Survey of the Inhibitory Effects of Glycine on Threonine Aldolase Activity of Yogurt Microorganisms[1]

Ronald H. Schmidt,* Laura B. Kennedy, Ellen B. McMullan, and Edward R. Mason

To evaluate the inhibitory effects of glycine on threonine aldolase activity (E.C. 2.1.2.1) of yogurt microorganisms, glycine was added to assay mixtures containing cell-free extracts of different strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and enzyme activity was determined by head-space gas chromatography. A glycine level of 25 μmol added to assay mixtures containing 125 μmol of threonine substrate resulted in a range of inhibition from 39.1 to 98.1% for *S. thermophilus* strains and from 2.1 to 18.9% for *L. bulgaricus* strains. In assays involving cell extracts of *S. thermophilus* MS1, addition of 5 μmol of glycine with increasing threonine levels from 0 to 200 μmol resulted in data trends resembling those of an allosteric inhibitor. A linear relationship between glycine level and inhibition of threonine aldolase for *S. thermophilus* MS1 was observed for glycine levels of 0–20 μmol at a threonine level of 125 μmol in assay mixtures. Glycine levels of 20 μmol or above resulted in almost complete inhibition of MS1 threonine aldolase activity.

Streptococcus thermophilus and *Lactobacillus bulgaricus* grow symbiotically during yogurt fermentation to produce lactic acid and acetaldehyde, which are responsible for the characteristic yogurt flavor (Law, 1981; Sandine and Elliker, 1970; Tamime and Deeth, 1980). However, the acetaldehyde level in yogurt products is highly variable (Tamime and Deeth, 1980; Schmidt et al., 1983), and the activity of threonine aldolase (E.C. 2.1.2.1) (Lees and Jago, 1976, 1978; Shankar, 1977; Wilkins et al., 1986a,b), the enzyme involved in the direct conversion of threonine to acetaldehyde and glycine, may play an important role in yogurt manufacture relative to elevated, as well as variable, levels of acetaldehyde in these products. While lactic acid bacteria, with very few exceptions, possess threonine aldolase (Lees and Jago, 1976, 1978), differences have been suggested regarding the nature of this activity in yogurt microorganisms. In previous investigations (Wilkins et al., 1986b), we observed that the *S. thermophilus* threonine aldolase may be more affected by growth conditions than that of *L. bulgaricus*. The enzyme activity of *S. thermophilus* decreased considerably as the growth temperature and incubation time increased. Moreover, it has been shown (Wilkins et al., 1986a) that the threonine aldolase of *S. thermophilus* MS1 is inhibited more dramatically by glycine in the assay mixture than is *L. bulgaricus* MR1. Other investigators (Raya et al., 1986) could not detect threonine aldolase activity in dialyzed cell extracts from *S. thermophilus* ATCC 19258 nor *S. thermophilus* Yop9, while activity was detectable by the same procedures in strains of *L. bulgaricus*. The objective of the present investigation was to examine the inhibitory effects of glycine on threonine aldolase activity for several strains of *S. thermophilus* and *L. bulgaricus* and to partially characterize the nature of the glycine inhibition in yogurt microorganisms.

MATERIALS AND METHODS

Microorganisms and Growth Conditions. *S. thermophilus* MS1 and *L. bulgaricus* MR1 and WRW were obtained from Microlife Technics, Sarasota, FL. *S. thermophilus* 20, 19987 and 3641 and *L. bulgaricus* UM were obtained from the University of Minnesota, St. Paul, MN. The conditions for propagation, harvesting, disintegration, and preparation of cell-free extracts have been previously discussed (Wilkins et al., 1986a).

