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INACTIVATION OF SERINE TRANSHYDROXYMETHYLASE AND THREONINE ALDOLASE ACTIVITIES

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SUMMARY

It is shown that 2-chloroacetaldehyde, iodoacetamide and bromopyruvate irreversibly inactivate the serine transhydroxymethylase (L-serine:tetrahydrofolate 5,10-hydroxymethyltransferase, EC 2.1.2.1) and threonine aldolase (L-threonine acetaldehyde-lyase, EC 4.1.2.5) activities in a coordinated fashion. Glycine, L-serine and DL-allothreonine partially protect both the activities against inactivation. These results confirm the conclusion of SCHIRCH AND GROSS[8] that the serine transhydroxymethylase and threonine aldolase activities from the rabbit liver may be the property of the same or very similar enzymes.

INTRODUCTION

The enzyme serine transhydroxymethylase (L-serine:tetrahydrofolate 5,10-hydroxymethyltransferase, EC 2.1.2.1) catalyses the condensation of glycine with a C_1 unit attached to tetrahydrofolate to give serine[1-3] according to the equation:

$$\text{Formaldehyde} + \text{tetrahydrofolate} \rightleftharpoons \text{tetrahydrofolate methylene} \xrightleftharpoons{\text{Glycine}} \text{tetrahydrofolate} + \text{serine} \quad (1)$$

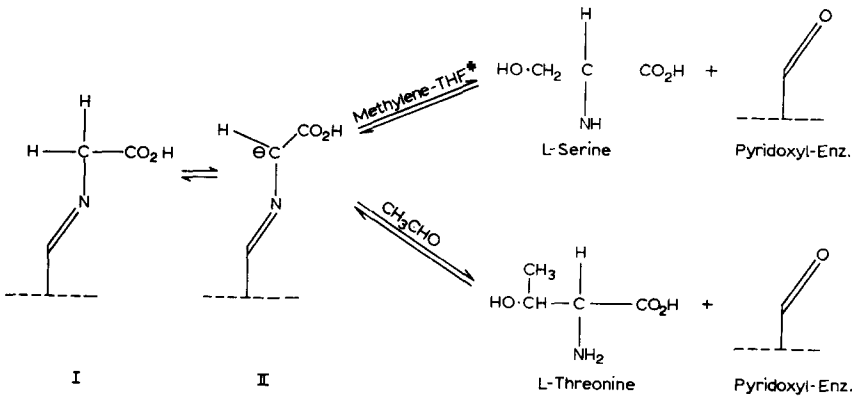
Another condensation reaction between glycine and acetaldehyde, which does not involve the participation of tetrahydrofolate has been attributed to threonine aldolase (L-threonine acetaldehyde-lyase, EC 4.1.2.5)[4] (Eqn. 2):

$$\text{Glycine} + \text{acetaldehyde} \rightleftharpoons \text{threonine (and/or allothreonine)} \quad (2)$$

Mechanistic studies suggest that the first event in these reactions is the formation of glycine–pyridoxal–enzyme complex[5,6] (I) which in the next step undergoes deprotonation to give the carbanion intermediate[7] (II). The latter type of intermediate (II) then either reacts in the serine transhydroxymethylase reaction with the C_1 unit of methylene tetrahydrofolate to give serine or in the threonine aldolase reaction with acetaldehyde to give threonine[7].

Recent kinetic and stereochemical evidence[7,8] has pointed to the possibility that the reaction of Eqn. 2 may also be catalysed by serine transhydroxymethylase

thus suggesting that methylene tetrahydrofolate and acetaldehyde may interchangeably participate in reaction with the carbanion species of the type (II) at the active site of the same enzyme. Such a possibility will have profound implications on the precise mechanism through which C_1 unit transfer between tetrahydrofolate and the carbanion (II) occurs[7].



