3. 1:2:3:5-Tetrachlorobenzene is very slowly metabolized. Only about 5 % is oxidized to and excreted as 2:3:4:6-tetrachlorophenol in 6 days. Some 14 % is eliminated unchanged in the faeces, 12 % in the breath and 23 % remains in the tissues after 6 days. There is evidence that some 9 % of the dose is dechlorinated and eliminated in the expired air as less chlorinated benzenes. Some 5 % of the dose may also be excreted in the urine as di- and tri-chlorophenols. Injected 1:2:3:5-tetrachlorobenzene is partly excreted as such in the faeces, probably via the bile.

4. 1:2:4:5-Tetrachlorobenzene appears to be the least readily metabolized of the three isomers. Only about 2 % is converted into 2:3:5:6-tetrachlorophenol in 6 days; 48 % of the dose was found in the tissues after 6 days, and 16 % was in the faeces and 2 % in the expired air. Dechlorination products could account for 15 % of the dose, about 10 % of the dose appearing in the expired air as less chlorinated benzenes and 5 % in the urine as di- and tri-chlorophenols. Dechlorination of the tetrachlorobenzenes is believed to occur in the gut, probably under the influence of bacteria.

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Glutamic–Alanine and Glutamic–Aspartic Transaminases of Wheat Germ

By D. H. CRUICKSHANK*
Botany School, University of Cambridge

and F. A. ISHERWOOD
Low Temperature Research Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research

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Since the discovery of the transamination reaction by Braunstein & Kritzmann (1937), transaminase systems have been studied in extracts from a number of plant and animal tissues and micro-organisms. This work has been reviewed by Braunstein (1947) and Cohen (1951, 1954). Results of many of the early investigations were conflicting, as crude enzyme preparations and non-specific quantitative methods were used. Recently much information on the properties of animal and microbial transaminases has been obtained with purified enzyme preparations and specific quantitative methods. There is, however, little precise information on the plant transaminases.

* Present address: Division of Food Preservation and Transport C.S.I.R.O., Botany School, University of Sydney, Australia.

In the present investigation a study has been made of some of the properties of two partially purified transaminase enzymes from wheat germ. Wheat germ was chosen as the plant material because Leonard & Burris (1947) had previously demonstrated that it contained active transaminase systems. The two transaminase systems were as follows:

(1) L-Glutamic acid + pyruvic acid ⇌ α-oxoglutaric acid + L-alanine

(catalysed by glutamic–alanine transaminase)

(2) L-Glutamic acid + oxaloacetic acid ⇌ α-oxoglutaric acid + L-aspartic acid

(catalysed by glutamic–aspartic transaminase)

These reactions were followed by the specific chromatographic methods recently developed by Isherwood & Cruickshank (1954*a*, *b* respectively).

MATERIALS AND METHODS

Enzyme preparation

Commercial wheat germ (kindly supplied by Dr J. Pace of the Research Association of British Flour Millers, Cereal Research Station, St Albans) was extracted three times with 1·5 vol. of ether and dried at room temperature. Removal of the fat caused the original flaky material to disintegrate into a powder. This defatted powder (15 g.) was suspended in 60 ml. of water in a stoppered bottle and the mixture agitated gently for 3 hr. by slowly rotating the bottle between rollers. The mixture was then centrifuged; the milky supernatant was adjusted to pH 5·7 with 2N-acetic acid and the liquid again centrifuged. The clear, brown supernatant was brought to pH 7 with 2N-NaOH and the liquid treated with saturated $(NH_4)_2SO_4$ (pH 7) at 5°. The fraction precipitated between 33 and 66% saturation was suspended in a few millilitres of water, dialysed for 15 hr. at 5° against 1% (w/v) KCl and finally adjusted to pH 7·5 or 8·0 with N-NaOH. No α-keto acids or amino acids could be detected in this preparation by the chromatographic methods referred to above.

Substrates

α-Keto acids. Commercial samples of α-oxoglutaric acid, oxaloacetic acid and pyruvic acid (as sodium pyruvate) were used. Chromatographic examination of their 2:4-dinitrophenylhydrazones showed that they were free from other keto acids. Immediately before use neutral 0·2M-solutions were prepared (with the addition of dil. NaOH if necessary) and these were diluted to 0·1M with 0·2M-phosphate buffer (KH_2PO_4–NaOH), pH 7·5 or 8·0.

