Reports

Prebiotic Synthesis of Methionine

Abstract. *Methionine has been shown to be a product of the action of a spark discharge on a simulated primitive earth atmosphere containing CH_4, N_2, NH_3, H_2O, and H_2S or CH_3SH. Acrolein has also been shown to be a product of the discharge and is proposed as an intermediate in the prebiotic synthesis of methionine and of glutamic acid, homocysteine, homoserine, and α,γ-diaminobutyric acid.*

Little attention has been given to the prebiotic synthesis of the sulfur amino acids cysteine and methionine. Heyns *et al.* (*1*) studied the action of a spark discharge on CH_4, NH_3, H_2O, and H_2S but did not find any sulfur-containing amino acids. Lu *et al.* (*2*) reported cysteine, cystine, and possibly methionine; the identifications were based only on paper chromatography. Choughuley and Lemmon (*3*) using an electron beam reported cysteic acid and cystamine in a similar experiment, but could find no cysteine. Sagan and Khare (*4*) reported obtaining cysteine by ultraviolet irradiation of a mixture of CH_4, C_2H_6, NH_3, H_2O, and H_2S; the identifications were based on results of autoradiography and the amino acid analyzer.

It has been claimed that methionine can be synthesized by the ultraviolet irradiation of NH_4SCN solutions (*5*). The identification was based only on coincidence of the radioactivity ($S^{14}CN^-$ was used) of the unknown with the ninhydrin color of known methionine on two-dimensional chromatography (*6*). We have repeated this experiment and were unable to detect any methionine ($< 10^{-7}$ mole of methionine per mole of NH_4SCN) (*7*).

Methionine might be considered "too complex" an amino acid to be synthesized in significant yield in an electric discharge reaction because of the large number of possible isomers. However, it was shown that quite large yields of α-hydroxy-γ-amino butyric acid and α,γ-diaminobutyric acid are obtained by the action of an electric discharge on a mixture of CH_4, N_2, H_2O, and traces of NH_3 (*8*). A reasonable precursor of these two amino acids is acrolein, which in the presence of CH_3SH, NH_3, and HCN might give methionine.

We therefore sparked a mixture of CH_4, N_2, H_2S, H_2O, and a trace of NH_3 (*9*) and found that methionine was synthesized in 0.03 percent yield based on the H_2S (2×10^{-4} percent, based on the carbon). The yields of glycine and alanine in the same experiment were 0.068 and 0.104 percent, respectively, based on the carbon.

It seemed likely that the limiting factor in this methionine synthesis was the formation of the thiomethyl group. We therefore sparked a mixture of CH_4, N_2, H_2O, CH_3SH, and a trace of ammonia and obtained 0.23 percent methionine. In a similar experiment, a mixture of CH_4, N_2, H_2O, and a trace of NH_3 was sparked, and the CH_3SH was added at the termination of the sparking. This gave an 0.63 percent yield of methionine, based on the sulfur.

The methionine in these experiments was separated from the other amino acids by the amino acid analyzer (*10*) without the use of ninhydrin. The methionine peak, which contained norvaline and alloisoleucine, was desalted and rerun on the analyzer, with the use of only the *p*H 3.28 citrate buffer. This procedure separated the methionine from the norvaline and alloisoleucine, and allowed quantification. The methionine peak was converted to the *N*-trifluoroacetyl *sec*-butyl ester (*11*) and chromatographed on a 50-m gas chromatographic capillary column with OV-225 as a stationary phase. The mass spectrum and gas chromatograph retention time of this unknown derivative agreed with the mass spectrum and retention time of an authentic sample of DL-methionine.

Acrolein was shown to be a product of the action of a spark discharge on a mixture of CH_4 and H_2O by means of an acrolein-specific fluorescent assay with *m*-aminophenol (*12*). The yield of acrolein was 0.04 percent, based on the methane. An alternate spectrophotometric assay with 4-hexylresorcinol (*13*), a method which is sensitive to both acrolein and propiolaldehyde, gave a combined yield of 0.11 percent. A propiolaldehyde yield of 0.07 percent is in agreement with the results of Dowler *et al.* (*14*).

