The Synthesis of Taurine from Sulfate
V. Regulatory Modifiers of the Chick Liver Enzyme System[1] (37629)

LENORA J. HILL[2] AND W. G. MARTIN

Agricultural Experiment Station, West Virginia University, Morgantown, West Virginia 26506

The enzyme system which catalyzes the synthesis of taurine from L-serine and 3′-phosphoadenosine-5′-phosphosulfate (PAPS) in chick liver has been described (1), and has also been shown in rat tissues with equal or higher activity (2). This enzyme system, which uses inorganic sulfate–sulfur (PAPS) to synthesize taurine, is referred to as the peak one (P_1) system (1). Most animals have the necessary enzymes to synthesize taurine from the sulfur amino acids via transsulfuration, the organic pathway. The activity of one metabolic pathway has frequently been observed to have a regulating effect on another which produces the same or a similar product. This research was conducted, using the P_1 enzyme system from chick liver, to ascertain the influence of transsulfuration intermediates on the *in vitro* activity of PAPS-sulfotransferase (EC 2.8.2).

Materials and Methods. The experimental subjects were white leghorn cockerels, obtained from a commercial hatchery at 1 day of age, fed a purified diet (2) for 2 or 3 wk and sacrificed by decapitation. Tissues were immediately excised, chilled and subjected to ammonium sulfate fractionation and column separation as described earlier (1).

The enzyme assay was performed using the basic reaction mixture for taurine synthesis (1) which contained Tris–HCl buffer, P_1 enzyme system, pyridoxal phosphate, L-serine, and PAP^{35}S. The PAP^{35}S was enzymatically prepared as reported earlier (3) and each preparation had a concentration of about 40 μmole/ml as determined spectrophotometrically at 260 nm by comparison to an ATP standard curve.

The specific activity of the PAPS-sulfotransferase is defined as the cpm PAP^{35}S incorporated into taurine-^{35}S/μg protein. The specific activity was determined in the presence of varying concentrations of test compounds, and the resulting activity divided by the activity in the absence of the compound was referred to as the activity ratio. The compounds tested as possible modifiers of the P_1 system were: D- and L-methionine, *S*-adenosyl L-methionine (SAM), homocysteine, cysteine, cysteine sulfinic acid (CSA), cysteic acid (CA), DL-ethionine, and isethionic acid. The test compounds were added to the basic reaction mixture at varying concentrations from 10 to 100 μM and from 4 to 10 replications of each treatment were performed.

Results. Preliminary results with the reaction mixture indicated that the highest and most reproducible activity was obtained when the ratio of P_1 protein:L-serine:PAP^{35}S was 200 μg:50 μM:0.5 ml (Table I). This combination is similar to that reported earlier (1), except that more P_1 protein was used in these experiments. Although increasing concentrations of L-serine slightly reduced the specific activity, 50 μM was used throughout these tests. The PAP^{35}S concentration selected, 0.5 ml or approximately 20 μM, was optimal and was used to enable comparison to be made to previous work.

That these levels of serine and enzyme protein were not in themselves limiting was shown by calculating the product conversion rates (Table II). The addition of individual components after the initial 10 min of reaction and measuring the activity after

[1] This manuscript is published with the approval of the Director of the West Virginia Agricultural Experiment Station, Morgantown, as Scientific Paper No. 1283.

[2] This work is in partial fulfillment of the requirements for the degree of MS in Agricultural Biochemistry, West Virginia Univ.

TABLE I. The Effects of Enzyme, L-Serine, and PAP^{35}S Concentrations on the Conversion of L-Serine and PAP^{35}S to ^{35}S-Taurine by the P_1 System.

Enzyme (μg protein)	50	100	150	200	300	400	500
AR[a]	1.00	1.32	1.63	1.75	1.84	1.71	1.76
L-Serine (μM)	10	30	50	80	100		
AR	1.00	0.99	0.96	0.85	0.80		
PAP^{35}S (ml)	0.1	0.3	0.5	0.7	0.9	1.0	
AR	1.00	4.24	6.11	6.72	8.05	7.64	

[a] AR, activity ratio is the ratio of the specific activity (net cpm taurine-^{35}S produced from PAP^{35}S/μg protein) of the higher variables to the lowest level.

an additional 10 min produced little change in activity.

When the activity of the P_1 system was tested by additions of possible modifiers, two trends were observed. With respect to methionine, the initial compound of the transsulfuration pathway, a slight positive response was observed at lower levels of the D-isomer (Fig. 1). A significant negative response was observed when L-methionine was added, and a marked reduction was seen at each of three levels of *S*-adenosyl-L-methionine (SAM).

