OXALACETIC ACID AS AMINO GROUP ACCEPTOR IN TRANSAMINATION*

by

P. S. CAMMARATA** AND PHILIP P. COHEN

Department of Physiological Chemistry, University of Wisconsin, Madison (U.S.A.)

INTRODUCTION

In a previous report from this laboratory[1] it has been demonstrated that the transamination of α-ketoglutaric acid is of wide scope, occurring in all mammalian tissues tested, and involving a large number of amino acids. This study did not consider the possibility of another keto acid acting as an amino group acceptor. BRAUNSTEIN AND KRITZMAN[2] in their original statement of the reaction claimed that many α-keto acids, as well as α-ketoglutaric acid, could act as amino group acceptors in transamination from any amino acid, but later restricted their claim to α-ketoglutaric, pyruvic, and oxalacetic acids. Specifically, they claimed to have evidence for the existence of an enzyme which catalyzed the transfer of the amino group of alanine to oxalacetic acid. GREEN, LELOIR AND NOCITO[3] succeeded in resolving the glutamic-oxalacetic and glutamic-pyruvic systems, but could find no evidence for the existence of an alanine-oxalacetic system. O'KANE AND GUNSALUS[4] concurred in this finding and demonstrated the existence of a coupled reaction system to explain the results of the Russian investigators[5]. More recently, KRITZMAN AND SAMARINA[6] reported a fractionation procedure which allegedly demonstrates the existence of a specific alanine-oxalacetic transaminase in pigeon liver. Using a sensitive spectrophotometric method[7], we have checked the possible existence of a general amino acid-oxalacetic transaminase in various tissues, and have repeated and tested KRITZMAN AND SAMARINA's fractionation of pigeon and chicken liver. Our studies indicate that of the amino acids tested, glutamic acid is the only one capable of transamination with oxalacetic acid.

METHODS AND PROCEDURES

Enzyme preparation. Pig heart extract was prepared according to our earlier method[1] except that it was dialyzed for twelve hours and used immediately. Rat liver and pigeon liver extracts were prepared by homogenizing one part of tissue with three parts of distilled water at 4° C. The homogenates were diluted with one part of 0.05 *M* phosphate buffer and then centrifuged for 15 minutes at 4000 r.p.m. The supernatant solution was then dialyzed for twelve hours. Pigeon liver and chicken livers were fractionated according to the method of KRITZMAN AND SAMARINA[6]. All dialyses were carried out against 0.01 *M* phosphate buffer, pH 7.4.

* Aided in part by grants from the Wisconsin Alumni Research Foundation and the Rockefeller Foundation.

** Present address, Department of Physiological Chemistry, Yale University, New Haven, Connecticut.

References p. 120.

Substrates. Oxalacetic acid was prepared according to the method of HEIDELBERGER AND HURLBERT[8]. Amino acids were used as supplied by the manufacturer.

Coenzymes. A solution of pyridoxal phosphate, kindly supplied by Dr W. W. UMBREIT, Merck Institute for Therapeutic Research, was routinely employed in all assays. The coenzyme of BRAUNSTEIN AND KRITZMAN[9] was prepared according to the directions of O'KANE AND GUNSALUS[5].

Assay conditions. The spectrophotometric method previously described by us[7] was employed to determine the formation or disappearance of the appropriate keto acid. In most cases, 1 ml of enzyme solution at the highest concentration which permitted an optical density determination to be made was incubated with 1 ml of pyridoxal phosphate solution (30 γ/ml) for twenty minutes at 38° C in the quartz cell of the Beckman Spectrophotometer. One ml of amino acid solution (10 μM/ml with respect to the L form) was then added and the mixture allowed to incubate for 10 minutes. 0.2 ml of α-keto acid solution (50 μM/ml) was then added and the reaction allowed to continue for at least an hour. All solutions were prepared in 0.05 M phosphate buffer, pH 7.4. The rate of change of concentration with time was determined using the formula $dC_3/dt = dD/dt \times 1/(K_3 + K_4 - K_1 - K_2)$, the symbols having the meanings previously assigned to them[7]. The values of the extinction coefficients and the description of the determination of dD/dt have been previously reported[7]. The blanks employed in the previous work were routinely employed. Because of the varying catalytic effects of different amino acids on the spontaneous decomposition of oxalacetic acid[10], an additional blank consisting of coenzyme, oxalacetic acid, boiled enzyme solution and amino acid was employed. Explicit experimental conditions are presented in Tables I and II.

RESULTS

The results of attempts to demonstrate general amino acid-oxalacetic transaminases in pig heart and rat liver extracts are presented in Table I. A survey of column 7, which gives the observed change in concentration of the substance named in column 6, in terms of micromoles per milligram protein per hour, reveals that of the five amino acids tested with heart muscle extract, and the three amino acids tested with rat liver extract, none shows any significant ability to transfer its amino group to oxalacetic acid in the presence of these extracts. Experiments 6 and 7 reveal a small and possibly significant amount of transamination, but since the enzyme source is undialyzed rat liver extract, the transamination can be accounted for by the high glutamic acid content of such extracts, and coupled transaminase systems, similar to those first suggested by GREEN *et al.*[3] and demonstrated by O'KANE AND GUNSALUS[5]. That this is probably the case is shown by experiment 8 in which dialysis of liver reduces the rate of synthesis to a practically negligible amount, and experiment 5 in which dialyzed heart preparations show no activity with phenylalanine. In previous work[1] it was shown that dialysis of liver preparations never completely eliminated a substance which in the presence of *Clostridium welchii, S.R.* 12 behaved as though it were glutamic acid. We thus explain the activity remaining after dialysis as being due to this residual glutamic acid.

