Our experimental results fit an equation of the form (3), and, therefore, agree with the theory of ternary complexes consisting of enzyme–acetaldehyde–coenzyme or enzyme–substrate–activator, according to the variables chosen.

$$V_{max}/v = 1 + K_A/[A] + K_B/[B] + K_{AB}/[AB] \quad (3)$$

In equation (3), A and B are substrates, or substrate and activator, respectively. K_{AB}, the complex constant, could be measured directly and the value obtained agreed well with the value of the product $K_A \times K_B$ whichever were the pair of variables chosen. The dissociation constant of each enzyme–substrate or enzyme–activator compound is not affected by the presence of the other reactants and therefore random formation of the active complex could take place if the system were operating in equilibrium conditions. Nevertheless, the possibility of compulsory order of binding of substrates under steady-state conditions cannot be ruled out entirely. The point will be discussed in more detail elsewhere.

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The enzymic synthesis of δ-aminolevulinic acid*

δ-Aminolevulinic acid has been demonstrated to be an intermediate in the synthesis of porphyrins[1,2] and the porphyrin-like moiety of vitamin B_{12}[3]. This aminoketonic acid arises biologically from the condensation of "active" succinate[4] and glycine. However, the details of this condensation have not yet been elucidated. This communication reports our finding that the supernatant fluid obtained on centrifugation of an extract of the photosynthetic bacterium *Rhodopseudomonas spheroides* for 30 min at 100,000 × *g* is capable of catalyzing the net synthesis of δ-aminolevulinic acid from succinate and glycine on addition of cofactors or from succinyl-coenzyme A and glycine.

LASCELLES has demonstrated[5] that not only are porphyrins synthesized by *R. spheroides* by the route previously elaborated for other cells, but also that porphyrins and porphobilinogen accumulate in the medium. We were encouraged to investigate this bacterium for the enzymes responsible for the synthesis of δ-aminolevulinic acid, for we found that δ-aminolevulinic acid was also present in the medium after the cells were suspended in medium I of LASCELLES[5]. LAVER AND NEUBERGER[6] recently reported that a particulate fraction obtained from erythrocytes of phenylhydrazine-treated chicks was capable of synthesizing this compound from succinate and glycine and that the amount synthesized was increased 2.5 fold on addition of both coenzyme A (CoA) and pyridoxal phosphate.

The strain 241C of *R. spheroides*, obtained from Professor R. Y. STANIER, which originally came from Professor C. V. VAN NIEL's collection, was grown in medium S of LASCELLES. After about 36 h, the bacteria were harvested and washed several times with 0.9% saline. The cells were ground with alumina, extracted with 0.1 *M* phosphate buffer, pH 6.9, containing 0.9% NaCl, and centrifuged for 30 min at 5000 × *g*. The supernatant fluid was then centrifuged for 30 min at 100,000 × *g*, and this latter supernatant fluid was then used as the initial enzyme system.

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On incubation of this fraction with glycine, succinate, CoA, and pyridoxal phosphate, both δ-aminolevulinic acid (*e.g.*, 0.05 to 0.1 μmole/mg protein/h) and porphyrins accumulated. Omission of the cofactors diminished the synthesis about 10-fold. The aminoketonic acid was determined by the method of SHUSTER[7] and further identified by paper chromatography. Surprisingly, the addition of α-ketoglutarate to the complete system inhibited the formation. The synthesis is markedly increased by the addition of ATP and also stimulated by the admission of light. Cysteine inhibits the synthesis.

δ-Aminolevulinic acid synthesis was also demonstrated by the isolation of a derivative of this acid after the addition of non-radioactive δ-aminolevulinic acid to the complete system which contained either radioactive glycine or succinate. However, in contrast to the avian system of LAVER AND NEUBERGER, the addition of the acid to our enzyme system at the start of the incubation resulted in the isolation of δ-aminolevulinic acid as the 2-methyl-3-carbethoxy-4-(3-propionic acid) pyrrole[8], whose radioactivity was low (16 counts/min), whereas the derivative isolated from the incubation mixture to which δ-aminolevulinic acid was added after the incubation period was highly radioactive (1500 counts/min). The radioactivity agreed with the calculated value.

It appears that the "active" succinate is succinylcoenzyme A, for the rate of δ-aminolevulinic acid formation is markedly greater from succinylcoenzyme A than from the components (succinate and CoA), even on addition of ATP (Fig. 1). Further, a particulate fraction (9.25 mg protein), obtained by centrifugation for 2.5 h at 100,000 × *g*, which synthesizes very little porphyrin, formed, in 45 min, 0.176 μmole δ-aminolevulinic acid from succinylcoenzyme A, whereas 0.031 μmole were synthesized from succinate, CoA and ATP, and 0.006 μmole from succinate and CoA.

It seems that part of the enzyme system responsible for δ-aminolevulinic acid synthesis is in very small particles, for on centrifugation of the above-described supernatant fluid for another 90 min at 100,000 × *g*, the bottom half was more enzymically active than the top half. However, the top fraction is still appreciably active after centrifugation for 3 h at 100,000 × *g*. We also found that extracts of *Rhodospirillum rubrum* catalyze the net synthesis of δ-aminolevulinic acid.

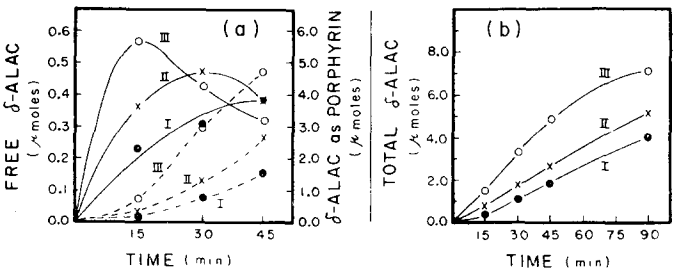

Fig. 1. Rates of synthesis of δ-aminolevulinic acid (δ-ALAC): Each flask contained glycine (150 μmoles), $MgCl_2$ (15 μmoles), pyridoxal phosphate (0.75 μmole), 4.5 ml 0.1 *M* phosphate buffer (pH 6.9) and 3.0 ml of extract (80.2 mg protein). In addition, flask I contained succinate (15 μmoles) and CoA (15 μmoles), flask II contained succinate (15 μmoles), CoA (15 μmoles) and ATP (5.0 μmoles), and flask III contained succinylcoenzyme A (15 μmoles). Final volume, 9 ml. (a) The solid lines represent determination of free δ-aminolevulinic acid and the dotted lines the amount of the compound as porphyrin. (b) The lines represent the total amounts of δ-aminolevulinic acid.

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