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Inhibition of glutamate decarboxylase by salicylate *in vitro*

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Salicylate inhibits the activity of glutamate decarboxylase from *Escherichia coli* by a reversible mechanism, but high concentrations of the drug denature the enzyme protein

Salicylate inhibits the activity of glutamate decarboxylase prepared from *Escherichia coli* and rat brain (Gould, Huggins & Smith, 1963; Gould & Smith, 1965). It was suggested that the mechanism of inhibition involved an irreversible combination of the drug with the enzyme protein. This mechanism has not been confirmed in the present work. High concentrations of salicylate denature, and lower concentrations cause a reversible inhibition of the bacterial enzyme.

EXPERIMENTAL

Materials

Type 1. Glutamate decarboxylase (L-glutamate-1-carboxylyase, EC 4.1.1.15) from *E. coli* was obtained from the Sigma Chemical Co., St. Louis, and the [^{14}C]salicylic acid from the Radiochemical Centre, Amersham, Bucks. Other chemicals were of analytical grade and deionized water was used throughout.

Measurement of glutamate decarboxylase activity

The decarboxylase activity was determined at 37° by the Warburg technique, using a Gilson Respirometer. The final concentrations were 1·25 mg/ml of glutamic acid and 0·5 mg/ml of glutamic decarboxylase in all experiments. All solutions were prepared in 0·1 M acetate buffer, pH 5·0. The reaction was started by the addition of glutamic acid from the side arm, except that in some experiments the addition of enzyme from the side arm was used to initiate the reaction. The activity was measured by the rate of evolution of carbon dioxide over a 5 or 10 min period and calculated as Qco_2 (μl of CO_2/mg of dry wt of protein/5 or 10 min).

Dialysis experiments

The enzyme preparation (5 mg) in 5 ml of either M or 0·1 M acetate buffer, pH 5·0, was dialysed against 50 ml of the same buffer at 0°; the dialysing medium was replaced ten times over a period of 48 h. Further samples of the enzyme suspensions were exposed for 15 h at 0° to either 10 μCi (10 μ mole) of [^{14}C]salicylate or to 0·25 M salicylate plus 10 μCi of radioactive salicylate and dialysed as described above. The radioactivity in triplicate samples (0·5 ml) of the mixture, before and after dialysis, were counted in a Beckman LS 200B scintillation counter, using as phosphor 5 ml of 0·4% 2,5-diphenyloxazole, 0·02% 1,4-bis-2(4-methyl-5-phenyl-oxazole)benzene and 60% naphthalene in 1,4-dioxan.

RESULTS AND DISCUSSION

The results in Table 1 show that salicylate inhibits the activity of glutamate decarboxylase from *E. coli* and the degree of inhibition becomes greater as the salicylate concentration is increased. Table 2 shows the effects of exposing the enzyme preparation to salicylate concentrations, ranging from 20 to 250 mM, for 1 h and then diluting with buffer before the reaction is started by the addition of the substrate. If the inhibitory effect of salicylate is irreversible, then the observed degree of inhibition should have been determined by the concentration of salicylate present at the preincubation stage, whereas if it is reversible, then the observed inhibition should depend on the salicylate concentration present in the diluted reaction mixtures. The results show that the inhibition produced by preincubation with 20 mM salicylate is completely reversible but that an increasing degree of irreversible inhibition occurs as the salicylate concentration is raised from 50 to 250 mM. Salicylate, therefore, inhibits glutamate decarboxylase *in vitro* by two separate mechanisms, one reversible and one irreversible. The concentration of the drug present in the reaction mixtures determines which type of inhibition predominates when the enzyme preparation is exposed to the drug for 1 h.

Some precipitation of the enzyme preparation was observed in the presence of salicylate concentrations greater than 150 mM, suggesting that chemical denaturation of the enzyme protein may have occurred. Further evidence supporting this mechanism is provided by the results in Table 3, which show that the degree of inhibition

Table 1. *Effect of salicylate on* E. coli *glutamate decarboxylase activity.* Each value represents the mean of six determinations. In the control experiment 93 μl of CO_2 were evolved during 10 min.