When the immediate precursors of taurine, CA and CSA, were added to the reaction mixture used in these experiments, no response was observed with CA. In contrast, when CA was added to the mixture containing 100 μg protein [one-half that used throughout and the same amount as used in previous work (1)], a significant stimulation in taurine-^{35}S synthesis from PAP^{35}S was obtained. The addition of CSA significantly reduced the activity of the system (Fig. 2).

The addition of the transsulfuration intermediates cysteine and homocystein produced marked reduction in activity at low concentrations and completely blocked activity at higher concentrations (Fig. 3). Addition of DL-ethionine, the methionine antimetabolite, gave decreased activity similar to that observed with L-methionine. The ethionine response was actually intermediate to that observed from D- and L-methionine. The metabolite of taurine, isethionate, gave a slight increase in activity at low concentrations and a decrease at higher levels.

Discussion. Intermediates of the transsulfuration pathway which channels methionine-*S* to cysteine and ultimately to taurine were tested as modifiers of the P_1 system which produces taurine from another sulfur source, PAPS. The L-isomer of methionine, SAM, homocysteine, cysteine, and CSA (essentially the entire pathway) produced strong negative modification of the enzymatic activity of the P_1 system. Only D-methionine and CA were observed to produce little change or a minor positive response. In previous work (4, 5), chicks were fed purified diets supplemented with varying

TABLE II. Product Conversion Rates of the P_1 System as Influenced by Additional Components Added to the Basic Reaction Mixture.

Serine (μM)	Enzyme (μg)	PAP^{35}S[a] (ml)	Corrected sp act[b]	Corrected cpm/ml[c]	% Conversion
50	200	0.5	672	41359	82.5
50 + 50	200	0.5	641	33750	78.7
50	200 + 200	0.5	699[d]	35337	85.9
50	200	0.5 + 0.5	1237.5	65136	75.9

[a] PAP^{35}S preparation contained 325,000 cpm.

[b] Sp act = cpm taurine-^{35}S produced/μg protein.

[c] cpm/ml = cpm/ml of reaction mixture.

[d] Sp act based on first 200 μg protein.

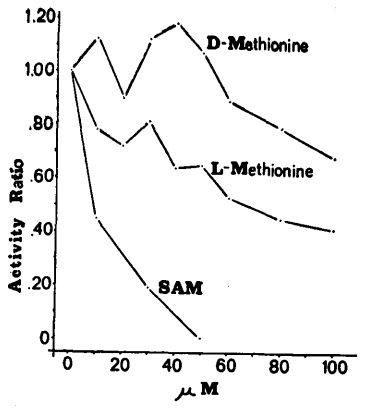

FIG. 1. The effects of varying concentrations (μM) of D- and L-methionine and *S*-adenosyl methionine (SAM) on the P_1 activity.

amounts of DL-methionine and increased taurine-^{35}S specific activities were routinely observed from injections of $^{35}SO_4^{2-}$. Possibly the L-isomer is selectively used for protein synthesis and SAM production, leaving the tissues with a higher concentration of the dietary D-isomer.

The production of taurine by the P_1 system is dependent upon the concentration of PAPS (1, 2) which is significantly increased in chick liver by the dietary supplementation of sulfate (5). It is known that cysteine is a negative modifier of ATP sulfurylase, the initial enzyme required for PAPS synthesis, in *E. coli* (6). In the chick fed a diet supplemented with cysteine liver taurine as well as taurine specific activity

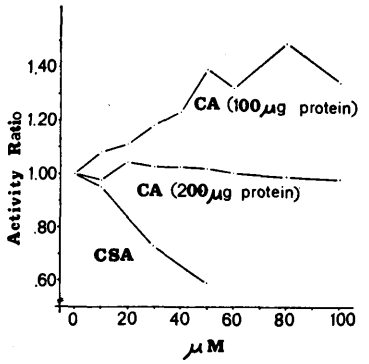

FIG. 2. The effects of varying concentrations (μM) of cysteic acid (CA) at enzyme protein levels of 100 and 200 μg, and cysteine-sulfinic acid (CSA), on the P_1 activity.

from $^{35}SO_4^{2-}$ decreased (7). The present experiments show that cysteine also negatively modifies the activity of the P_1 system for which PAPS is a cosubstrate. The presence of homocysteine produced the same negative response as cysteine, which suggests that both compounds may be acting as sulfhydryl oxidizing agents to the P_1 system. The activity of the P_1 system has been shown to be dependent on sulfhydryl groups (1).