* THF = tetrahydrofolate

We envisaged, that if serine transhydroxymethylase also catalyses the reaction of Eqn. 2, then alkylating agents derived from acetaldehyde may interfere with its activity. In this communication it is shown that 2-chloroacetaldehyde irreversibly inactivated an enzyme preparation towards the serine transhydroxymethylase as well as the threonine aldolase activities in a colinear fashion.

EXPERIMENTAL

Partially purified serine transhydroxymethylase from rabbit liver acetone powder was prepared by the method described by SCRIMGEOUR AND HUENNEKENS[9] and assayed by Nash's method described by the same authors[9]. The specific activity of the enzyme was 0.35 μmole of formaldehyde consumed per min per mg of protein. The enzyme protein was determined by the Biuret method[10].

A 100-fold purified serine transhydroxymethylase was prepared from the soluble fraction of rabbit liver homogenate by a modification of the method of FUJIOKA[11] as follows. Rabbit livers were homogenized in 5 vol. 0.25 M sucrose solution. The homogenate was passed through a layer of gauze and then centrifuged at 700 $\times$ g for 10 min to separate the nuclear fraction. The mitochondria were removed by centrifugation at 10 000 $\times$ g for 10 min.

Step 1

The 10 000 $\times$ g supernatant (700 ml) was treated with 175 g of solid $(NH_4)_2SO_4$, the precipitated protein was removed by centrifugation and discarded. The supernatant was then treated with 70 g of $(NH_4)_2SO_4$ and the resulting precipitate was collected by centrifugation and dissolved in 0.05 M potassium phosphate buffer (pH 7.1) containing 1 mM EDTA.

Step 2

The enzyme solution was then diluted with distilled water to give a protein concentration of about 10 mg/ml. To this solution were added 0.2 vol. of 0.5 M potassium phosphate buffer (pH 6.5) and L-serine to a final concentration of 20 mM. The mixture was then heated at 63° for 3 min and then rapidly cooled to below 5°. The denatured protein was removed by centrifugation.

Step 3

The supernatant was then treated with $(NH_4)_2SO_4$ as in Step 1. The precipitate obtained was dissolved in 0.05 M potassium phosphate buffer (pH 7.1) containing 1 mM EDTA. The enzyme solution was dialysed overnight against 0.01 M potassium phosphate buffer (pH 7.1) containing 1 mM EDTA and 1.4 mM 2-mercaptoethanol.

Step 4

The dialysed enzyme solution containing 218 mg of protein was then poured onto calcium phosphate gel (7 mg dry wt. of calcium phosphate per mg of protein) which had been equilibrated with 8 μM pyridoxal phosphate and 1.4 mM 2-mercaptoethanol. The mixture was stirred until most of the activity was adsorbed to the gel then the supernatant was discarded after centrifugation. The desorption of the enzyme activity was achieved by elution using different ionic concentrations of potassium

TABLE I

PURIFICATION OF SERINE TRANSHYDROXYMETHYLASE

Fraction	*Volume (ml)*	*Protein (mg/ml)*	*Total units*	*Specific activity (units/mg protein)*	*Yield (%)*	*Ratio of specific activities serine transhydroxymethylase/aldolase*
10 000 × *g* supernatant	700	25.0	350	0.02	(100)	4.34
1st $(NH_4)_2SO_4$	90	44.7	273	0.068	78	4.5
Heat treatment	556	2.0	245	0.22	70	4.4
2nd $(NH_4)_2SO_4$	20	10.9	136	0.63	50	4.3
Calcium phosphate treatment				0.7–2.0		4.2

phosphate buffer (pH 7.1) containing 8 μM pyridoxal phosphate and 1.4 mM 2-mercaptoethanol. The gel was first stirred with 10 ml of 0.05 M buffer, the supernatant was collected by centrifugation, assayed for enzyme activity and protein content. This was repeated three times using the same buffer which removed about 80 mg of protein. The gel was then extracted six times with 5 ml samples of 0.1 M buffer. The protein content per ml of these portions were: 2.0, 2.0, 1.6, 1.6, 1.4 and 0.6 mg, respectively. The respective specific activities being 0.7, 0.9, 1.37, 2.0, 1.33 and 0.4 units per mg. Table I summarizes the typical purification procedure.