α-Amino acids. Commercial preparations were used. These were found by paper chromatography to be free from other amino acids. For enzyme studies the amino acids were prepared as 0·2M neutral solutions and diluted to 0·1M immediately before use with 0·2M-phosphate buffer (KH_2PO_4–NaOH), pH 7·5 or 8·0.

Pyridoxal phosphate. A sample of the calcium salt of pyridoxal phosphate was kindly provided by Dr E. F. Gale, F.R.S., Department of Biochemistry, University of Cambridge. Immediately before use, 40 μg. was dissolved in 1 ml. of 0·2M-phosphate buffer, pH 7·5 or 8·0.

Procedure

To small tubes were added 1 vol. (0·2–0·4 ml.) of 0·2M-phosphate buffer (KH_2PO_4–NaOH), 1 vol. of 0·1M-α-amino acid solution and 1 vol. of transaminase preparation, and the mixture was incubated at 25° for 10 min.; 1 vol. of 0·1M-α-keto acid solution was then added. To test the effect of inhibitors or coenzymes this procedure was slightly modified. Enzyme solution (2 vol.) was incubated for 15 min. at 25° with 2 vol. of inhibitor or coenzyme and 2 vol. of phosphate buffer and then 1 vol. of 0·2M-α-amino acid and 1 vol. of 0·2M-α-keto acid were added. Control experiments were carried out in which the enzyme preparation, phosphate buffer and water were incubated with and without addition of each α-amino acid or α-keto acid.

At specified times samples were removed for analysis. For the estimation of α-keto acids, 0·1 ml. of the enzyme digest was added to 0·5 ml. of 0·01M-2:4-dinitrophenylhydrazine in 0·2N-HCl in ethanol contained in a stoppered centrifuge tube. The α-keto acids were then estimated by the method of Isherwood & Cruickshank (1954*a*). For the quantitative determination of the α-amino acids, a sample (0·1 ml.) of the enzyme reaction mixture was added to 0·05 ml. of 0·3N-acetic acid. The mixture was centrifuged and samples of the supernatant were used for the estimation of the α-amino acids by the method of Isherwood & Cruickshank (1954*b*).

Results have been expressed as micromoles of reactant present in 1 ml. of enzyme digest. The percentage transamination (% *T*), used to express transaminase activity, is defined as the percentage of the initial substrate transaminated during a specified time. This has been determined from the amount of substrate utilized or the amount of reaction product formed: the results should be identical whichever method is used.

RESULTS

The purified enzyme preparation contained only glutamic–alanine and glutamic–aspartic transaminases. A preliminary examination of the crude aqueous extract of wheat germ showed that other transaminases were present, for transamination occurred to a small extent between α-oxoglutaric acid and valine, leucine, phenylalanine, tyrosine and tryptophan. These other transaminases were lost during the purification procedure described above.

Measurements in the glutamic–alanine system

Preliminary experiments showed that there was no significant change in any of the reactants (reaction 1) when incubated singly with the enzyme preparation and that the value obtained for rate of transamination was the same whichever component was determined. Some results are given in Table 1.

The results indicated that the glutamic–alanine transamination system was not being influenced by side reactions involving the formation or destruction of any of the reactants, and that the estimation of any one of the reactants would serve as a measure of the progress of the reaction.

Progress curves for the forward and reverse reactions are shown in Fig. 1. The points on the curves were obtained by estimating α-oxoglutaric acid and pyruvic acid at each specified time.

The initial rates for the forward and reverse reactions were almost equal: at 7·5 min. 27·4% of the L-glutamic acid had been converted into α-oxoglutaric acid and 26·6% of the L-alanine into pyruvic acid. Equilibrium was reached in 30 min., the system remaining unchanged after incubation for a further 30 min. The equilibrium was slightly in favour of the formation of alanine and α-oxoglutaric acid, the separate determination of pyruvic

Table 1. *Disappearance of substrate and formation of reaction product of a glutamic–alanine transaminase system of wheat germ*

Concentrations are expressed as μmoles of reactant/ml. of digest; % *T* is the percentage of initial added keto (or amino) acid transaminated. Temp. 25°; pH 7·5.

