ovomucoid-Sepharose column, caused only a 40% reduction of the growth of *B. cinerea* hyphae. This experiment indicated that UDA is a major proteinaceous antifungal agent in stinging nettle rhizomes. Furthermore, immunocytochemical localization studies have shown that UDA is distributed predominantly throughout the cortex of rhizomes and in the outer exodermis cell layer of roots, whereas the lectin is absent from stems and leaves of *Urtica dioica* plants (*20*). The occurrence of UDA at the periphery of the underground organs is consistent with a possible role in the defense against potentially pathogenic fungi.

Apical growth of fungal hyphae depends on a delicate balance between chitin synthesis and selective hydrolysis of preformed chitin chains (*21*). The suggestion of Mirelman *et al.* (*3*), that chitin-binding lectins can disturb this balanced growth by binding or crosslinking chitin chains, may also apply to UDA. However, the particular small size of this lectin may render it more accessible to the active sites involved in cell wall morphogenesis, and hence, more active in inhibiting hyphal growth than larger chitin-binding lectins such as WGA or potato lectin (molecular masses of 36 kD and 100 kD, respectively). UDA could prove promising for possible applications in the genetic engi-neering of disease-resistant plants because of its simplicity as a single gene product, its distinct antifungal properties, and its restricted distribution in the plant kingdom.

REFERENCES AND NOTES

1. M. E. Etzler, *Annu. Rev. Plant Physiol.* **36**, 209 (1985).
2. H. Rüdiger, *Bio Sci.* **34**, 95 (1984).
3. D. Mirelman *et al.*, *Nature* **256**, 414 (1975).
4. M. Mishkind *et al.*, *J. Cell Biol.* **92**, 753 (1982).
5. R. Barkai-Golan, D. Mirelman, N. Sharon, *Archs. Microbiol.* **116**, 119 (1978).
6. R. Brambl and W. Gade, *Physiol. Plant.* **64**, 402 (1985).
7. A. Schlumbaum *et al.*, *Nature* **324**, 365 (1986).
8. W. J. Peumans, M. De Ley, W. F. Broekaert, *Fed. Exp. Biol. Soc. Lett.* **177**, 99 (1983).
9. N. Shibuya *et al.*, *Arch. Biochem. Biophys.* **249**, 215 (1986).
10. M. P. Chapot, W. J. Peumans, A. D. Strosberg, *Fed. Exp. Biol. Soc. Lett.* **195**, 231 (1986).
11. UDA was isolated from stinging nettle rhizomes as described by Peumans *et al.* (*8*). However, the lectin was further purifed by high-performance gel filtration on a Superose-12 column (Pharmacia), with phosphate buffered saline (PBS) as a running buffer. The lectin fraction was subsequently subjected to affinity chromatography on ovomucoid Sepharose. Washing buffer was PBS and desorbent 50 mM acetic acid. UDA was dialyzed against water, lyophilized, and stored at 4°C.
12. J. Molano *et al.*, *Anal. Biochem.* **83**, 648 (1977).
13. W. F. Broekaert, J. Van Parijs, A. K. Allen, W. J. Peumans, *Physiol. Mol. Plant Pathol.* **33**, 319 (1988).
14. J. S. Price and R. Storck, *J. Bacteriol.* **124**, 1574 (1975).
15. R. J. Cohen, *Plant Sci.* **43**, 93 (1986).
16. F. B. Abeles *et al.*, *Plant Physiol.* **47**, 129 (1970).
17. S. Bartnicki-Garcia and E. Lippman, in *Handbook of Microbiology*, A. I. Laskin and H. A. Lechevalier, Eds. (CRC Press, West Palm Beach, FL, ed. 2, 1978), vol. 4, pp. 87–252.
18. Isolation of WGA was done as described by W. J. Peumans, H. M. Stinissen, and A. R. Carlier [*Planta* **154**, 562 (1982)]. Potato lectin was purified as describing R. J. Owens, and D. H. Northcote [*Phytochemistry* **19**, 1861 (1980)].
19. J. Van Parijs, unpublished results.
20. D. Lambrechts and J.-P. Verbelen, unpublished results.
21. V. Farkas *Microbiol. Rev.* **43**, 117 (1979).
22. O. D. Dhingra and J. B. Sinclair, in *Basic Plant Pathology Methods* (CRC Press, Boca Raton, FL, 1985).
23. Culture of fungi and collection of spores was done according to published protocols [see Broekaert *et al.* (*13*) for *T. hamatum, T. viride, B. cinerea*, and *Phycomyces blakesleeanus*, and Dhingra and Sinclair (*22*) for *Phoma betae, C. lindemuthianum, Phytophthora erythroseptica*, and *S. nodorum*.
24. Supported in part by grants of the National Fund for Scientific Research (Belgium); W.J.P. is senior research associate, and W.F.B. research assistant of this fund; J.V.P. received a fellowship of the Belgian Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw. We thank A. Van Laere and J. Vanderleyden for discussion.