Enzyme assay

The assay mixture contained in a total volume of 1.2 ml: glycine, 50 μmoles;

formaldehyde, 2.5 μmoles; pyridoxal phosphate, 0.03 μmole; tetrahydrofolate, 2 μmoles; potassium phosphate buffer (pH 7.5), 120 μmoles and 0.1 to 0.5 mg of enzyme protein. The control contained no glycine. The assay mixtures were incubated at 37° under N_2 for 10 min and the reaction was terminated by 0.3 ml of 20% trichloroacetic acid. The protein was removed by centrifugation and 0.5 ml of supernatant was added to 2.0 ml of Nash's reagent and the colour developed at 37° for 45 min. After the addition of 7.5 ml of water the colour was measured spectrophotometrically at 430 nm.

The threonine aldolase activity was measured spectrophotometrically at 25° by following the rate of oxidation of NADH by acetaldehyde and subsequent decrease of absorbance at 340 nm. The reaction mixture contained in a total volume of 2.0 ml; alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1), 0.05 mg; NADH, 0.24 μmole; DL-allothreonine, 40 μmoles; potassium phosphate buffer (pH 7.4), 120 μmoles and 0.1 to 0.5 mg of serine transhydroxymethylase.

Materials

DL-Tetrahydrofolate was prepared by catalytic hydrogenation of folic acid over platinum oxide as described by HATEFI *et al.*[12]. 2-Chloroacetaldehyde was purchased from Fluka A.G., Switzerland as a 45% solution in water. Calcium phosphate gel was prepared as described by KEILIN AND HARTREE[13]. After the final washing with water the gel was centrifuged and resuspended in 0.01 M potassium phosphate buffer (pH 7.1) to give a suspension containing 50 mg dry wt. of gel per ml of buffer and stored at 0–5°.

RESULTS AND DISCUSSION

Partially purified serine transhydroxymethylase prepared from the acetone powder of rabbit liver by the method of SCRIMGEOUR AND HUENNEKENS[9] (5 mg) in a final volume of 2 ml was treated with 10 mM 2-chloroacetaldehyde at 37°, samples were removed at various time intervals and the residual activity for the synthesis of serine assayed. Fig. 1 shows the time dependent inactivation of the serine transhydroxymethylase activity. When the preincubation mixture in addition to 10 mM 2-chloroacetaldehyde also contained one of the following: 25 mM glycine, 25 mM serine or 20 mM DL-allothreonine, the enzyme activity was partly protected against inactivation. The threonine aldolase activity could not be measured in the presence of 2-chloroacetaldehyde since the latter compound acted as an efficient substrate for alcohol dehydrogenase[14] used in the coupled enzyme assay system. The difficulty was overcome as follows: serine transhydroxymethylase (10 mg) in a total volume of 4 ml was treated with 10 mM 2-chloroacetaldehyde at 37°, 1-ml samples of the incubation mixture were removed at various time intervals and subjected to dialysis against 1 mM potassium phosphate buffer (pH 7.4) for 2 h at 1°. The non-diffusable material was then separately assayed for the serine transhydroxymethylase and threonine aldolase activities as shown in Fig. 2 (Curve B). In another set of experiments when preincubation with 10 mM 2-chloroacetaldehyde was carried out in the presence of 25 mM glycine, 25 mM serine or 20 mM DL-allothreonine both the enzyme activities were partially protected against inactivation (Fig. 2, Curves D, C and E, respectively). That the dialysis used for the removal of 2-chloroacetaldehyde did not adversely