Substrate	Incubation period (min.)	Glutamic acid (μmoles/ml.)	% *T*	Pyruvic acid (μmoles/ml.)	% *T*	α-Oxoglutaric acid (μmoles/ml.)	% *T*	Alanine (μmoles/ml.)	% *T*
Glutamic acid + pyruvic acid	0	25·0	—	25·0	—	—	—	—	—
	10	17·5	30·0	16·8	32·8	8·0	32·0	8·8	35·2
Alanine + α-oxoglutaric acid	0	—	—	—	—	25·0	—	25·0	—
	10	7·8	31·2	8·0	32·0	17·3	30·8	16·8	32·8

Table 2. *Disappearance of substrate and formation of reaction product of a glutamic–aspartic transaminase system of wheat germ*

Concentrations are expressed as μmoles of reactant/ml. of digest; % *T* is the percentage of initial added keto (or amino) acid transaminated. Temp. 25°; pH 8.

Substrate	Incubation period (min.)	Glutamic acid (μmoles/ml.)	% *T*	Oxaloacetic acid (μmoles/ml.)	% *T*	α-Oxoglutaric acid (μmoles/ml.)	% *T*	Aspartic acid (μmoles/ml.)	% *T*
Glutamic acid + oxaloacetic acid	0	25·0	—	25·0	—	—	—	—	—
	10	14·3	42·8	12·1	50·0	11·5	46·0	10·5	42·0
Aspartic acid + α-oxoglutaric acid	0	—	—	—	—	25·0	—	25·0	—
	10	6·3	25·2	5·5	22·0	18·8	24·8	19·0	24·0

and α-oxoglutaric acids giving practically the same figure for the equilibrium mixture (about 55 % of the L-glutamic acid was changed). The equilibrium constant for reaction (1),

$$K = \frac{[\text{α-oxoglutaric acid}]\,[\text{L-alanine}]}{[\text{L-glutamic acid}]\,[\text{pyruvic acid}]},$$

was calculated after determining all four reactants, and was found to be 1·4 at pH 7·5.

Measurements in the glutamic–aspartic system

Preliminary experiments showed that there were no significant changes in the concentrations of L-glutamic, L-aspartic or α-oxoglutaric acids when they were incubated singly with the enzyme preparation. However, oxaloacetic acid was slowly converted into pyruvic acid and carbon dioxide in aqueous solution (with KH_2PO_4–NaOH buffer, pH 8) and this instability meant that a perfect equilibrium as described in reaction (2) could not be reached in practice. In the present experiments the loss in oxaloacetic acid due to non-enzymic decarboxylation to pyruvic acid was found to be 2–4 % in 10 min. and a similar figure was obtained when oxaloacetic acid was incubated with the enzyme preparation. Some preliminary results for the transamination reaction are described in Table 2. These have been corrected for the slight loss due to non-enzymic decarboxylation mentioned above but it is noticeable that even after this correction the % *T* based on oxaloacetic acid

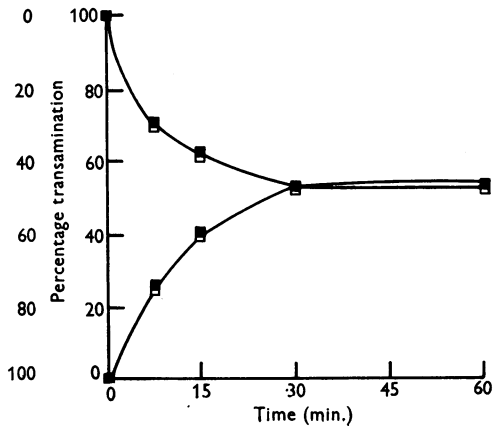

Fig. 1. Rates of forward and reverse reactions catalysed by glutamic–alanine transaminase. Ordinate numbers on the right show % *T* measured by pyruvic acid disappearance (or α-oxoglutaric acid formation) in reaction (1), with the substrates glutamic acid + pyruvic acid. Ordinate numbers on the left show % *T* measured by α-oxoglutaric acid disappearance (or pyruvic acid formation) in (1), with the substrates alanine + α-oxoglutaric acid. Substrate concn. 0·025 M; temp. 25°; pH 7·5. □, α-Oxoglutaric acid; ■, pyruvic acid.