Threonine Aldolase Activity. Head-space gas chromatography (HSGC) was used as previously described (Wilkins et al., 1986a) to assay for threonine aldolase activity. Assay mixtures were prepared by placing 23 μmol of sodium phosphate buffer (pH 7.0), 125 μmol of threonine, and 250 μL of cell-free extract (CFE) or whole-cell suspension in a total volume of 2.0 mL. In experiments in which several strains of yogurt microorganisms were surveyed for threonine aldolase activity, glycine was added to the assay mixture at levels of 0, 25, and 50 μmol. For experiments in which the effect of glycine on threonine aldolase of *S. thermophilus* MS1 was more specifically examined, glycine levels of 0, 2, 3, 5, 10, 20, 50, and 100 μmol were added to assay mixtures containing 125 μmol of threonine substrate, or threonine was used at levels ranging from 0 to 200 μmol while glycine was added to 0 or 5 μmol. Data are reported for threonine aldolase activity as specific activity (nanomoles of acetaldehyde per minute per

Table I. Glycine Effect on Threonine Aldolase Activity of Cell-Free Extracts for Different Strains of *S. thermophilus* (ST) and *L. bulgaricus* (LB)

microorganism[a]	Gly level[b]	sp act.[c]	rel inhibn[d]
ST-MS1	0	7.75	
	25	0.15	98.1
	50	0.11	98.5
ST-20	0	3.63	
	25	2.21	39.1
	50	0.89	75.5
ST-19987	0	11.60	
	25	0.49	95.8
	50	0.39	96.6
ST-3641	0	14.90	
	25	0.41	97.3
	50	0.28	96.6
LB-MR1	0	6.32	
	25	6.19	2.1
	50	4.24	32.9
LB-UM	0	4.77	
	25	4.09	14.0
	50	3.64	23.8
LB-WRW	0	22.80	
	25	18.50	18.9
	50	4.61	79.8

[a] Strains of *S. thermophilus* and *L. bulgaricus*. [b] Glycine (micromoles) added to assay mixtures containing 125 μmol of threonine substrate. [c] Specific activity in cell-free extracts expressed as nanomoles of acetaldehyde per minute per milligram of protein in cell extract. [d] Percent inhibition relative to assay with no glycine.

Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611.

[1] Florida Agriculture Experiment Station Journal Series No. 9478.

0021-8561/89/1437-1215$01.50/0

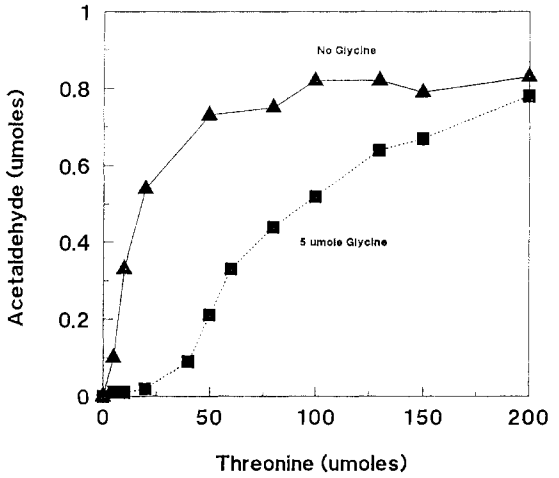

Figure 1. Effect of glycine at 5 μmol in assay mixtures containing threonine substrate ranging to 200 μmol on the threonine aldolase activity of cell-free extracts for *S. thermophilis* MS1.

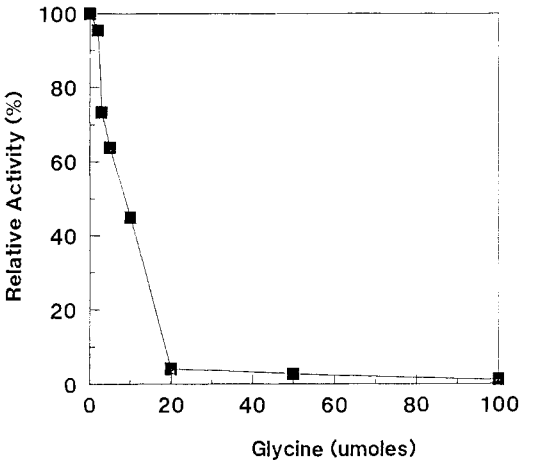

Figure 2. Effect of increasing the glycine from 0 to 100 μmol in assay mixtures containing 125 μmol of threonine substrate on the threonine aldolase activity of cell-free extracts for *S. thermophilus* MS1.

milligram of protein in cell extract) and are means from duplicate trials.