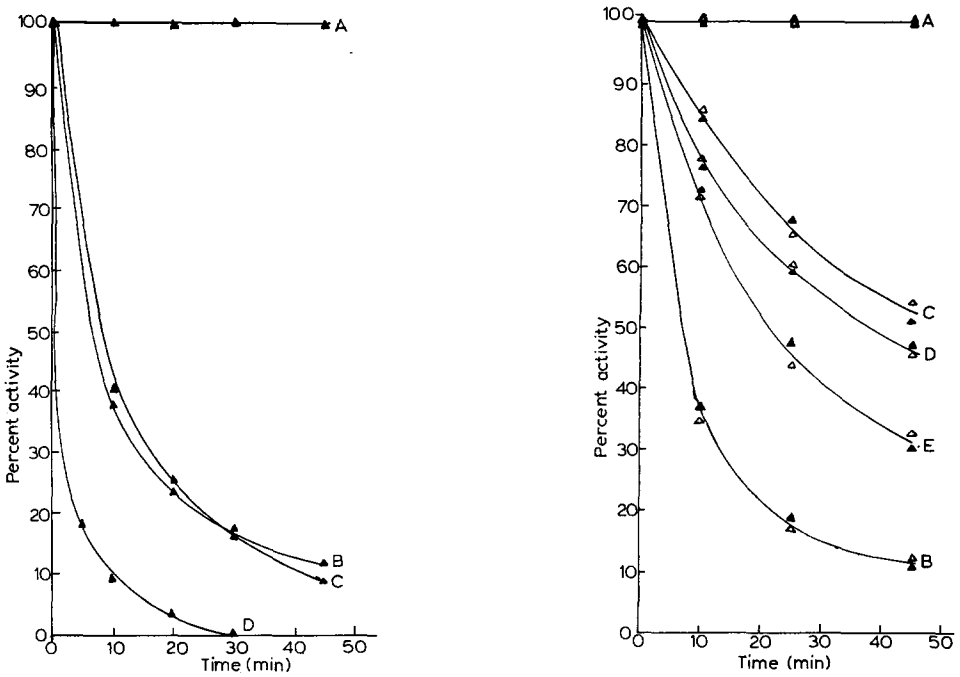
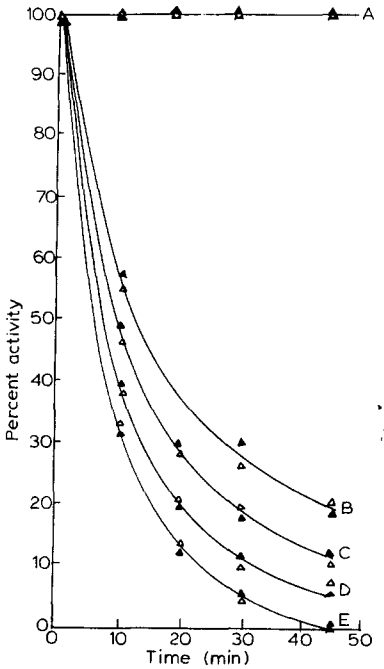


Fig. 1. Time course of the inactivation of the serine transhydroxymethylase activity by 2-chloroacetaldehyde, iodoacetamide and bromopyruvate. Enzyme was preincubated in the presence or absence of inhibitor, samples were removed at various time intervals and the residual serine transhydroxymethylase activity assayed as described in EXPERIMENTAL. Curve A, no addition; Curve B, preincubated with 10 mM 2-chloroacetaldehyde; Curve C, preincubated with 10 mM iodoacetamide; Curve D, preincubated with 5 mM bromopyruvate.

Fig. 2. Time course of the inactivation of serine transhydroxymethylase and threonine aldolase activities by 2-chloroacetaldehyde in the absence or presence of glycine, serine or DL-allothreonine. Enzyme (from acetone powder) was preincubated as below and at various time intervals samples were removed and processed for the determination of the serine transhydroxymethylase and threonine aldolase activities as described in RESULTS. Curve A, no addition; Curve B, preincubated with 10 mM 2-chloroacetaldehyde; Curve C, preincubated with 10 mM 2-chloroacetaldehyde and 25 mM L-serine; Curve D, preincubated with 10 mM 2-chloroacetaldehyde and 25 mM glycine; Curve E, preincubated with 10 mM 2-chloroacetaldehyde and 20 mM DL-allothreonine. ▲—▲, serine transhydroxymethylase activity; △—△, threonine aldolase activity.

effect either enzymic activity is shown by the result of Curve A, Fig. 2 and Curve A, Fig. 3.