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Fig. 3. Synergism between UDA and tobacco chitinase. Hyphal growth of *T. hamatum* was measured (as described in the legend to Fig. 1) at different concentrations of UDA (O), tobacco chitinase (△), and UDA and tobacco chitinase combined at a 2-to-1 mass ratio (▲). Relative hyphal growth is expressed as a percentage of the hyphal growth of control cultures; data are means of three independent experiments. Ratios of standard errors to means were 11% or less. Protein concentration is presented on a logarithmic scale.

Was Adenine the First Purine?

ALAN W. SCHWARTZ AND C. G. BAKKER

Oligomerization of HCN (1 molar) in the presence of added formaldehyde (0.5 molar) produced an order of magnitude more 8-hydroxymethyladenine than adenine or any other biologically significant purine. This result suggests that on the prebiotic Earth, nucleoside analogs may have been synthesized directly in more complex mixtures of HCN with other aldehydes.

FORMALDEHYDE AND HCN ARE THE simplest and most plausible starting materials for prebiotic syntheses on the early Earth (*1–3*). Formaldehyde is expected to have been ubiquitous, because of the ease of its photochemical formation from CO_2 and water vapor, as well as its formation from methane and water vapor by electric discharge, and HCN is produced efficiently by electric discharge in nitrogen-methane mixtures. In dilute aqueous solution at pH 9.2, HCN oligomerizes to produce a complex range of products (*4, 5*). Formaldehyde has been shown to accelerate this oligomerization (*6*). Adenine and traces of uracil have been identified among the products under other conditions, such as acid hydrolysis, that hydrolyze more complex precursors (*7*). Investigation of unhydrolyzed oligomerization mixtures prepared from HCN in the absence of formaldehyde has revealed the presence of the adenine precursor adenine-8-carboxamide (*8*). In this report we show, however, that the purine formed most in oligomerizing solutions of HCN to which formaldehyde has been added is 8-hydroxymethyladenine (HMA), rather than adenine-8-carboxamide or adenine itself.

We synthesized HMA according to a known procedure (*9*) and determined its chromatographic behavior on two high-performance liquid chromatographic (HPLC) columns: Aminex A25 (Bio-Rad) in 0.1M sodium formate (pH 4.0) and Aminex A6 in 0.1M NH₃ containing 0.02M ammonium carbonate (pH 10.0). Both columns were operated at 60°C. A fresh solution of formaldehyde (1M), prepared by sublimation from paraformaldehyde, was mixed with an
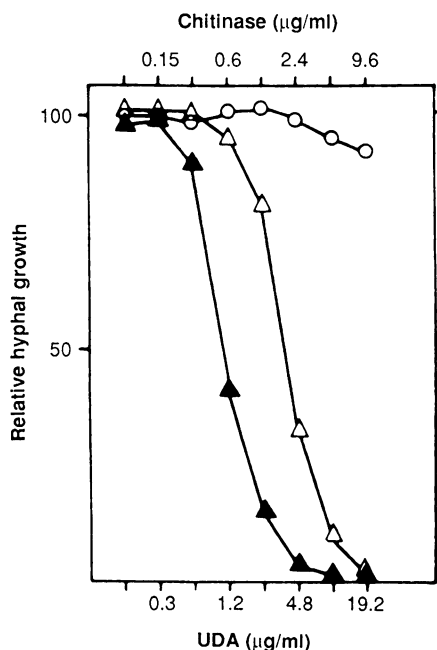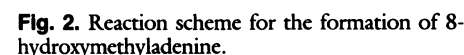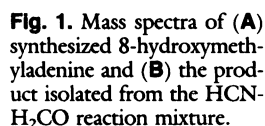
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equal volume of $2M$ NaCN. The pH of the solution so obtained was adjusted to 9.2 (the pK_a of HCN) by addition of HCl. Formaldehyde reacts nearly quantitatively with HCN to form glycolonitrile. The solution therefore contained $0.5M$ HCN/CN$^-$ and $0.5M$ glycolonitrile and was allowed to stand for 9 months at room temperature. A similar solution was prepared and analyzed immediately to serve as a blank. A sample of the 9-month reaction mixture was also adjusted to pH 8.5 with HCl and hydrolyzed in a sealed vial at 110°C for 24 hours.