On the basis of these results we propose the model shown in Fig. 1 for the prebiotic synthesis of methionine.

A model experiment was conducted to determine whether methionine could be synthesized from acrolein under the dilute conditions expected in the primitive ocean (*15*). A mixture of acrolein ($8 \times 10^{-4}M$), HCN ($4 \times 10^{-3}M$), NH_3 ($2.5 \times 10^{-3}M$), and CH_3SH ($5 \times$

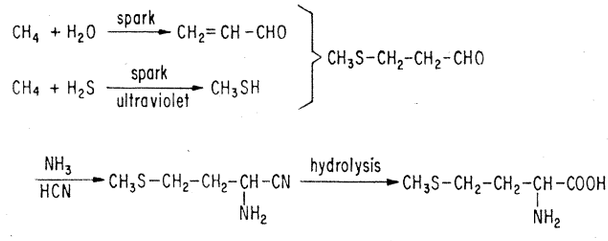

Fig. 1. Proposed model for prebiotic synthesis of methionine.

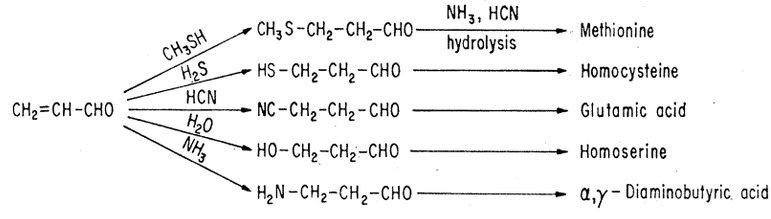

Fig. 2. Prebiotic synthesis of amino acids from acrolein.

$10^{-4}M$) was added to a deaerated solution of NH_4Cl ($7.5 \times 10^{-3}M$, final *p*H 8.7) and the solution was kept for 28 days. The mixture was hydrolyzed with 3*M* HCl, desalted, hydrolyzed again with 3*M* HCl, and quantitated on the amino acid analyzer. The yields were 15 percent methionine, 0.5 percent glutamic acid, 0.5 percent α,γ-diaminobutyric acid, and 13 percent α-hydroxy-γ-aminobutyric acid, based on the added acrolein. The same experiment omitting the CH_3SH gave 1.5 percent glutamic acid and 0.8 percent α,γ-diaminobutyric acid. These results show that CH_3SH adds to acrolein in preference to NH_3 or HCN under the conditions of the experiment. The relative yields of the amino acids in the primitive ocean would depend on the concentrations of CH_3SH, HCN, and NH_3 as well as the temperature and hydrolytic conditions.

It appears likely that acrolein was a key intermediate in prebiotic amino acid synthesis, being a precursor not only of methionine but also of glutamic acid, homocysteine, homoserine, α,γ-diaminobutyric acid, and α-hydroxy-γ-aminobutyric acid (Fig. 2).

There have been a number of speculations that methionine is not a "primitive" amino acid (*16*). These speculations are based primarily on the fact that methionine has only one codon, and that methionine could not be a primitive amino acid if it was very unstable. Methionine is indeed unstable in the presence of air (*17*), but seems to be quite stable under anaerobic conditions (*18*). Our results indicate that substantial amounts of methionine may have been present in the primitive ocean; therefore the possibility of methionine being a primitive amino acid cannot be excluded.