FUJIOKA[11] has recently reported the purification of cytoplasmic serine transhydroxymethylase from rabbit liver. Using a modification of the method a 100-fold purified serine transhydroxymethylase was prepared. Under our assay condition the activity of the 10 000 × *g* supernatant was 0.02 unit per mg of protein and of the final preparation was 2.0 units per mg of protein. The activities reported by FUJIOKA[11] for similar stages are 0.057 and 7.4 units per mg of protein, respectively. At each stage of purification the ratio of serine transhydroxymethylase to the threonine aldolase activities was found to be constant (Table I) thus confirming the previous result of SCHIRCH AND GROSS[8]. The effect of 2-chloroacetaldehyde on the cytoplasmic serine



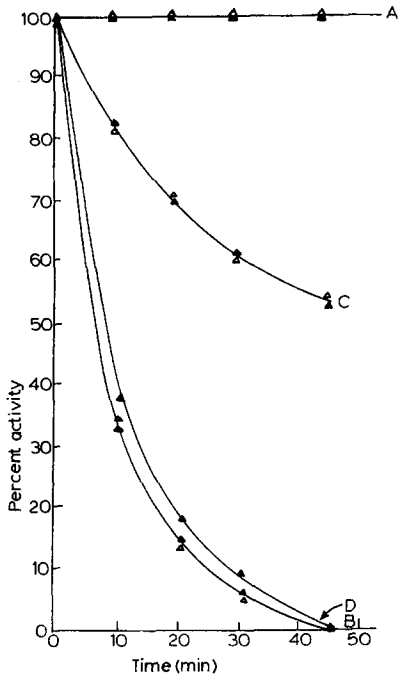


Fig. 3. Time course of inactivation of serine transhydroxymethylase and threonine aldolase activities at different steps of purification by 2-chloroacetaldehyde. Enzyme from each step was preincubated as below and at various time intervals samples were removed and processed for the determination of serine transhydroxymethylase and threonine aldolase activities as described in RESULTS and Fig. 2. Curve B, 10 000 × *g* supernatant (Step 1, Table I) enzyme preincubated with 15 mM 2-chloroacetaldehyde; Curve C, 3.4-fold purified enzyme (Step 2) preincubated with 15 mM 2-chloroacetaldehyde; Curve D, 30-fold purified enzyme (Step 4) preincubated with 10 mM 2-chloroacetaldehyde; Curve E, 100-fold purified enzyme (Step 5) preincubated with 7.5 mM 2-chloroacetaldehyde; Curve A represents the enzyme from each step preincubated and processed as above but without 2-chloroacetaldehyde. ▲—▲, serine transhydroxymethylase activity; △—△, threonine aldolase activity.

Fig. 4. Time course of inactivation of serine transhydroxymethylase and threonine aldolase activities of the 100-fold purified enzyme by 2-chloroacetaldehyde in the absence or presence of glycine or L-alanine. Enzyme was preincubated as above and at various time intervals samples were removed and processed for the determination of the serine transhydroxymethylase and threonine aldolase activities as described in RESULTS. Curve A, no addition; Curve B, preincubated with 7.5 mM 2-chloroacetaldehyde; Curve C, preincubated with 7.5 mM 2-chloroacetaldehyde and 25 mM glycine; Curve D, preincubated with 7.5 mM 2-chloroacetaldehyde and 25 mM L-alanine. ▲—▲, serine transhydroxymethylase activity; △—△, threonine aldolase activity.