estimations was slightly higher for the forward reaction and lower for the reverse reaction (reaction 2) than the % T from estimations of the other reactants. This indicated that the rate of oxaloacetic acid decomposition was greater in the complete reaction mixture than that observed in the presence of the enzyme alone. It appears that the presence of glutamic or aspartic acids and, to a lesser extent, α-oxoglutaric acid increases the non-enzymic breakdown (Nisonoff, Henry & Barnes, 1952). Though an active glutamic–alanine enzyme was present in the extract used, the amount of alanine formed from the pyruvic acid produced in this way was not detectable on a paper chromatogram at 10 min. By comparison the % T of glutamic acid was of the order of 42 %, which meant that the rate of the side reaction was relatively small under the experimental conditions.

The results in Table 2, based on initial reaction velocities, show that the forward reaction was about twice as fast as the reverse reaction.

Complete progress curves for the forward and reverse reactions are shown in Fig. 2. These were based on estimations of α-oxoglutaric acid and oxaloacetic acid.

The forward reaction was 5–10 % more rapid if measured by disappearance of oxaloacetic acid than if measured by α-oxoglutaric acid formation. In the reverse reaction the rate of disappearance of α-oxoglutaric acid was higher than the rate of formation of oxaloacetic acid. Equilibrium was reached for both reactions in 30 min., but the observed position of equilibrium depended on whether oxaloacetic acid or α-oxoglutaric acid was determined. It was 75 % in favour of the forward reaction, if oxaloacetic acid formation and disappearance were measured, and 68 % if α-oxoglutaric acid was determined. Small changes detected in the period from 30 to 60 min. were probably caused by the decarboxylation of the oxaloacetic acid: control experiments showed that the non-enzymic destruction of oxaloacetic acid increased from 7 to 13 % during the period 30–60 min. Calculation of the equilibrium constant was made by directly estimating the concentration of each of the reactants after 30 and 60 min. The relatively slow change in the oxaloacetic acid concentration was not sufficient to affect seriously the equilibrium concentrations of the various reactants. The equilibrium constant

$$K = \frac{[\alpha\text{-oxoglutaric acid}]\,[\text{L-aspartic acid}]}{[\text{oxaloacetic acid}]\,[\text{L-glutamic acid}]}$$

was 5·0, with initial substrate concentrations 0·025 M, pH 8 and temperature 25°.

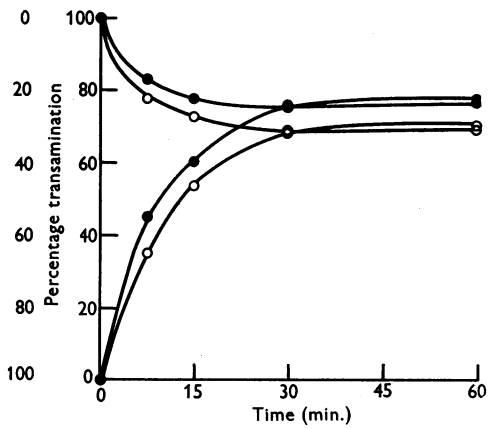

Fig. 2. Rates of forward and reverse reactions catalysed by glutamic–aspartic transaminase. Ordinate numbers on the right show % T measured by oxaloacetic acid disappearance (or α-oxoglutaric acid formation) in reaction (2), with the substrates glutamic acid + oxaloacetic acid. Ordinate numbers on the left show the % T measured by α-oxoglutaric acid disappearance (or oxaloacetic acid formation) in (2), with the substrates aspartic acid + α-oxoglutaric acid. Substrate concn. 0·025 M; temp. 25°; pH 8. ○, α-Oxoglutaric acid; ●, oxaloacetic acid.

Enzyme properties

Pyridoxal phosphate. Addition of pyridoxal phosphate (10 μg. of the calcium salt to 1 ml. of the reaction mixture) to digests containing the purified enzyme from wheat germ increased the glutamic–aspartic enzyme activity by 50–80 %, depending on the treatment to which the enzyme had been subjected during purification, but did not affect the glutamic–alanine enzyme activity at all. The difference between the two enzymes was probably due to the difficulty of resolving the apoenzyme from coenzyme in the latter. Attempts to resolve the enzyme complexes by dialysis against distilled water, aq. 0·002 N-NH_3 soln. or 1 % KCl for 72 hr. were inconclusive, as the enzymes were largely irreversibly inactivated by the treatments.

