in which nucleoli begin to dissolve) permits structurally normal mitosis; thus, treatment with actidione must take effect in somewhat less than 2 minutes.

The primary consideration, in the interpretation of these results, is whether or not this delay in anaphase was caused by the inhibition of protein synthesis during late prophase. Actidione might interact with spindle proteins to cause the delay, but this explanation is probably incorrect, since actinomycin D, which is a specific inhibitor of RNA synthesis and is chemically unrelated to cycloheximide, causes a similar delay in the transition from metaphase to reconstruction if treatment is initiated during interphase (*3*). It can also be argued that this delay is caused by a general and unspecific reduction in metabolism and that the effect is most pronounced during prophase, when spindle organization is most active. This argument cannot, however, explain the abrupt and permanent blockage in early prophase, before nucleolar dissolution, whereas mitosis and nuclear reconstruction go to completion in the absence of protein synthesis after nucleolar dissolution, nor can it explain why RNA synthesis is not drastically inhibited along with protein synthesis during interphase (*2*). Arguments that the delay in anaphase is caused by effects other than an inhibition of protein synthesis are, therefore, of secondary importance, and the most probable explanation of these data is that the effect is related to an inhibition of protein synthesis. The essential structural units for mitosis and nuclear reconstruction must, however, be completed prior to the total blockage of prophase because the entire process is completed and morphologically normal even though the duration of anaphase is extended. It is, therefore, reasonable to propose that the proteins that are synthesized between late prophase and prometaphase are involved in the transformation of chemical energy into the mechanical work of mitosis. These proteins must have a purely catalytic function, because they limit the duration of mitosis but are not necessary for its completion. An earlier investigation suggested that the messenger RNA molecules that encode the information for these proteins are more sensitive to inhibition by actinomycin D than are the messenger RNA molecules that determine the essential structural proteins for mitosis (*3*). All of these experiments point out the necessity for distinguishing between the structural factors required for completely normal mitosis and the factors that regulate the duration of the process.

In summary, the essential structural proteins for mitosis and nuclear reconstruction are completed before the dissolution of the nucleolus in prophase. Proteins which determine the duration of the transition from metaphase to nuclear reconstruction are synthesized from late prophase to prometaphase. It is proposed that these proteins are concerned with the transformation of chemical energy into the mechanical work of mitosis.

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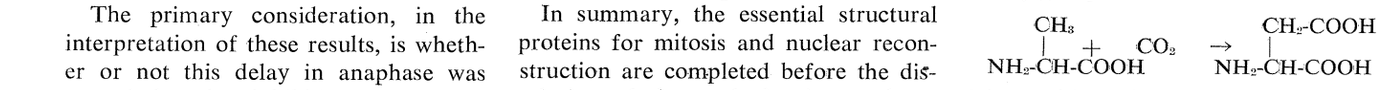
Synthesis of Amino Acid Residues with Reactive Side Chains under Simple Conditions

Abstract. *The side chains of amino acid residues can be converted from nonreactive to reactive species. Under the influence of electrical discharge, alanine is converted to aspartic acid in the presence of CO_2, for example. The possible implications of these findings to events taking place on primitive Earth are discussed.*

Amino acid residues, such as lysine and aspartic acid, with reactive side chains play an important role in biological processes. A study was undertaken to establish means by which amino acid residues with carboxyl and amino function in their side chains could be synthesized. The substitution of methyl hydrogens with more polar functions has been demonstrated by the sparking of an aqueous solution of acetic acid in the presence of nitrogen with the production of glycine (*1*). If a carboxyl group were added to the side chain of alanine (at β-methyl), aspartic acid would result.



The addition of an amino group to the β-methyl group of alanine would give α, β-diaminopropionic acid:

$$NH_2\,CH(CH_3)\,COOH + (NH_2) \rightarrow NH_2CH(CH_2\,NH_2)\,COOH + \tfrac{1}{2}H_2$$

The latter reaction is similar to the production of glycine from acetic acid already cited. A possible mechanism for this reaction has been discussed (*1*). Diaminopropionic acid is used here as a model compound for an amino acid containing an ω-amino group in its side chain.