transhydroxymethylase was studied as follows: serine transhydroxymethylase (2–3 units) was treated with chloroacetaldehyde at 37°, samples were removed at various time intervals and processed for the determination of serine transhydroxymethylase and threonine aldolase activities as above. The result in Fig. 3 shows that at all stages of purification the enzyme was inactivated against both serine transhydroxymethylase and threonine aldolase activities in a coordinated fashion. As expected the inhibitory effect of 2-chloroacetaldehyde improved with the purification of the enzyme. Once again when the preincubation mixture also contained 25 mM glycine both the enzymic activities were partially protected against inactivation (Fig. 4). That this

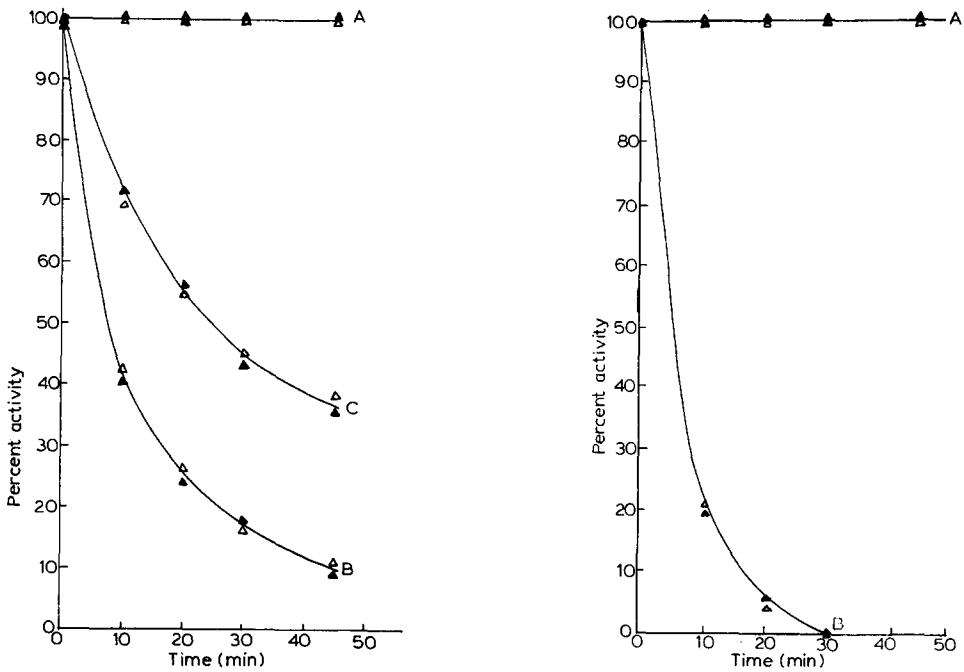


Fig. 5. Time course of inactivation of serine transhydroxymethylase and threonine aldolase activities by iodoacetamide in the presence or absence of glycine. Enzyme was preincubated as below and at various time intervals samples were removed and processed for the determination of the serine transhydroxymethylase and threonine aldolase activities as described in RESULTS except that the samples were not subjected to dialysis. Curve A, no addition; Curve B, preincubated with 10 mM iodoacetamide; Curve C, preincubated with 10 mM iodoacetamide and 25 mM glycine. ▲—▲, serine transhydroxymethylase activity; △—△, threonine aldolase activity.

Fig. 6. Time course of inactivation of the serine transhydroxymethylase and threonine aldolase of the purified enzyme by 1 mM bromopyruvate. Serine transhydroxymethylase (2 mg) in a total volume of 3 ml was treated with 1 mM bromopyruvate at 37°. Samples were removed at various time intervals and processed for the determination of serine transhydroxymethylase and threonine aldolase activities as described in RESULTS. Curve A, no addition; Curve B, preincubated with 1 mM bromopyruvate. ▲—▲, serine transhydroxymethylase activity; △—△, threonine aldolase activity.

protection was not due to destruction of 2-chloroacetaldehyde by its reaction with the amino group of glycine was established as follows. The possible reaction of the amino group of an amino acid with 2-chloroacetaldehyde was studied titrimetrically by following the rate of formation of HCl using an automatic titrimeter connected to a pH meter and a titrigraph. No reaction was detected for up to 2 h. In another experiment the enzyme and 2-chloroacetaldehyde were preincubated with 25 mM of either glycine or L-alanine (which is not a substrate) and the result in Fig. 4 shows that the latter compound did not protect the enzyme against inactivation thus suggesting little if any reaction of the inhibitor with L-alanine.

