $\Delta G^*$ Value of 26.3 kJ/mol was calculated from the Arrhenius plot. The $K_m$ values for L-aspartate and 2-oxoglutarate at pH 7.3 were 25 and 100 mmol/L, respectively. Sodium maleate and glutamate acted as inhibitors of the enzyme activity. The $K_i$ values for sodium maleate with L-aspartate or 2-oxoglutarate were 8.0 and 4.0 mmol/L, respectively. An inhibitory effect was observed with 1 mm Hg$^2+$ ions (1 mmol/L). The activity of the enzyme was diminished by only 12% in the absence of pyridoxal 5'-phosphate.

Aspartate aminotransferase (AAT; EC 1.6.1.1) is wide-spread in nature. It catalyzes the following reaction:

$$L\text{-aspartate} + 2\text{-oxoglutarate} \rightleftharpoons \text{oxaloacetate} + L\text{-glutamate}$$

In animal and plant cells, AAT isoenzymes occur in mitochondria and the cytosol. In microorganisms, it was extensively studied in E. coli (Gelfand and Steinberg 1977; Mavrides and Orr 1975; Powell and Morrison 1978), Klebsiella aerogenes ((Paris and Magasanik 1981), Pseudomonas aeruginosa (Whittaker et al. 1982), Saccharomyces cerevisiae (Yagi et al. 1982) and Brevibacterium linens 47 (Lee and Desmazeaud 1985).

At present no reference to aspartate aminotransferase in lactic acid bacteria exists. Rollan et al. (1985) reported the properties of L-aspartate-ammonia lyase, EC 4.3.1.1) in Lactobacillus murinus. Lactobacilli were not found to contain all the enzymes involved in the citric acid cycle and this paper is the first report of the properties of aspartate aminotransferase in Lactobacillus murinus.


** To whom the correspondence should be sent.

1 Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
2 Members of the investigator's career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.
MATERIALS AND METHOD

Microorganisms. Lactobacillus murinus strain CNRZ313 (ATCC 35020) was obtained from the Centre National de la Recherche Zootechnique (France). The strain was isolated by Raibaud et al. (1973) and described as a new species by Hemme et al. (1980).

Culture medium and growth conditions. The basal medium (LAPTg) for the growth of this microorganism (Raibaud et al. 1961) has the following composition (in %): yeast extract 1, peptone 1.5, tryptone 1, glucose 1, Tween 80 0.1 %. The pH was adjusted to 6.8 with NaOH (0.2 mol/L).

Cells were grown in LAPTg medium in precultures of 10 mL, 100 mL and a final culture of 2 L. After being cultured for 4.5 h (end of exponential growth) at 37 °C, the cells were harvested by centrifugation (7000 g, 10 min) in a MSE refrigerated centrifuge.

Cell-free extract. The cells were washed twice in 0.1 % 2-mercaptoethanol. They were then suspended at 20 °C (W/V) in the same solution and ruptured using a French press. Cell debris was removed by centrifugation (20 000 g, 20 min, 4 °C).

Enzyme activity. The enzyme was assayed by spectrophotometric estimation of oxaloacetic acid (Green et al. 1945).

The reaction mixture (3 mL) had the following composition: sodium aspartate 20 μmol, 2-oxoglutarate 100 μmol, phosphate buffer (30 mmol/L; pH 7.3) and the enzymic extract. The change in absorbance per min at 280 nm was followed in a Gilford spectrophotometer. One unit of aspartate transaminase is that quantity of enzyme which produces 1 μmol of oxaloacetate per min. Specific activity is expressed as units per milligram of protein.

Protein content was determined according to Lowry's method using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The growth of L. murinus was not altered by the addition of L-aspartate (Rollán et al. 1985).

Optimum of pH. Fig. 1 shows that the pH optimum for AAT activity was 7.3. The assay was unsatisfactory above pH 8.0 because of extensive breakdown of oxaloacetate to pyruvate. Since the enzyme was stable, and the substrates do not ionize throughout this pH range, the variation in activity must be due to the ionization of some group(s) on the enzyme. For Streptococcus faecalis R AAR, Lichstein et al. (1945) report that the rate of reaction is slightly dependent on the pH within the range of 5.7 to 9.5 in phosphate buffer (0.1 mol/L). AAT of E. coli has a pH optimum at 8.5 (Lichstein and Cohen 1945). In Brevibacterium linens, Lee and Desmazeaud (1985) found the pH optimum to lie between 8.5 and 9.0.

Effect of temperature. The optimal temperature for the AAT activity was 40 °C. The rate of transamination decreased rapidly above this temperature. For E. coli AAT, Lichstein and Cohen (1945) report the optimal temperature at 32 °C, and in B. linens, Lee and Desmazeaud (1985) found an optimal temperature of 37—40 °C. A plot of the logarithm of the activities between 30 to 50 °C versus the reciprocal of the absolute temperature yielded a straight line from which the activation energy was calculated as 26.3 kJ/mol (Fig. 2).