frared spectrum of the synthetic acid was identical with that of *d*-dehydroabietic acid, m.p. 171°, from natural sources.

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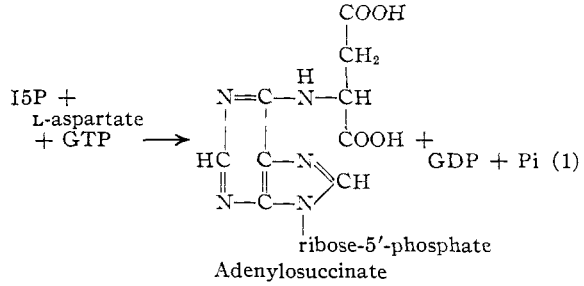
INVOLVEMENT OF GUANOSINE TRIPHOSPHATE IN THE SYNTHESIS OF ADENYLOSUCCINATE FROM INOSINE-5′-PHOSPHATE[1]

Sir:

Our studies on the amination of a pyrimidine nucleotide[2] led us to an investigation of the amination of a purine nucleotide to determine whether the reactions are similar. With an enzyme purified about 40-fold from extracts of *Escherichia coli* B, evidence now has been obtained for the synthesis of adenylosuccinate, a compound first prepared and characterized by Carter and Cohen,[3] from I5P[4] and L-aspartate in a reaction involving GTP, as illustrated in equation (1). Adenylosuccinate



Adenylosuccinate

isolated from reaction (1) was cleaved by an extract of *E. coli* B to yield A5P (equation (2)), a reaction first described by Carter and Cohen[3] with an enzyme from yeast.

$$\text{Adenylosuccinate} \longrightarrow \text{A5P} + (\text{fumarate}) \quad (2)$$

With the partially purified enzyme preparation A5P, guanosine-5′-phosphate, GDP, and the di- and triphosphates of adenosine, cytidine, uridine, and inosine were incapable of replacing GTP. L-Asparagine, D-aspartate, L-glutamate, and L-glutamine could not substitute for L-aspartate (each 7×10^{-4} *M*). D-Aspartate did not inhibit the synthesis of adenylosuccinate.

The stoichiometry of the reaction was studied with the partially purified enzyme (Table I). I5P was identified by its absorption spectrum (peak at 249 mμ, λ250/λ260 = 1.60, λ280/λ260 = 0.21, at *p*H 2), GDP and GTP by their absorption spectra (peaks at 256 mμ, λ250/λ260 = 0.99, λ280/λ260 = 0.68, at *p*H 2), and by their molar ratios of guanine, pentose, acid-labile P, and total P of 1.00:1.02:1.02:2.01, and 1.00:0.94:1.94:2.90, respectively. Adenylosuccinate was identified by its absorption spectrum[3] (peak at 267 mμ, λ250/λ260 = 0.64, λ280/λ260 = 0.68, at *p*H 2; peak at 269 mμ, λ250/λ260 = 0.60, λ280/λ260 = 0.81, at *p*H 12). Further, when L-aspartate labeled with C^{14} in both carboxyl groups was used as a substrate, as shown in the table, it was incorporated into adenylosuccinate without dilution. Likewise, in an experiment with 8-C^{14}-labeled I5P (37,200 c.p.m./μmole), the specific activity of the adenylosuccinate (36,600 c.p.m./μmole) was the same as the substrate. Using the molar extinction coefficient found by Carter and Cohen[3] (E_M 267 mμ at *p*H 1 = 16.9×10^3), the product yielded molar ratios of pentose and total P of 0.99 and 0.96, respectively. No Pi was liberated during incubation in 1 *N* H_2SO_4 in a boiling water-bath for 15 minutes, but from 0.107 μmole of product, 0.109 μmole of Pi was released by 5′-nucleotidase.[5] No detectable diazotizable amine reaction[6] occurred with the product. Incubation of the product, with an extract of *E. coli* B, yielded a radioactive compound (88% of the counts) whose anion-exchange chromatographic behavior was indistinguishable from that of authentic A5P.

TABLE I

STOICHIOMETRY OF ADENYLOSUCCINATE SYNTHESIS

The reaction mixture (29.4 ml.) contained 4.2 ml. of glycine buffer (1 *M*, *p*H 8.0), 1.68 ml. of $MgCl_2$ (0.1 *M*), 1.68 ml. of C^{14}-carboxyl-labeled-L-aspartate (0.01 *M*, 147,000 c.p.m./μmole), 0.85 ml. of I5P (0.01 *M*), 1.42 ml. of GTP (0.0059 *M*), and 4.2 ml. of the enzyme preparation (containing 1.85 mg. of protein). An aliquot of the reaction mixture (15 ml.) was placed immediately in a boiling water-bath for 2.5 minutes, the remainder was incubated at 37° for 50 minutes, and then heated for 2.5 minutes in a boiling water-bath.

	0 min. μmoles	50 min. μmoles	μmoles	Δ Total c.p.m.	Specific activity c.p.m./μmole
I5P[a,b]	3.43	1.75	−1.68	0	
GTP[c]	3.39	1.63	−1.76	0	
L-Aspartate[d]	6.87	5.21	−1.66	−244,020	147,000
Adenylosuccinate[e]	0.00	1.61 (1.66)[g]	+1.61 (+1.66)	+227,180	141,100
GDP[c]	0.00	1.72	+1.72	0	
Pi[f]	0.39	2.08	+1.69		

[a] Anion-exchange chromatography of aliquots of the reaction mixtures (12 ml.) gave complete separations of aspartic acid and each of the nucleotides. [b] Estimated spectrophotometrically at 250 mμ. [c] Estimated spectrophotometrically at 260 mμ. [d] Estimated by radioactivity measurements. [e] Estimated spectrophotometrically at 267 mμ. [f] Estimated by the method of C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925), before chromatography. [g] Values in parentheses were determined by optical density measurements at 280 mμ before chromatography.

This work, in progress at the time Abrams and Bentley[7] reported on the conversion of I5P to adenosine-5-phosphate with rabbit bone marrow extracts, is in agreement with their results.

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(2) I. Lieberman, THIS JOURNAL, **77**, 2661 (1955).

(3) C. E. Carter and L. H. Cohen, *ibid.*, **77**, 499 (1955).

(4) Abbreviations used: Inosine-5′-phosphate, I5P; adenosine-5′-phosphate, A5P; guanosine diphosphate, GDP; guanosine triphosphate, GTP; Inorganic orthophosphate, Pi.

(5) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.* **188**, 665 (1951).

(6) J. M. Ravel, R. E. Eakin and W. Shive, *ibid.*, **172**, 67 (1948).

(7) R. Abrams and M. Bentley, THIS JOURNAL, **77**, 4179 (1955).