A number of other alkylating agents such as chloroacetate, chloroacetamide, iodoacetate, iodoacetamide and bromopyruvate were also investigated as possible inhibitors for the enzyme. Of these iodoacetamide and bromopyruvate irreversibly inactivated both the serine transhydroxymethylase and threonine aldolase activities

in a manner similar to that noted for 2-chloroacetaldehyde (Figs. 5 and 6). Other compounds had no significant inhibitory action against either of the enzyme activities.

In view of the fact that alkylating agents structurally less closely resembling the substrate such as iodoacetamide and bromopyruvate could replace chloroacetaldehyde, it is not possible to ascertain whether or not the inactivation noted in the present work involved the modification of the active site. It is, though, noteworthy that a plot of the half-life for inactivation *versus* the reciprocal of the inhibitor

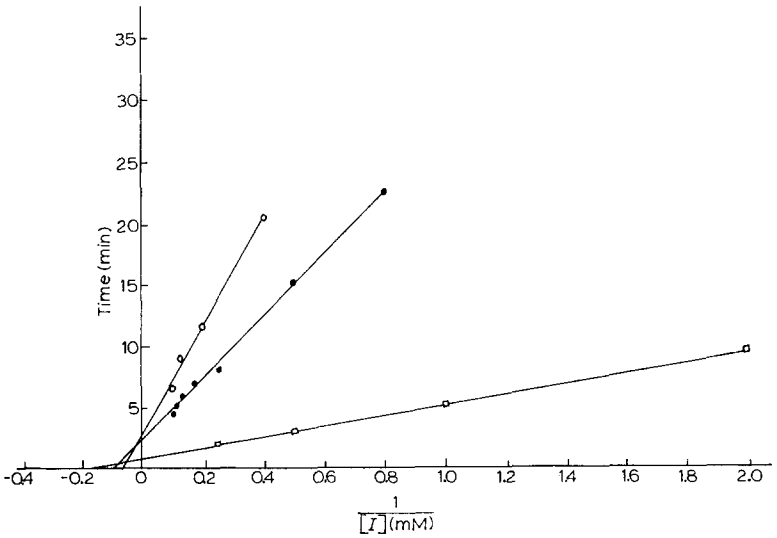


Fig. 7. Inactivation half-time as a function of the reciprocal of inhibitor concentration. Inactivation half-times were obtained by preincubating the enzyme with various concentrations of each of the inhibitors and assaying the residual serine transhydroxymethylase activity at various time intervals and then plotting log percentage activity as a function of preincubation time[15]. ●—●, 2-chloroacetaldehyde; ○—○, iodoacetamide; □—□, bromopyruvate.

concentration gave a straight line not passing through zero (Fig. 7). Thus the inactivation obeys saturation kinetics providing evidence that the inhibitors (2-chloroacetaldehyde, iodoacetamide and bromopyruvate) formed a dissociable complex prior to inactivation[15].

The main feature of the present study is the demonstration that 2-chloroacetaldehyde and iodoacetamide inhibit serine transhydroxymethylase activity, and the factors contributing to the inhibition also influence the threonine aldolase activity in an identical manner. The experiments confirm the earlier view of SCHIRCH AND GROSS[8], that serine transhydroxymethylase and threonine aldolase are the property of the same or very similar enzymes, and endorse (but do not prove) the hypothesis of JORDAN AND AKHTAR[7] regarding the mechanism of action of serine transhydroxymethylase.

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