To investigate the possibility of converting alanine to a residue with a reactive side chain, an aqueous solution of 0.01*M* L-alanine was prepared. The solution was "sparked" for 1 hour as described (*1*); longer periods of sparking led to decomposition. A mixture of carbon dioxide and nitrogen (1 : 1) was blown through the chamber during the process, and a transformer able to supply 9000 volts was used. The reaction vessel was cooled by a constant air stream blown across it. The solution was mixed by a magnetic stirrer. Portions of the product were chromatographed on Whatman No. 1 paper with appropriate standards in parallel; with separate sheets, the following solvent systems were used: a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 5, by volume, organic phase); a mixture of *t*-butanol, 2-butanone, formic acid, and water (4 : 3 : 2 : 1, by volume); and a mixture of isopropanol and water (4 : 1, by volume). Products and standards were located by ninhydrin. The production of aspartic acid was definitely established. A trace of α,β-diaminopropionic acid was observed. Another portion of the product was chromatographed; a sample was eluted at the R_F of aspartic acid and analyzed by the ninhydrin colorimetric procedure (*2*). The yield was 5 percent.

When a solution of α-aminobutyric acid was similarly sparked, glutamic acid was produced, presumably by the addition of CO_2. A surprising result of this experiment was that aspartic acid was also produced, in about twice the yield of the glutamic acid. This could

have resulted from carbon-carbon cleavage, yielding a methyl radical as a side product and an alanyl radical which then reacted with the carbon dioxide. A similar synthesis of glutamic acid from alanine and acetic acid is conceivable.

The original reagents gave no extraneous chromatographic spots. No aspartic acid was observed when CO_2 was bubbled through a solution of alanine for 1 hour that was not subjected to sparking.

Another reactive side-chain function of interest would be the sulfhydryl group of cysteine. The addition of SH (from H_2S) to alanine could conceivably lead to the synthesis of cysteine:

$$NH_2CH(CH_3)COOH + H_2S \rightarrow NH_2CH(CH_2SH)COOH + H_2$$

A solution of 0.01*M* alanine was sparked in the presence of 0.01*M* sodium sulfide in an atmosphere of nitrogen. When sparking of an aqueous solution is carried out in the presence of nitrogen, a drop in *p*H results, presumably as a result of the production of nitric acid (*1*). When a solution of sodium sulfide is made acidic, H_2S is evolved. In my experiment, a decrease from *p*H 10 to *p*H 5 was observed during the course of sparking for 1 hour, and this decrease was taken to indicate that H_2S had probably been produced, as desired. Chromatography failed to show cysteine as a product although a couple of new spots, as yet unidentified, were observed. If cysteine had indeed been synthesized, it may be that oxidation during processing prevented its detection.

Up to this point, only the reactions of the side chains of amino acid monomers had been studied to determine whether the desired transformations to reactive groups were possible. To determine whether the observed phenomena are also applicable to residues already part of a peptide chain, the effect of sparking on selected di- and tripeptides was considered. The synthesis of aspartyl residues was selected for study. The peptides, alanylglycine, alanylglycylglycine, and leucylalanine, were separately sparked under CO_2 for 1 hour as before. Each product was then hydrolyzed in 6*N* HCl and chromatographed. By comparison with a standard, aspartic acid was detected in each sample. The chromatograms of the ninhydrin-positive products, with leucylalanine as an example, gave the following types of