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6. An elemental analysis for C, H, N, and S is reported by Steinman *et al.* (*5*), but this was done on the authentic methionine sample and not on the unknown.
7. This experiment was performed as follows. One liter of 0.1*M* NH_4SCN held at 0°C was irradiated for 3 hours with a low-pressure Hanovia 4-watt ultraviolet lamp. The solution exhibited a milkiness described by Steinman *et al.* (*5*). Analysis on the Beckman-Spinco amino acid analyzer gave 24 peaks with yields from 3.1×10^{-4} to $\sim 10^{-5}$ mole per mole of NH_4SCN (on the basis of a 100 percent color yield). There was no detectable methionine.
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9. In a typical experiment we used a 3-liter flask with two tungsten electrodes. Then 100 ml of 0.05*M* NH_4Cl was added, the flask and solution were deaerated, and 5.4×10^{-5}, 5.4×10^{-4}, 1.6×10^{-2}, and 6.5×10^{-2} mole of H_2S (or CH_3SH), NH_3, N_2, and CH_4, respectively, were added as gases. The system was then sparked for 48 hours; a tesla coil was used as the discharge source.
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18. Methionine solutions at *p*H 5, 7, and 9 ($3 \times 10^{-3}M$) were degassed by multiple freezing and thawing and heated at 118°C for 29 days. No loss (< 5 percent) of methionine was detectable.
19. Supported by NSF grant GB 25048. The gas chromatograph–mass spectrometer was obtained, in part, with NSF grant GP 18245. We thank G. Pollock for the OV-225 gas chromatography column.

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Genetic Mapping of a Murine Leukemia Virus–Inducing Locus of AKR Mice

Abstract. *The chromosomal location of one of the two murine leukemia virus–inducing loci of AKR mice has been determined. The locus, which appears to be the integrated genome of the virus, is designated* Akv-1, *and is on linkage group I, 12 map units from* Gpi-1, *with gene order* c–Gpi-1–Akv-1. *This identification of a closely linked gene whose phenotype is independent of virus expression should facilitate analysis of the biologic importance of the* Akv-1 *locus.*

The high-leukemic mouse strain AKR (*1*) is characterized by lifelong infection with murine leukemia virus (MuLV), with virus being first detectable during late embryonic or early postnatal life (*2*). We have shown that two phenomena are responsible for this: that the potentially infectious MuLV genome is present, in unexpressed form, in all cells of the AKR embryo, and that it can undergo spontaneous induction, resulting in liberation of infectious virus which can infect the uninduced cells (*3*). The induction rate is immensely increased by treatment of the cells with either 5-iododeoxyuridine (IdU) or 5-bromodeoxyuridine (*4*). In recent experiments we have found that up to 50 percent of the cells of certain tissue culture lines of AKR cells initiate synthesis of viral antigen or antigens, and as many as 2 percent produce infectious virus within a few days after IdU treatment.

Low-leukemic strains of mice also contain MuLV genetic material, which may be manifested by appearance of small amounts of infectious virus late in life, or only by appearance of virus-specific antigens (*5*, *6*). In the case of one low-leukemic strain, BALB/c, it has been shown that the MuLV genome is present in all cells (*7*), as in AKR. The IdU induction of tissue cultures of embryo cells from low-leukemic strains gives rise to two patterns of response, both of which are different from that of AKR cells. Either little or no viral antigen or virions are produced (NIH Swiss mice, for example) (*8*), or, as in the case of BALB/c, relatively large amounts of viral antigens (*8*) and virions (*7*) are induced; but the virus particles are far less infectious than those from AKR cultures.

By studies of the genetic transmission of the high-virus phenotype, we have demonstrated that AKR mice possess two independently segregating chromosomal loci, either of which leads to the appearance of MuLV early in life (*9*, *10*). These loci also appear to confer the ability to synthesize infectious MuLV after induction with IdU. All available evidence is compatible with these loci being chromosomally integrated viral genetic determinants.

One of these virus-inducing loci, referred to as V_1, has been shown to be on linkage group I, about 30 map units from the loci for the genes for albino (*c*) and the β chain of hemoglobin (*Hbb*) genes (*9*). The other locus, V_2, has not been mapped.

Analysis of these loci has been facilitated by their isolation in backcross lines (*9*). In the first backcross (BC1) generation C57BR × (C57BR × AKR)-