The results of attempts to demonstrate a specific alanine-oxalacetic transaminase (aspartico-alanine aminopherase in the nomenclature of the Russian workers) are presented in Table II. Once again, a survey of column 7 reveals that no significant activity exists in pig heart, rat liver, pigeon liver, and chicken liver extracts. Neither was any activity found in any of the fractions obtained by following KRITZMAN AND SAMARINA's fractionation scheme. To eliminate the possibility of approaching the reaction from an unfavorable side of the equilibrium, the system was tested using aspartic acid plus pyruvic acid, as well as alanine plus oxalacetic acid. Contrary to KRITZMAN AND SAMARINA's assertions, when glutamic acid was used as amino donor to oxalacetic acid, activity was found in all fractions, and this activity is probably the reason for the observations reported by them.

References p. 120.

TABLE I

AMINO ACID-OXALACETIC TRANSAMINASES IN MAMMALIAN TISSUES

Exp. #	*Enzyme source*	*L-Amino acid*	*Keto acid*	*Wave length*	*Observation*	$\frac{dC_3}{dt/m\mu\ protein}$
1	Pig heart (3.0)	*Iso*leucine	OAA	290 mμ	OAA disappearance	0.00
2	Pig heart (3.0)	Methionine*	OAA	290 mμ	OAA disappearance	0.00
3	Pig heart (3.0)	Leucine	OAA	290 mμ	OAA disappearance	0.00
4	Pig heart (10.0)	Tyrosine	OAA	310 mμ	HPA formation	0.00
5	Pig heart (10.0)	Phenylalanine	OAA	310 mμ	PPA formation	0.00
6	Undialyzed rat liver (9.25)	Tyrosine	OAA	310 mμ	HPA formation	0.05
7	Undialyzed rat liver (9.25)	Phenylalanine	OAA	310 mμ	PPA formation	0.03
8	Dialyzed rat liver (9.25)	Tyrosine	OAA	310 mμ	HPA formation	0.02
9	Dialyzed rat liver (9.25)	Leucine	OAA	290 mμ	OAA disappearance	0.00
10	Dialyzed rat liver (9.25)	Tyrosine	α-KG	310 mμ	HPA formation	4.50

* DL form used

Reaction systems contained 10 μM L-amino acid, 10 μM keto acid, 30 γ pyridoxal phosphate, and tissue extract as indicated, all in a total volume of 3.2 ml. Incubation time, one hour; pH 7.4. OAA, oxalacetic acid; α-KG, α-ketoglutaric acid; PPA, phenylpyruvic acid; HPA, *p*-hydroxyphenyl pyruvic acid. Figures in parenthesis refer to concentration of protein in mg/ml.

TABLE II

ASSAY FOR A SPECIFIC ALANINE-OXALACETIC TRANSAMINASE

Exp. #	*Enzyme source*	*L-Amino acid*	*Keto acid*	*Observation*	$\frac{dC_3}{dt/m\mu\ protein}$
1	Pig heart extract (1.0)	Alanine	OAA	OAA disappearance	0.00
2	Rat liver extract (1.0)	Alanine	OAA	OAA disappearance	0.00
3	Pigeon liver extract (1.0)	Aspartic	Pyruvic	OAA formation	0.01
4	Pigeon liver pH 1 ppt. (1.0)	Aspartic	Pyruvic	OAA formation	0.00
5	Above + "coenzyme" (1.0)	Aspartic	Pyruvic	OAA formation	0.60
6	Pigeon liver extract (0.73)	Alanine	OAA	OAA disappearance	0.00
7	Chicken liver extract (0.75)	Alanine	OAA	OAA disappearance	0.00
8	Chicken liver extract (0.75)	Glutamic	OAA	OAA disappearance	10.10
9	Chicken liver pH 1, ppt. (0.50)	Alanine	OAA	OAA disappearance	0.02
10	Chicken liver pH 1, ppt. (0.50)	Glutamic	OAA	OAA disappearance	4.23
11	Chicken liver pH 1, supernate (0.70)	Alanine	OAA	OAA disappearance	0.00
12	Chicken liver pH 1, supernate (0.70)	Glutamic	OAA	OAA disappearance	0.17
13	Chicken liver pH 5, ppt. (0.65)	Alanine	OAA	OAA disappearance	0.01
14	Chicken liver pH 5, ppt. (0.65)	Glutamic	OAA	OAA disappearance	2.80
15	Chicken liver pH 5, supernate (0.54)	Alanine	OAA	OAA disappearance	0.00
16	Chicken liver pH 5, supernate (0.54)	Glutamic	OAA	OAA disappearance	10.50

Conditions and symbols are identical with those of Table I. Reactions measured at 280 mμ. "Coenzyme" refers to the BRAUNSTEIN AND KRITZMAN factor prepared from pig heart according to O'KANE AND GUNSALUS[5].