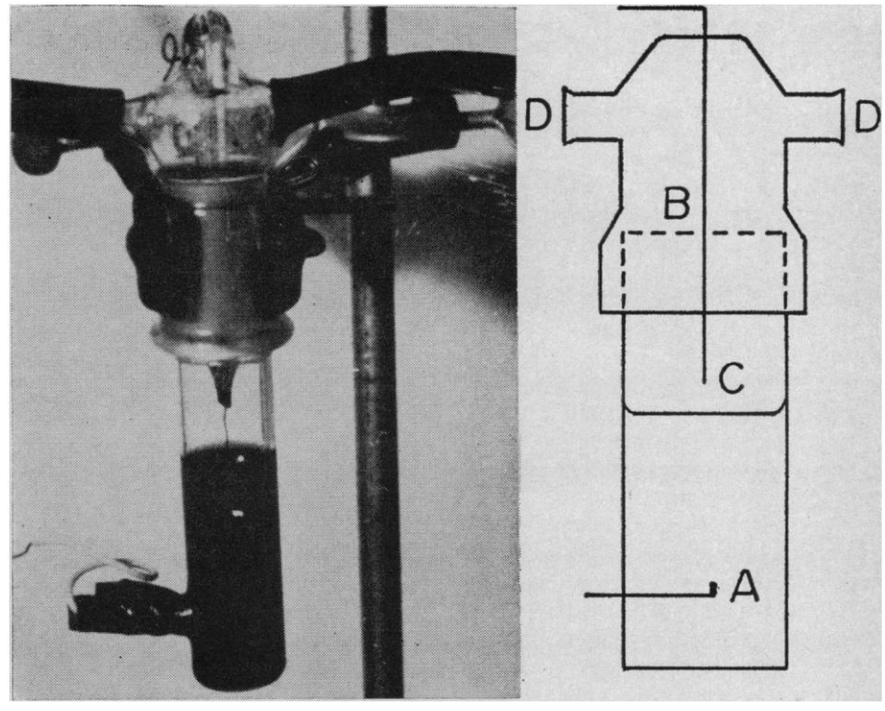


Fig. 1. Photograph and schematic drawing of sparking vessel with submerged electrode (*A*), suspended electrode (*B*), liquid surface (*C*), and ventilation ports (*D*).

data with a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 5) as solvent. The sample of the dipeptide through which CO_2 had been bubbled showed only leucine (R_{Leu} = 1.00; R_F relative to leucine) and alanine (R_{Leu} = 0.58) upon hydrolysis. The sample which was similarly treated, but which was also sparked, yielded leucine, alanine, and aspartic acid (R_{Leu} = 0.26) after hydrolysis, as judged by comparison with appropriate standards. Before hydrolysis, this sparked product contained the dipeptide as well as small amounts of leucine, alanine, aspartic acid, and a prominent yellow spot (R_{Leu} = 0.42) which did not appear in the hydrolyzate. (Many peptides exhibit yellow spots upon ninhydrin treatment.) The free aspartic acid appearing before hydrolysis could have resulted from degradation of the dipeptide during sparking or by reaction of the small amount of free alanine detected in the original leucylalanine preparation. The sparked dipeptide also displayed traces of a couple of other substances before hydrolysis.

Conceivably the observations reported here have application to the elucidation of primitive biogenesis. It is not certain to what degree electrical discharge promoted chemical evolution, but it has been pointed out that the results observed with the use of one form of energy (such as electrical discharge) could also be expected from others (such as ultraviolet light, probably the major source of primordial energy) in the context of prebiological phenomena (*1, 3*).

The production of aspartic acid, for example, under simple conditions that may have existed on primitive Earth has been reported (*1, 4*). However, the transformations noted here could represent a means involved in the appearance of peptides containing reactive side-chain functions. Suggestions have been made concerning the mechanism of amino acid polymerization under possible primitive Earth conditions. These include mediation by anhydrous heating (*5*), by polymetaphosphates (*6*), and by dicyanamide in aqueous solution (*7*), among others. One problem that has not been thoroughly worked out is how α-peptide bonds between amino acids also containing carboxyl and amino groups in their side chains, such as aspartic acid and lysine, are preferentially formed. In other words, there would appear a priori to be just as much likelihood for aspartic acid to form a β-peptide as an α-peptide, for example.

One system which has been proposed that could overcome such a dilemma involves first the formation of a polymer of amino acids with nonreactive

(nonpolar) side chains (*8*). These side chains would then react with appropriate reagents to produce reactive (polar) functions. It was shown that the combination of polyglycine with formaldehyde yields seryl residues and with acetaldehyde yields threonyl residues (*8*). Thus, the presence and potential interference of the hydroxyl group during the process of formation of peptide bonds is overcome.