RESULTS AND DISCUSSION

Glycine Effect on Threonine Aldolase Activity of Different Strains of Yogurt Microorganisms. Data for the effect of glycine in the assay mixture on threonine aldolase activity of different strains of yogurt microorganisms are presented in Table I. While activity was somewhat varied between the different strains, there was no apparent overall difference in amount of enzyme activity between strains of *S. thermophilus* and those of *L. bulgaricus*. However, the data suggest that strains of *S. thermophilus* are more inhibited by glycine than those of *L. bulgaricus*. With the exception of *S. thermophilus* 20, the enzyme activity of all *S. thermophilus* strains showed greater than 95% inhibition at a glycine level of 25 μmol or higher. For the strains of *L. bulgaricus* examined, however, the inhibition by 25 μmol of glycine ranged from 2.1 to 18.9%. Addition of 50 μmol of glycine to assays involving *L. bulgaricus* resulted in inhibition ranging from 23.8 to 79.8%.

Specific Effects of Glycine on Threonine Aldolase of *S. thermophilus* MS1. Data for effects of 5 μmol of glycine added to assay mixtures for *S. thermophilus* MS1 while threonine increased from 0 to 200 μmol are presented in Figure 1. Threonine aldolase activity, in the presence of 5 μmol of glycine, did not increase substantially with increased substrate levels from 0 to 50 μmol. However, at threonine levels above 50 μmol, the enzyme activity generally increased. The threonine aldolase activity at 200 μmol of threonine in experiments with and without glycine was similar. The data trends suggest that glycine is an allosteric inhibitor of threonine aldolase in this organism. Allosteric inhibition by glycine for threonine aldolase of *Streptococcus lactis* subsp. *diacetylactis* DRC3 has been previously suggested (Lees and Jago, 1976).

From data presented in Figure 2, it is apparent that glycine inhibition of *S. thermophilus* MS1 threonine aldolase approaches linearity at glycine levels ranging from 0 to 20 μmol with threonine in the assay mixture at 125 μmol. Almost complete inhibition was observed at glycine levels of 20 μmol or above.

Data presented suggest that the inhibition of threonine aldolase activity of yogurt microorganisms by glycine varies with different strains. Such information suggests that the acetaldehyde level in yogurt not only is a function of the level of enzyme activity in the starter culture used but also could be related to qualitative differences in enzyme activity as affected by the level of glycine in the products.

Registry No. Glycine, 56-40-6; threonine aldolase, 9029-83-8; acetaldehyde, 75-07-0.

LITERATURE CITED

Law, B. A. *Dairy Sci. Abstr.* **1981**, *43*, 143.
Lees, G. J.; Jago, G. R. *J. Dairy Res.* **1976**, *43*, 75.
Lees, G. J.; Jago, G. R. *J. Dairy Sci.* **1978**, *61*, 1205.
Raya, R. R.; De Nadra, M. C. M.; De Ruiz Holgado, A. P.; Oliver, G. *Milchwissenschaft* **1986**, *41*, 397.
Sandine, W. E.; Elliker, P. R. *J. Agric. Food Chem.* **1970**, *18*, 557.
Shankar, P. A. Ph.D. Dissertation, University of Reading, U.K., 1977.
Schmidt, R. H.; Davidson, S. M.; Bates, R. P. *J. Food Sci.* **1983**, *48*, 1556.
Tamime, A. Y.; Deeth, H. C. *J. Food Prot.* **1980**, *43*, 939.
Wilkins, D. W.; Schmidt, R. H.; Kennedy, L. B. *J. Agric. Food Chem.* **1986a**, *34*, 150.
Wilkins, D. W.; Schmidt, R. H.; Shireman, R. B.; Smith, K. L.; Jezeski, J. J. *J. Dairy Sci.* **1986b**, *69*, 1219.

Received for review November 18, 1988. Accepted April 3, 1989.