Final concn of salicylate (mM) in reaction mixture	5	10	15	20	50	100	150	250
Inhibition (%) ± s.e.	0	9 ±2·6	13 ±3·0	20 ±3·4	56 ±2·1	85 ±1·8	99 ±0·5	99 ±0·4

Table 2. *Effect of preincubating enzyme with salicylate for* 1 *h.* The enzyme preparation was preincubated at room temperature (20°) with salicylate for 1 h, diluted 1 to 20 with 0·1M acetate buffer, pH 5·0 and there action started by tipping the glutamate from the side arm into an aliquot of the diluted mixture contained in the centre well of the Warburg flask. The evolution of CO_2 was followed manometrically for 10 min and inhibitions were calculated from the Q_{CO_2}/10 min (91 μl), observed with an enzyme preparation treated as above, except that it was preincubated in the absence of salicylate.

Salicylate concentration (mM)		Theoretical inhibition (%)		
Preincubated with enzyme	In diluted mixture	Irreversible	Reversible	Observed Inhibition (%)
20	1	20	0	0
50	2·5	56	0	16
100	5·0	85	0	70
150	7·5	99	5	85
250	12·5	99	12	99

Table 3. *Effects of preincubating enzyme with salicylate with time.* Experimental details were as described in Table 2. The results are given as percentage inhibitions calculated from corresponding control experiments in which the enzyme preparation was incubated in the absence of the drug.

Salicylate concn (mM) used for preincubation	Time of preincubation (h)			
	1	6	24	48
20	0	0	7	13
50	16	24	42	50
100	70	76	82	86

increased as the period of preincubation of the enzyme preparation with the drug was extended. Denaturation could explain the complete loss of activity which occurred when glutamate decarboxylase was exposed for 15 h to 250 mM salicylate but not the reported binding of radioactive salicylate to the enzyme protein (Gould, Huggins & Smith, 1963). The glutamate decarboxylase preparation was therefore exposed to either 250 mM salicylate plus 10 μmole of [^{14}C]salicylate or to 10 μ mole of the radioactive salicylate for 15 h at 0° and dialysed as described in the Experimental section. The average counts/min were 63,000 $\pm$ 1500 per ml in the dialysis sac at the beginning of the experiments. With and without carrier salicylate the corresponding figure at the end of dialysis was 100 $\pm$ 15 counts/min while outside the dialysis sac there were 90 $\pm$ 10 counts/min ml^{-1}. These results show that negligible binding occurred either with the denatured enzyme protein preincubated with 250 mM salicylate or when the enzyme protein was exposed to only a tracer amount of radioactive salicylate (10 μ mole). We are unable to explain the discrepancy between the experimental results of the earlier work and of the present investigation. Neither substitution of 3 M acetate buffer, pH 5·0, which was used in the previous experiments, nor continuous stirring of the contents of the dialysis sac during the dialysis, affected the removal of the radioactivity from the enzyme preparation.

It must be concluded that salicylate does not irreversibly combine with glutamate decarboxylase but that the drug chemically denatures the enzyme protein, the extent of denaturation depending on the salicylate concentration and on the time of exposure. Very high concentrations of the drug (250 mM) caused practically complete denaturation within an hour (Table 2) whereas the effect did not become evident with 20 mM salicylate until the preincubation period was extended to 24 h. The present results also show (Table 2) that part of the inhibitory effect of salicylate is due to a reversible mechanism and it is known from previous work (Gould & others, 1963) that this does not involve competition with the substrate.

REFERENCES

GOULD, B. J., HUGGINS, A. K. & SMITH, M. J. H. (1963). *Biochem. J.*, **88**, 346–349.
GOULD, B. J. & SMITH, M. J. H. (1965). *J. Pharm. Pharmac.*, **17**, 15–18.