Effect of pH. The effect of pH on transaminase activity (measured as % T in 10 min., under standard conditions) is shown in Fig. 3.

The optimum pH of the glutamic–alanine system was approximately 7·5 but there was little change between pH 7 and 8. The glutamic–aspartic transaminase showed maximum activity between pH 8·0 and 8·5.

Effect of temperature. The effect of temperature on enzyme activity (measured as % T in 10 min. under standard conditions) is shown in Fig. 4. The optimum temperature for each reaction was between 40° and 50°. The fact that very little difference in activity was found between 40° and 50°, under the experimental conditions used, suggested that inactivation of the enzymes was proceeding at 50°.

Table 3. *Effect of some inhibitors on glutamic–alanine and glutamic–aspartic transaminase activity*

Substrate concn. 0·025 M; temp. 25°; pH 7·5; incubation period 10 min.

Inhibitor	Final concn. (mM)	Substrate: Glutamic acid + pyruvic acid — α-Oxoglutaric acid formed (μmoles/ml. of digest)	Substrate: Glutamic acid + pyruvic acid — Inhibition (%)	Substrate: Glutamic acid + oxaloacetic acid — α-Oxoglutaric acid formed (μmoles/ml. of digest)	Substrate: Glutamic acid + oxaloacetic acid — Inhibition (%)
—	—	7·5	—	8·8	—
Iodoacetate	1·0	7·5	0	8·0	9
Malonate	10·0	7·0	7	8·8	0
KCN	1·0	4·5	40	7·0	20
$AgNO_3$	0·1	5·0	33	8·8	0
$AgNO_3$	1·0	0·0	100	5·3	40
Semicarbazide	1·0	6·2	18	7·1	19
Semicarbazide	2·0	2·3	70	3·5	60
Hydroxylamine	1·0	0·8	90	1·4	84

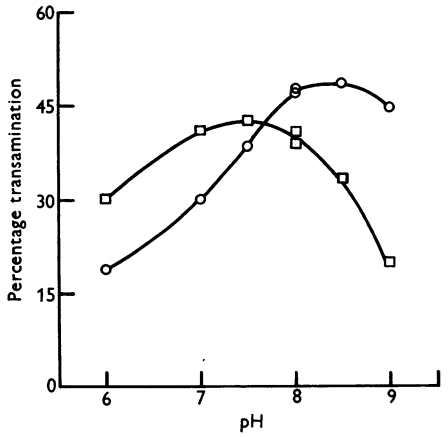

Fig. 3. Effect of pH on transaminase activity. % T measured by α-oxoglutaric acid formed. Substrate concn. 0·025 M; temp. 25°; incubation period 10 min.; pH 6–8, 0·05 M-phosphate buffer (KH_2PO_4–NaOH); pH 8–9, 0·05 M-sodium borate–potassium chloride buffer ($Na_2B_4O_7$–KCl). □, Glutamic–alanine transaminase, substrates glutamic acid + pyruvic acid; ○, glutamic–aspartic transaminase, substrates glutamic acid + oxaloacetic acid.

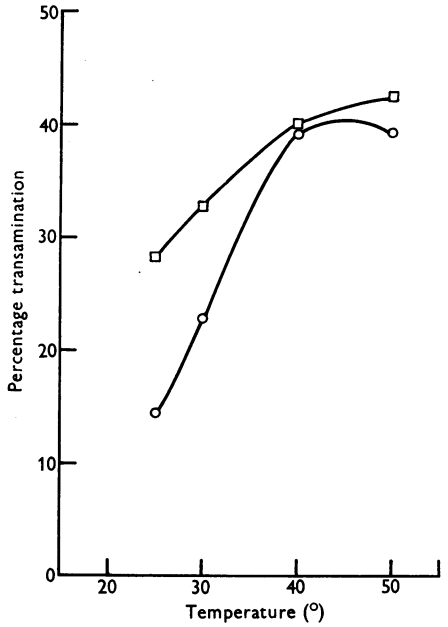

Fig. 4. Effect of temperature on transaminase activity % T was measured by α-oxoglutaric acid formed. Substrate concn. 0·25 M; incubation period 10 min. □, Glutamic–alanine transaminase, substrates glutamic acid + pyruvic acid, pH 7·5; ○, glutamic–aspartic transaminase, substrates glutamic acid + oxaloacetic acid, pH 8.