It should be noted that only a few milligrams of protein were precipitated at pH 1, both with pigeon liver and chicken livers as the enzyme source. The appearance of any considerable amount of precipitate at this low pH which still retains vigorous enzymic activity would be surprising. In experiment # 5, Table II, BRAUNSTEIN AND KRITZMAN'S coenzyme rather than pyridoxal phosphate was added with the same effect on the system as the addition of glutamic acid. This is not surprising since the glutamic acid content of heart muscle extracts is known to be high[5].

References p. 120.

DISCUSSION

All attempts to demonstrate the existence of general transaminases capable of effecting the transfer of amino groups to oxalacetic acid have yielded negative results. While it is conceivable that some variation of conditions would make possible the demonstration of activity, it is felt that the use of incubation conditions analogous to those employed in demonstrating known transaminase systems, plus the use of a sensitive assay method makes it appear likely that enzymes capable of catalyzing the transfer of amino groups from amino acids to oxalacetic acid do not exist in mammalian heart or in mammalian and bird liver. Specifically, the existence of an alanine-oxalacetic transaminase in mammalian heart and liver, as well as in fractionated and unfractionated bird liver, is not supported by the data presented here. It is of interest to note that oxalacetic acid was found to be inactive as an amino group acceptor from leucine and phenylalanine by ROWSELL[11] who used a washed insoluble particle preparation of rat liver as a source of enzyme and filter paper chromatography as a method of assay.

SUMMARY

1. Extracts of pig heart and rat liver have been tested for their ability to catalyze the transfer of amino groups of seven amino acids to oxalacetic acid. With the exception of glutamic acid, no transaminase activity was observed.

2. KRITZMAN AND SAMARINA's fractionation scheme for the isolation of a purified "aspartico-alanine aminopherase" has been applied to pigeon and chicken livers. No activity could be demonstrated. The glutamic-oxalacetic transaminase system, however, was found to be active in all the fractions tested.

RÉSUMÉ

1. Nous avons examiné des extraits de coeur de porc et de foie de rat en vue de leur capacité ue catalyser le transfert de groupes amino de sept acides aminés à l'acide oxalacétique. Sauf chez l'acide glutamique, nous n'avons observé aucune activité de transaminase.

2. Nous avons appliqué le schéma de fractionnement de KRITZMAN ET SAMARINA pour l'isolement d'une "aspartico-alanine aminopherase" à des foies de pigeon et de poule. Aucune activité n'a pu être démontrée. Cependant le système de transaminase glutamique-oxalacétique s'est montré actif dans toutes les fractions étudiées.

ZUSAMMENFASSUNG

1. Extrakte aus Schweineherzen und Rattenleber wurden auf ihre Fähigkeit die Übertragung der Aminosäuregruppen von sieben Aminosäuren auf Oxalessigsäure zu katalysieren untersucht. Mit Ausnahme der Glutaminsäure konnte keine Transaminaseaktivität beobachtet werden.

2. Das Fraktionsschema von KRITZMANN UND SAMARINA zur Isolierung einer gereinigten "Aspartico-Alanin Aminopherase" wurde auf Tauben- und Hühnerleber angewandt. Es konnte keine Aktivität festgestellt werden. Das Glutamin-Oxalessigsäure-Transaminasesystem jedoch zeigt sich in allen untersuchten Fraktionen aktiv.

REFERENCES

1 P. S. CAMMARATA AND P. P. COHEN, *J. Biol. Chem.*, 187 (1950) 439.
2 A. E. BRAUNSTEIN AND M. G. KRITZMAN, *Enzymologia*, 2 (1937) 129.
3 D. E. GREEN, L. F. LELOIR AND NOCITO, *J. Biol. Chem.*, 161 (1945) 559.
4 D. E. O'KANE AND I. C. GUNSALUS, *J. Biol. Chem.*, 170 (1947) 425.
5 D. E. O'KANE AND I. C. GUNSALUS, *J. Biol. Chem.*, 170 (1947) 433.
6 M. G. KRITZMAN AND O. P. SAMARINA, *Doklady Akad. Nauk. S.S.S.R.*, 63 (1948) 171.
7 P. S. CAMMARATA AND P. P. COHEN, *J. Biol. Chem.*, 193 (1951) 45.
8 C. HEIDELBERGER AND R. B. HURLBERT, *J. Am. Chem. Soc.*, 72 (1950) 4704.
9 A. E. BRAUNSTEIN AND M. G. KRITZMAN, *Biokhimiya*, 8 (1943) 1.
10 S. P. BESSMAN AND E. C. LAYNE JR., *Arch. Biochem.*, 26 (1950) 25.
11 E. V. ROWSELL, *Nature*, 168 (1951) 104.

Received August 5th, 1952