Samples were analyzed directly on the A25 column. The blank solution showed no peaks corresponding to any of the products of interest. We could detect no more than a trace of adenine-8-carboxamide, either before or after hydrolysis at pH 8.5. Peaks corresponding in retention time to HMA and to adenine were collected from the A25 column, concentrated, and reanalyzed on Aminex A6. We isolated the material on a preparative scale and refractionated the final product to verify the identification of HMA. Retention times on both columns were identical to the standard, as was the ultraviolet absorption spectrum. Mass spectroscopy (Fig. 1) confirmed the identification.

A plausible mechanism for the synthesis of HMA is given in Fig. 2. This pathway is consistent with previous work on the pathway of adenine formation in HCN solutions (8), as well as with a mechanism that has been proposed to explain the catalysis of HCN oligomerization by formaldehyde (7).

We detected 47 μmol of HMA and 3 μmol of adenine per liter of reaction mixture before hydrolysis. These concentrations increased by about 25% after hydrolysis without altering the relative proportions of the two products. The totals of HMA and adenine present after hydrolysis correspond to a yield of 0.03% based on the total HCN in the solution (including glycolonitrile), or 0.06% based on the available HCN that was originally present. This amount is comparable to yields of adenine reported previously after acid hydrolysis of $1M$ solutions of HCN in the absence of formaldehyde (10).

The question of the ease of synthesis of purines and pyrimidines is an important one in the context of theories of the origins of life. Although HCN oligomerization is often cited as a source for purines and pyrimidines on the prebiological Earth, it is important to realize that none of the five bases that occur in nucleic acids has been identified among the initial oligomerization products formed in dilute solution. The detection of adenine requires an additional hydrolysis step; usually hydrolysis in 3 to $6M$ HCl at 100 to 110°C for up to 18 hours. Such conditions cannot be considered to be prebiotic (11). The only attempt to apply a gentler hydrolytic procedure reported in the literature is the use of pH 8.5 at 110°C, which results in the (qualitative) detection of adenine (10). It should be noted in this connection that all of the purines and pyrimidines that have been reported in meteorites have similarly been found only after acid hydrolysis [for review, see (12)].

An even more important consideration in evaluating such studies, however, is the question of the possible role of side reactions with other components of the presumed prebiotic milieu. In earlier work, we investigated the pathway to adenine formation in unhydrolyzed oligomerization mixtures prepared from pure HCN and showed that a major precursor is the compound adenine-8-carboxamide (8). It has now become clear that the presence of formaldehyde shifts the reaction pathway so that HMA rather than adenine-8-carboxamide is formed. It is highly unlikely that pure solutions of HCN ever existed on the primitive Earth. Since some of the most probable "contaminants" of the prebiotic environment would have been formaldehyde and other aldehydes, the question arises as to the possible role of this pathway in chemical evolution. Recent speculations (13) concerning the origins of the first RNA molecules, together with the demonstration that nucleic acid analogs are potentially capable of acting as templates for oligomerizations (14), provide an incentive to search for more primitive roots of the system. Among the more interesting structures that have been studied are acyclic analogs of nucleosides in which ribose has been replaced at the N-9 position of adenine or guanine by a derivative of glycerol (15, 16). Although such compounds provide a number of theoretical advantages in comparison with nucleosides (13), no prebiotic synthesis has yet been achieved. Inspection of the reaction scheme shown in Fig. 2 suggests that the substitu-

Fig. 1. Mass spectra of (A) synthesized 8-hydroxymethyladenine and (B) the product isolated from the HCN-H_2CO reaction mixture.

