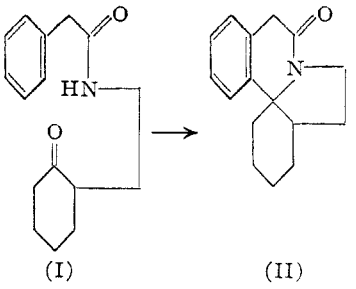

(I) (II)

hexahydroindole [b.p. 123–124° (0.4 mm.); *Anal.* Calcd. for $C_{16}H_{21}N$: C, 84.52; H, 9.31. Found: C, 84.33; H, 9.45; oily salts; obtained by treating β-phenylethyl bromide with hexahydroindole] to erythrinane, led only to unchanged starting material.

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ENZYMATIC REDUCTION OF β-ASPARTYL PHOSPHATE TO HOMOSERINE

Sir:

The enzymatic formation of β-aspartyl phosphate from L-aspartate and ATP[1] recently has been reported.[2] A synthetic preparation of this substance now has been obtained, and found to be enzymatically reduced to homoserine in a TPN-dependent system obtained from yeast. The phosphorylation and subsequent reduction of the β-carboxyl group of aspartate to a hydroxyl group appear, in view of recent isotope and genetic work, to represent intermediate steps in the biosynthesis of threonine. The latter was demonstrated by such experiments to arise from aspartate via homoserine.[3,4]

The starting material for BAP[1] synthesis was carbobenzoxy-L-aspartyl-α-benzyl ester β-chloride.[5] To replace the chloride with phosphate this compound was shaken in ether with monosilver phosphate,[6] and the carbobenzoxy and benzyl groups were then removed by hydrogenation over palladium black in cold potassium bicarbonate solution. Because of its extreme lability the product was not further purified. It was characterized by the β-asparthydroxamic acid, identified chromatographically, formed on reacting with hydroxylamine. Enzymatic identification was made by its ability to transfer phosphate to ADP.[2] This substance is approximately 30% hydrolyzed in 30 minutes at 30° in aqueous solutions from *p*H 4 to 10. In such solutions at 15° it is relatively stable for several hours.

The enzyme preparation used in these experiments was an extract of baker's yeast partially purified by heating to 55°, discarding the precipitate, and dialyzing 15 hours against cold potassium bicarbonate buffer.

Table I shows the equivalent disappearance of BAP and formation of homoserine in the complete enzyme system. No homoserine appears when the BAP or enzyme is destroyed by brief heating prior to addition. On prolonged incubation complete transformation of BAP to homoserine occurs.

TPN and glutamate markedly increase the extent of the reduction, presumably through the TPN-linked yeast glutamic dehydrogenase[7] which is very active in the protein preparation. Glutamate may be replaced by glucose plus the glucose dehydrogenase of ox liver.[8] Though the latter reduces both DPN and TPN, only TPN serves to reduce BAP in its presence.

TABLE I

Homoserine was qualitatively identified by paper chromatography in water-saturated phenol with 0.3% NH_3, and in the two alternate solvent systems recommended by Redfield.[9] It was further identified by conversion to its DNP-derivative,[10] which was chromatographed on paper.[11] Quantitative estimation of homoserine was made by eluting the DNP-homoserine from paper chromatograms and analyzing spectrophotometrically at 362 mμ. BAP was determined as acyl phosphate.[12] The complete system contained in 1.0 ml. 100.0 μM. TEA-chloride buffer, 0.16 μM. TPN (63% pure), 5.0 μM. TEA-glutamate, 2.7 μM. BAP, and 0.27 ml. of enzyme. The *p*H was 7.9 and the incubation was at 15° for 60 minutes.

Experiment	BAP utilized, μM.	Homoserine formed, μM.
Complete system	1.4	1.4
Omit TPN	0.4	0.4
Omit glutamate	0.5	0.5
Heated enzyme used (100°, 5 min.)	0.0	−0.1
Heated BAP used (100°, 10 min., neutral solution)	...	0.1

The enzyme preparation used here causes a rapid splitting of BAP in the presence of potassium arsenate, a point of similarity with 3-phosphoglyceraldehyde dehydrogenase which also catalyzes a reduction and an arsenolysis of an acyl phosphate.[13,14] Both arsenolysis and reduction are inhibited by iodoacetate.

We are grateful to Drs. J. P. Greenstein and A. Meister for gifts of several compounds, and for assistance during the synthesis of BAP.

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(1) Abbreviations used are BAP (β-aspartyl phosphate), ADP (adenosine diphosphate), ATP (adenosine triphosphate), TPN (triphosphopyridine nucleotide), DPN (diphosphopyridine nucleotide), DNP (dinitrophenyl), and TEA (triethanolamine).

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