The temperature coefficient (Q_{10}) was calculated for each reaction from the ratio % T (α-oxoglutaric acid) at 35°/% T (α-oxoglutaric acid) at 25°. The Q_{10} of reactions (1) and (2) was 1·3 and 2·1 respectively.

Inhibitors. The effects of different inhibitors on the activity of the two enzymes is listed in Table 3.

The glutamic–alanine transaminase was completely inhibited by mM-$AgNO_3$ but only 30% inhibited by 0·1 mM-$AgNO_3$. The glutamic–aspartic transaminase was less sensitive to the presence of Ag^+ ions. Iodoacetate (mM) and malonate (10 mM) did not affect the transaminase activity.

DISCUSSION

The present study indicates that the glutamic–alanine enzyme from wheat germ has many properties similar to those of the corresponding enzymes from animals and micro-organisms. The equilibrium constant for the reaction, 1·4 at pH 7·5 and 25°, is close to the latest figure of 1·53 at pH 7·4 and 25° reported by Krebs (1953) for the

animal enzyme. Lénárd & Straub (1942) reported 1·43 and Cohen (1940) 1·0 for this enzyme from animal sources. The enzyme showed its maximum activity at pH 7·5, but there was only a small change in activity between pH 7 and 8. Kritzmann (1939) records 7·4 for plant and animal transaminase systems and Rautanen (1946) 6·9 for the glutamic–alanine enzyme from many plant tissues. The effect of temperature on the rate of the transamination reaction is complicated by the inactivation of the enzyme above 50°, but if this is ignored an apparent optimum was found between 40° and 50° under the experimental conditions used. Rautanen (1946) reported a figure of 41°, but a longer incubation period was used to measure the initial rate of the reaction. The effect of pyridoxal phosphate on the purified enzyme was small, though it seems clear that this compound is the coenzyme for transaminase enzymes from animal tissues and micro-organisms (Lichstein, Gunsalus & Umbreit, 1945; Schlenk & Snell, 1945; Green, Leloir & Nocito, 1945). Attempts to resolve the purified enzyme into apoenzyme and coenzyme by dialysis against various aqueous solutions were only partially successful, and it seems that the plant enzyme must be much less easily split without denaturation of the protein moiety than some of the animal enzymes. The inhibition of the glutamic–alanine transaminase from wheat germ by silver nitrate and potassium cyanide, and the lack of inhibitory effect with iodoacetate and malonate, are in general agreement with the results reported for animal transaminases (Braunstein, 1947). The main differences between the enzyme from wheat germ and that from animal sources is the sensitivity of the former to hydroxylamine and semicarbazide. This suggests that an aldehyde or keto group is present in the enzyme complex and that if it is attached to pyridoxal phosphate, the link between the apoenzyme and the coenzyme must be different in the plant and animal enzymes.

The properties of the glutamic–aspartic enzyme from wheat germ were similar to those of the enzymes extracted from animal tissue and from micro-organisms. The equilibrium constant at pH 8 and 25° was 5·0. Figures of 5·4 at pH 7·5 and 25°, 6·7 at pH 7·4 and 25° and 7·8 at pH 7·4 and 37·5°, have been reported by Darling (1945), Krebs (1953) and Nisonoff, Barnes & Enns (1953) respectively for animal enzymes. The enzyme showed optimum activity between pH 8·0 and 8·5. For the corresponding enzyme in oat embryo (Albaum & Cohen, 1943), *Escherichia coli* (Lichstein & Cohen, 1945) and *Streptococcus faecalis* (Lichstein *et al.* 1945) optimum activity was shown at pH 8·6, 8·5 and somewhere between 5·7 and 9·5 respectively. The activity of the enzyme reached an apparent maximum between 40° and 50°, as with the glutamic–alanine enzyme from wheat germ. Cohen (1940) reported a figure of 40° for the animal enzyme. The effect of pyridoxal phosphate on the purified enzyme from wheat germ suggests that a considerable resolution into apoenzyme and coenzyme had occurred during the isolation procedure. This contrasts sharply with our experience with the glutamic–alanine enzyme, which did not split at all readily, but is similar to the observations of O'Kane & Gunsalus (1947) on pig heart-muscle transaminases. They found that the glutamic–aspartic enzyme was resolved by heating at 56° followed by ammonium sulphate precipitation, whereas the glutamic–alanine enzyme was not split. The sensitivity of the glutamic–aspartic enzyme from wheat germ to hydroxylamine and semicarbazide is similar to that of the glutamic–alanine enzyme and is much greater than with the corresponding animal enzymes (Braunstein, 1947). The configuration of the enzyme complex is probably different for the two groups of enzymes.