The response to CSA and CA was clearly different. The synthesis of taurine from PAPS by the P_1 system was significantly decreased by additions of low concentrations of CSA, while only minor stimulations were observed with CA in the reaction mixture containing 200 μg enzyme protein. When the

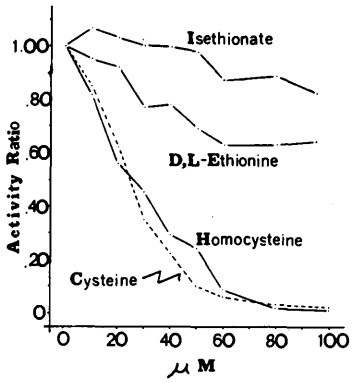

FIG. 3. The effects of varying concentrations (μM) of cysteine, homocysteine, DL-ethionine, and isethionate on the P_1 activity.

mixture contained 100 μg P_1 protein a highly significant stimulation in the synthesis of taurine-^{35}S from PAP^{35}S was observed. Since CA is postulated to be an enzyme-bound intermediate in the P_1 system, an explanation of this observation may be that added CA significantly improved the catalytic effectiveness of the P_1 system by creating a more optimal intermediate-enzyme concentration. The P_1 system has been shown to decarboxylate CA without L-serine or PAPS present (1). Since the reaction goes to completion during the early stages, and enzyme activity is lost thereafter, the addition of CA may have increased the production rate of taurine by increased stability of the system.

It has been reported that chick liver con-

tains higher decarboxylase activity toward CA than to CSA (8), and that CSA strongly inhibits the decarboxylation of CA (8, 9). The CSA/CA decarboxylase described by others is precipitated by 0.5 saturation with ammonium sulfate, while the P_1 system is soluble after the liver homogenate is 0.7 saturated with ammonium sulfate. It appears that the decarboxylase activity of the P_1 system, specific for CA and inhibited by CSA, is different from the precipitable CSA/CA decarboxylase which is generally CSA specific.

Isethionate, the deaminated product of taurine, produced a slight increase in P_1 activity at low concentrations and only mild decreases at higher concentrations. When chicks were fed a purified diet supplemented with small amounts of isethionate, the taurine concentration of the liver, heart, and intestine was doubled, indicating increased synthesis, increased tissue retention or a combination of these (10). It is likely that isethionate may influence one or more of the enzymes of the organic pathway by a feedback mechanism which would contribute to the tissue total taurine, which appears to arise continuously from both sulfur sources.

Although more work is required before the relevance of this pathway to taurine biosynthesis is determined, it appears that it may work in tandem with the transsulfuration organic pathway to maintain tissue taurine concentrations of the animal. These data suggest the possible existence of a switchover mechanism for taurine synthesis.

Summary. The activity of the enzyme system which produces taurine from PAPS and serine in chick liver has been tested for regulation by intermediates of the transsulfuration pathway. The L-isomer of methionine, SAM, homocysteine, cysteine, and CSA produced significant decreases in the activity of this enzyme system. Low concentrations of D-methionine, and isethionate, a product of taurine deamination, gave a slight enhancement of enzyme activity. Cysteic acid, a postulated enzyme-bound intermediate of this enzyme system, produced increased activity at low enzyme–protein concentration but no effect at higher concentrations. These data suggest the existence of a switchover mechanism in the biosynthesis of taurine in the animal from these two sulfur sources.

1. Sass, N. L., and Martin, W. G., Proc. Soc. Exp. Biol. Med. **139**, 755 (1972).

2. Martin, W. G., Sass, N. L., Hill, L. J., Tarka, S. M., and Truex, R. C., Proc. Soc. Exp. Biol. Med. **141**, 632 (1972).

3. Sass, N. L., and Martin, W. G., Anal. Biochem. **38**, 559 (1970).

4. Miraglia, R. J., and Martin, W. G., Proc. Soc. Exp. Biol. Med. **132**, 640 (1969).

5. Martin, W. G., Poultry Sci. **51**, 608 (1972).

6. Pasternak, C. A., Ellis, R. J., and Jones-Mortimer, M. C., Biochem. J. **96**, 270 (1965).

7. Martin, W. G., Miraglia, R. J., Spaeth, D. G., and Patrick, H., Proc. Soc. Exp. Biol. Med. **122**, 841 (1966).

8. Jacobsen, J. G., Thomas, L. L., and Smith, L. H., Biochim. Biophys. Acta **85**, 103 (1964).

9. Simonnet, G., Chapeville, F., and Fromageot, P., Bull. Soc. Chim. Biol. **42**, 891 (1960).

10. Tarka, S. M., MS thesis, West Virginia Univ. (1973).

Received May 29, 1973. P.S.E.B.M., 1973, Vol. 144.