TRANSAMINASE ACTIVITY OF DIAPHORASE, PHOSPHORYLASE A AND SEVERAL DEHYDROGENASES*

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In the course of a study of the γ-aminobutyrate-α-ketoglutarate transaminase of mouse brain it was found that purification of the enzyme also resulted in the concomitant extensive purification of a component with lactic dehydrogenase activity. These findings, to be reported in detail elsewhere, suggested the possibility that also in some other instances transaminase and dehydrogenase and possibly other activities might be associated with the same or closely associated proteins. The present report shows that diaphorase, phosphorylase a, and several crystalline dehydrogenases do, indeed, catalyze transamination reactions and that several other types of crystalline or highly purified enzymes and proteins do not.

MATERIALS AND METHODS

The transaminase activities of the preparations were assayed by determining the rate of glutamate-α-ketoglutarate exchange employing α-ketoglutarate-5-C^{14} and by measuring the formation of labeled glutamate from the labeled α-ketoglutarate and nonradioactive aspartic acid,

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γ-aminobutyric acid, or α-alanine. Incubations were performed in capped, round-bottom centrifuge tubes with shaking in the Dubnoff incubator for one hour at 37° C. All incubation mixtures had a final volume of one ml and contained: 50 μM of amino donor; 50 μM of α-ketoglutarate-5-C^{14} (0.2 μc); 60 μM of 0.2M borate buffer, pH 8.2; and 0.1 or 0.2 mg of enzyme. All of the substances were tested in the presence of 100 μg of pyridoxal phosphate and in the absence of the coenzyme. Suitable blanks were run in which the enzyme or amino donor was omitted or boiled enzyme preparations were employed. Such blanks gave closely concordant, extremely small values and always were subtracted from the values obtained from the corresponding experiment containing active enzyme and amino donor. The reaction was stopped by the addition of one drop of concentrated HCl and heating for 3 minutes at 100° C. After adjusting the pH to between 3 and 4 by the addition of dilute alkali, the reaction mixture was transferred with several washings onto 7 x 1 cm columns of Dowex 50 x 8 in the H^+ form. All of the unreacted α-ketoglutarate could be washed out with 30 ml of water. The labeled glutamic acid formed was eluted with 25 ml of 2N NH_4OH, and in the case of the experiment with the commercial muscle lactic dehydrogenase the glutamic acid was isolated and identified. The eluate was taken to dryness in the air stream of a fan and 2 ml of 1M hydroxide of Hyamine 10-X in methanol was added. One ml of this sample was pipetted into a counting vial. Toluene (15 ml), containing 0.3% 2,5-diphenyloxazole and 0.01% 1,4-bis-2(5-phenyloxazolyl)-benzene in toluene, was added to each vial and the vials were counted in a Packard Tri-Carb liquid scintillation counter.

It was found that crystalline preparations of phosphorylase a and of lactic, alcohol, and glutamic dehydrogenases, as well as a highly purified diaphorase preparation catalyzed both the glutamate-α-keto-glutarate exchange and the glutamate-oxalacetate transaminase reactions (Table I). In several instances there was a marked activation by pyridoxal phosphate. Alanine-α-ketoglutarate transaminase activity was

TABLE I

Comparison of Transaminase Activity of Diaphorase, Phosphorylase a, and Three Dehydrogenases With That of Purified Aspartate-α-Ketoglutarate Transaminase

Enzyme	Source	Molecular Weight	Glutamate-αKG Exchange (a)		Aspartic-αKG Transaminase		Alanine-αKG Transaminase	
			μM/10mM enzyme/hour					
			$-B_6$ (b)	$+B_6$	$-B_6$	$+B_6$	$-B_6$	$+B_6$
Lactic dehyd. (1,c)	Beef heart	1.35×10^5	288	1,091	0	553	403	541
" " (1)	Rabbit muscle		0	0	0	0	0	0
" " (2)	Rabbit muscle		0	1,404	0	428	0	0
" " (3)	Beef heart		903	1,156	0	156	1,147	1,231
Alcohol dehyd. (4)	Yeast	1.51×10^5	75	1,501	0	768	0	0
Glutam. dehyd. (2,d,e)	Beef liver	1.0×10^6	0	1,450	0	1,093	0	0
Diaphorase (2)	Pig heart	7.0×10^4	22	459	0	491	0	0
Phosphorylase a (3)	Rabbit muscle	4.95×10^5	2,342	4,165	165	300	6,511	6,563
Aspartate-αKG Transaminase (2f)	Pig heart	1.1×10^5	15,352	18,154	8,100	17,081	0	0

(1) Kindly given to us by Prof. N. O. Kaplan of Brandeis University (see Cahn, Kaplan, Levine and Zwilling (1962))
(2) Biochemica (Boehringer) through California Corporation for Biochemical Research
(3) Sigma Chemical Co.
(4) Crystalline lyophilized enzyme - Worthington Biochemical Co.
(a) αKG: α-ketoglutarate; (b) B_6: Pyridoxal Phosphate; (c) 2 hr. incubation-activity expressed for 1 hr; (d) crystalline suspension in ammonium sulfate; (e) ammonium sulfate-free preparation in 50% glycerol; (f) .1 mg enzyme used for incubation

found in the "heart" type of lactic dehydrogenase and in the phosphorylase a. No γ-aminobutyrate-α-ketoglutarate transaminase activity was found in any of the preparations tested and no L-glutamic acid decarboxylase activity could be found in the lactic dehydrogenase and glutamic dehydrogenase preparations tested. Results obtained with a purified glutamate-oxalacetate transaminase are shown in Table I for comparative purposes. This latter preparation had no lactic and no glutamic dehydrogenase activity. It is interesting that Kaplan's pure "heart"-type lactic dehydrogenase possesses good transaminase activity, while the pure "muscle" type does not. Commercial crystalline lactic dehydrogenases prepared either from rabbit muscle or from beef heart and known to contain a mixture of the various lactic dehydrogenase hybrids also were found to have transaminase activity.

No transaminase activity, whatsoever, could be detected by the above methods of testing in the following crystalline enzymes or proteins: ribonuclease, trypsin, pepsin, desoxyribonuclease, insulin, bovine serum albumin, and polylysine. Highly purified preparations of L-amino acid oxidase (snake venom) and cytochrome c and crude yeast hexokinase also showed no activity.

CONCLUSIONS

It is well known that some single enzymatic proteins are capable of catalyzing reactions with more than one substrate. Struck and Sizer (1960) and Fisher and McGregor (1961) demonstrated that one protein in presence of DPN or TPN was able to carry out the dehydrogenation of L-glutamic acid, L-leucine and L-alanine, the reaction of the latter proceeding approximately at 1% to 2% of the rate found for glutamic acid. Novogrodsky, Nishimura and Meister (1963) showed that the transamination of aspartic acid with α-ketoglutarate and the β decarboxylation of aspartic acid are catalyzed by one protein. In both of the latter studies more than one substrate appears to be acted upon by a single

protein associated with a single cofactor. The presently reported experiments suggest the possibility that single catalytic proteins may catalyze one or another different type of reaction with differing substrates and diverse cofactors, and open the possibility that many environmental factors, among which is the availability of coenzymes, may actually determine which of several reactions is catalyzed by a particular protein and, therefore, the role in the cellular economy which the protein plays. Further work is required to rule out unequivocally the possibility of enzymatic contamination in the present results. However, this seems unlikely since the proteins tested were prepared from different sources by widely divergent procedures.

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