The separation and purification of the glutamic–alanine and glutamic–aspartic transaminases of heart muscle have been reported from several laboratories (Meister, 1955), so that it is clear that reactions (1) and (2) are catalysed by two different enzymes in the animal. In the present work on wheat-germ extracts, we have not separated the individual enzymes but there seems little doubt that two separate enzymes are also responsible for catalysing reactions (1) and (2) in the plant. Treatment of the original extract with ammonium sulphate gave a fraction in which the glutamic–aspartic enzyme was largely in the apoenzyme form whereas the glutamic–alanine enzyme was still unresolved. The effect of silver nitrate on this fraction from wheat-germ extract also suggested that two different enzymes were responsible. The glutamic–alanine enzyme was inhibited at lower concentrations of Ag^+ ion than the glutamic–aspartic enzyme. If a single protein had been responsible for both enzyme activities the effect of Ag^+ ions might be expected to be similar for both enzymes.

SUMMARY

1. The transamination reactions

L-glutamic acid + pyruvic acid ⇌ α-oxoglutaric acid + L-alanine

(catalysed by glutamic–alanine transaminase)

and

L-glutamic acid + oxaloacetic acid ⇌ α-oxoglutaric acid + L-aspartic acid

(catalysed by glutamic–aspartic transaminase)

have been studied with partially purified enzyme preparations from wheat germ.

2. The glutamic–alanine transaminase has an equilibrium constant of 1·4 at 25° and pH 7·5, and an optimum pH of 7·5.

3. The glutamic–aspartic enzyme has an equilibrium constant of 5·0 at 25° and pH 8 and an optimum pH between pH 8·0 and 8·5.

4. The effect of pyridoxal phosphate and some inhibitors on the transaminases suggests that the two systems may be catalysed by two different enzymes, as are the animal glutamic–alanine and glutamic–aspartic transaminase systems.

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The Nature of the Link Between Protein and Carbohydrate of a Chondroitin Sulphate Complex from Hyaline Cartilage

By HELEN MUIR
Medical Unit, St Mary's Hospital, London, W. 2 and Postgraduate Medical School, Ducane Road, London, W. 12

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Chondroitin sulphate from hyaline cartilage has been prepared by several procedures which give products with physical properties differing according to the method used for extraction. Successively larger quantities can be extracted from fresh cartilage by water (Partridge, 1948*b*), neutral salts such as 30 % potassium chloride (Einbinder & Schubert, 1950) or 10 % calcium chloride (Meyer & Smyth, 1937; Blix & Snellman, 1945) and by alkali, which has been used extensively (Mörner, 1889; Levene & La Forge, 1913; Jorpes, 1928; Fürth & Bruno, 1937; Bray, Gregory & Stacey, 1944; Mathews, Roseman & Dorfman, 1951). Shrinkage of collagen in cartilage by heat-treatment increases the yield of water-soluble chondroitin sulphate (Partridge, 1948*b*), as does very prolonged extraction with water (Shatton & Schubert, 1954). The yield from alkaline extractions is high, but extraction with neutral salt gives much smaller yields and the remaining polysaccharide can be extracted only with alkali (Blix & Snellman, 1945; Einbinder & Schubert, 1950). By making a solution of potassium chloride mildly alkaline with potassium carbonate Einbinder & Schubert (1950) increased the yield of chondroitin sulphate considerably. Alkali is therefore particularly effective in releasing soluble chondroitin sulphate from cartilage, but the polysaccharide is obtained in a comparatively degraded form as judged by the relatively low molecular weight of such preparations, although the values depend partly on the method of measurement. Values ranging from 975