Apparently little has been done to elucidate means by which other reactive residues could have originated without interference with polymerization to α-peptides. To demonstrate that a peptide bond could easily form with the γ-carboxyl group of glutamic acid, the following experiment was performed. An aqueous solution was prepared containing 0.01*M* glycine (^{14}C-labeled), 0.01*M* L-cysteine, 0.01*M* L-glutamic acid, 0.1*N* HCl (final concentrations) and 2 mg of bentonite, a clay mineral that increases the yield of synthetic peptide (*9*). To the mixture, which was stirred with a magnetic stirrer, successive portions of sodium dicyanamide solution were added at 2-minute intervals, so as to bring the total concentration of the condensing agent to 0.12*M*. The solution was then neutralized with NaOH, and the clay was removed by centrifugation. A portion of the supernatant was chromatographed on Whatman No. 1 paper, with a standard of reduced glutathione (γ-glutamyl-cysteinyl-glycine) run in parallel, the solvent used being a mixture of isopropanol, formic acid, and water (65 : 1 : 34, by volume). The standard was located by spraying with *o*-tolidine (*10*), and a radioactive product of similar R_F, as observed by autoradiography, was eluted. Standard glutathione was added to the eluate which was then spotted on another sheet of Whatman No. 1 paper. Chromatography was carried out in the first dimension with a mixture of *n*-butanol, acetic acid, and water (25 : 6 : 25, by volume) as solvent. The second dimension was resolved by electrophoresis in borate buffer at *p*H 9.2, with 5.8 volt/cm applied voltage. The tolidine spray indicated that the shape and position of the carrier glutathione was the same as that of one of the labeled products that had been observed with x-ray film. Thus, under conditions where the γ-peptide bond may not be desirable, as usual with peptide synthesis, its production could pose problems. Similarly, in the pyrocondensation of aspartic acid, at least 33 percent of the peptide linkages formed were β-linkages (*11*).

The aforesaid means for the production of reactive side chains could have contributed, at least in part, to the formation of compounds needed for the ultimate appearance of living organisms. The overall scheme is based primarily on the production of polymers of amino acid residues with nonpolar side chains, such as alanine and glycine. This event could have taken place by any of a number of demonstrated experimental methods. These units, by interaction with nitrogen, water, and carbon dioxide, are convertible to residues with carboxyl and amino functions in their side chains. As part of a previously formed peptide chain, these residues, now transformed to species with reactive side chains, would not have interfered with the original polymerization process. Such a system is simple enough to suggest that it could have contributed to prebiological chemical evolution.

The study of the synthesis of α,β-diaminopropionic acid is not altogether out of place in the context of prebiological events since α,γ-diaminobutyric acid and α,δ-diaminovaleric acid have been found in bacterial peptides (*12*). It is conceivable that lysine (α,ϵ-diaminocaproic acid) is the product of a line of evolution that originally began with α,β-diaminopropionic acid.

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Activity Rhythms and Adiurnal Light-Dark Control

Abstract. *The running-wheel activity pattern of mature male rats was successfully synchronized to light-dark cycles as long as 48 hours and as short as 16 hours. Even after 6 months' exposure to "days" longer than the normal 24 hours, the animals returned promptly to circadian rhythmicity when placed under free-running conditions of continuous dark. That such rhythms also reappeared when the light condition of the 36-hour cycle was reduced from 660 to 33 lumens per square meter suggests that brightness may be the critical factor in the unexpectedly broad range of entrainment demonstrated.*

Many circadian (near-24-hour) rhythms in living organisms "entrain" (that is, modify their spontaneous frequencies so as to synchronize) with imposed environmental cycles, particularly of light and dark (LD) (*1*). In parallel to natural conditions, most observations of physiological and behavioral entrainment have been made on diurnal (precisely 24-hour) schedules. In order to contribute to the understanding of the intrinsic timing mechanism, and particularly to explore the possibility of modifying its control, attempts have been made to establish entrainment to adiurnal (non-24-hour) LD cycles. As an appropriate and convenient dependent variable, the activity pattern of the rodent has been the choice of a number of investigators. The limited success of these efforts, against the background of other plant and animal experiments, has suggested the proposition that the more complex the organism, the greater the resistance to entrainment to period lengths deviating considerably from 24 hours (*2, 3*). Bruce (*3*, p. 38), for example, found it impossible to synchronize the hamster's wheel-running activity to "days" shorter than 23 or longer than 25 hours. In mice, even when departures from 24 hours were made in 1-hour steps, with extended habituation permitted at each stage, Tribukait (*4*) was unable to stretch the limits of entrainability beyond 21 and 27 hours (spring-cage activity). While the directly observed