Fig. 2. Reaction scheme for the formation of 8-hydroxymethyladenine.

tion of more complex carbonyl compounds for formaldehyde (H_2CO) may lead to the direct formation of acyclic nucleoside analogs at the C-8 position of adenine. Glycoaldehyde, for example ($CHO\text{-}CH_2OH$), may produce a dihydroxyl analog of potential interest in chemical evolution studies.

REFERENCES AND NOTES

1. S. L. Miller and L. E. Orgel, *The Origins of Life on the Earth* (Prentice-Hall, Englewood Cliffs, NJ, 1974).
2. A. W. Schwartz, *Naturwissenschaften* **70**, 373 (1983).
3. R. Stribling and S. L. Miller, *Origins Life* **17**, 261 (1987).
4. J. Oro, *Biochem. Biophys. Res. Commun.* **2**, 407 (1960).
5. R. A. Sanchez *et al.*, *J. Mol. Biol.* **30**, 223 (1967).
6. A. W. Schwartz and M. Goverde, *J. Mol. Evol.* **18**, 351 (1982).
7. A. W. Schwartz *et al.*, *Origins Life* **14**, 91 (1984).
8. A. B. Voet and A. W. Schwartz, *Bioorg. Chem.* **12**, 8 (1983).
9. C. V. Z. Smith, R. K. Robins, R. L. Tolman, *Chem. Soc. Perkin. Trans. Part 1* **1973**, 1855 (1973).
10. J. P. Ferris *et al.*, *J. Mol. Evol.* **11**, 293 (1978).
11. The HCN concentrations used in this and in other studies can similarly not be regarded as "prebiotic." Solutions of 0.1 to 1M are chosen to produce a measurable set of products within a reasonable time. That such concentrations would not have been possible in a prebiotic ocean can be appreciated from the fact that the total nitrogen now present at the surface of the Earth would produce no more than a 0.2M solution if totally converted to HCN and dissolved in the present oceans. High concentrations of HCN can, however, be produced by freezing very dilute solutions, and this process has been proposed to have operated on the prebiotic Earth (*1*).
12. J. R. Cronin, S. Pizzarello, D. P. Cruikshank, in *Meteorites and the Early Solar System*, J. F. Kerridge and M. S. Matthews, Eds. (Univ. of Arizona Press, Tucson, 1988), pp. 819–857.
13. G. F. Joyce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4398 (1987).
14. J. Visscher, C. G. Bakker, R. van der Woerd, A. W. Schwartz, *Science* **244**, 329 (1989).
15. A. W. Schwartz and L. E. Orgel, *Science* **228**, 585 (1985).
16. J. Visscher and A. W. Schwartz, *J. Mol. Evol.* **28**, 3 (1988).
17. Supported in part by National Aeronautics and Space Administration grant NGR 05067. We thank P. Daas for synthesizing 8-hydroxymethyladenine.

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Fig. 1. Structures of ligands, substrates, and affinity labels.

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Generation of a Catalytic Antibody by Site-Directed Mutagenesis

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A hybrid Fv fragment of the dinitrophenyl-binding immunoglobulin A (IgA), MOPC315, has been generated by reconstituting a recombinant variable light chain (V_L) produced in *Escherichia coli* with a variable heavy chain (V_H) derived from the antibody. The Tyr^{34} residue of V_L was substituted by His in order to introduce a catalytic imidazole into the combining site for the ester hydrolysis. The His mutant Fv accelerated the hydrolysis of the 7-hydroxycoumarin ester of 5-(2,4-dinitrophenyl)-aminopentanoic acid 90,000-fold compared to the reaction with 4-methyl imidazole at pH 6.8 and had an initial rate that was 45 times as great as that for the wild-type Fv. The hydrolyses of aminopropanoic and aminohexanoic homologs were not significantly accelerated. Thus a single deliberate amino acid change can introduce significant catalytic activity into an antibody-combining site, and chemical modification data can be used to locate potential sites for the introduction of catalytic residues.

TWO GENERAL APPROACHES HAVE emerged for the development of selective biological catalysts: genetic modification of enzyme active sites and chemical modification of biological or synthetic receptors with catalytic groups. These efforts have not only resulted in the generation of novel catalysts but have also provided a greater understanding of the mechanisms of enzymatic reactions. Recently, we and others have demonstrated that antibody-combining sites are also attractive starting points for the generation of selective catalysts, since they bind ligands with high affinity and specificity (*1*). A number of strategies have been applied to the generation of antibodies that catalyze a variety of reactions with rate enhancements of 10^2 to 10^6 (*2–5*). Site-directed mutagenesis should prove useful for increasing the activity of these catalytic antibodies, or for the stepwise evolution of antibody-combining sites into efficient selective catalysts. We describe the substitution of a catalytic His residue for Tyr at position 34 of the light chain of the 2,4-dinitrophenyl (DNP)–binding antibody MOPC315 (*6, 7*). Tyr^{34L} was previously shown to be in close proximity to the binding site by affinity labeling experiments (*8*). The His mutant binds DNP ligands one-half to one-eighth as tightly as the wild type, but catalytically hydrolyzes DNP-coumarin ester
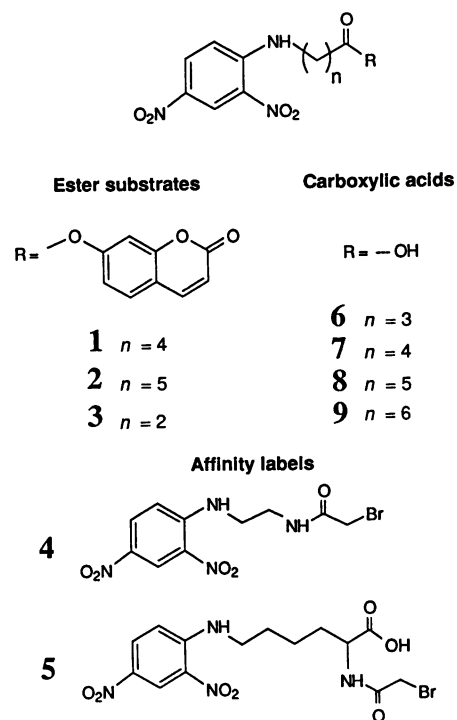
1 45 times as rapidly, with a rate enhancement of ~90,000 compared to 4-methylimidazole.

The MOPC315 antibody binds a variety of DNP ligands with association constants of 10^3 to $10^7 M^{-1}$ (*8, 9*) and can be proteolyzed with pepsin to yield functional Fab' or Fv fragments (*10*). The Fv fragment (26 kD) is a heterodimer consisting of two peptides, V_H (14 kD) and V_L (12 kD), and contains all of the sequences necessary for folding of the binding domain and recognition of the DNP hapten. Although the atomic coordinates for MOPC315 have not been determined, magnetic resonance spectroscopy (*11–13*) and affinity labeling (*8*) studies have provided some information concerning binding site structure.

Because imidazole acts as a nucleophilic catalyst for the hydrolysis of carboxylate esters in aqueous solutions, introduction of a His at the appropriate position in the combining site of MOPC315 should result in a catalytic antibody with specific hydrolytic activity towards DNP-containing esters. Data from chemical modification experiments were used to target residues for substitution with His. Although 14 potentially reactive side chains occur in the hypervariable region (2 His, 2 Lys, 3 Arg, and 7 Tyr), DNP-containing affinity labels alkylate primarily two residues, Tyr^{34L} and Lys^{52H} (*4, 8*). The reactivity of each residue strongly depends on the number of atoms between the DNP ring and the electrophilic carbon of the affinity reagent; Tyr^{34L} is alkylated

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