synthesized until after fertilization (*11*).

Studies of this type can identify mutagens and determine the risk associated with exposure to known mutagens. Exposure of the fetus to low doses of mutagens may not cause immediate, obvious effects such as morbidity or birth defects, but can have severe consequences when the otherwise normal female offspring reach puberty.

Caution must be exercised when data gathered from animal experiments are extrapolated to the situation in humans. However, many children have been born to mothers treated with azathioprine (*2, 12*), and these children should be observed carefully to determine whether their reproductive function has been adversely affected by the drug.

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References and Notes

1. M. K. Jensen and M. Soborg, *Acta Med. Scand.* **179**, 249 (1966); M. K. Jensen, *ibid.* **182**, 445 (1967); *Int. J. Cancer* **5**, 147 (1970).
2. D. E. Leb, B. Weisskopf, B. S. Kanovitz, *Arch. Intern. Med.* **128**, 441 (1971); H. V. Price, J. R. Salaman, K. M. Laurence, H. Langmaid, *Transplantation* **21**, 294 (1976).
3. G. B. Elion, S. Callahan, S. Beider, G. H. Hitchings, R. W. Rudles, *Cancer Chemother. Rep.* **14**, 93 (1961).
4. E. M. Gonzalez, K. Krejczy, R. A. Malt, *Surgery* **68**, 254 (1970); M. A. Lea, D. Sasovetz, A. Musella, H. P. Morris, *Cancer Res.* **30**, 1994 (1970); D. Malamud, E. M. Gonzalez, H. Chiu, R. H. Malt, *ibid.* **32**, 1226 (1972).
5. J. P. Scannell and G. H. Hitchings, *Proc. Soc. Exp. Biol. Med.* **122**, 627 (1966).
6. J. M. Clark, *Mutat. Res.* **28**, 87 (1975).
7. T. G. Baker, *Proc. R. Soc. London Ser. B* **158**, 417 (1963).
8. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960), p. 111.
9. J. H. Githens, J. G. Rosenkrantz, S. M. Tunnock, *J. Pediatr.* **66**, 959 (1965); J. G. Rosenkrantz, J. H. Githens, S. M. Cox, D. L. Kellum, *Am. J. Obstet. Gynecol.* **97**, 387 (1967).
10. B. Mintz and E. S. Russell, *Anat. Rec.* **122**, 443 (1955); B. Mintz, *J. Cell. Comp. Physiol.* **56**, 31 (1960).
11. J. L. Sirlin and R. G. Edwards, *Exp. Cell Res.* **18**, 190 (1959); G. T. Rudkin and H. A. Griech, *J. Cell Biol.* **12**, 169 (1962).
12. J. A. Board, H. M. Lee, D. A. Draper, D. M. Hume, *Obstet. Gynecol.* **29**, 318 (1967); J. J. Kaufman, W. Dignam, W. E. Goodwin, D. C. Martin, R. Goldman, M. H. Maxwell, *J. Am. Med. Assoc.* **200**, 162 (1967); R. M. Caplan, J. B. Dossetor, G. B. Manghan, *Am. J. Obstet. Gynecol.* **106**, 644 (1970); G. D. Lower, L. E. Steven, J. S. Najarian, K. Reemsta, *ibid.* **111**, 1120 (1971); I. R. Merkatz, G. H. Schwartz, D. S. David, K. H. Stenzel, R. R. Riggio, J. C. Whitesell, *J. Am. Med. Assoc.* **216**, 1749 (1971); I. Penn, E. Makowski, W. Doegemueller, C. G. Halgrimson, T. E. Starzl, *ibid.*, p. 1755; L. K. Merrill, J. A. Board, H. M. Lee, *Obstet. Gynecol.* **41**, 270 (1973); C. J. Cote, H. J. Meuwissen, R. J. Pickering, *J. Pediatr.* **85**, 324 (1974); E. T. Jacob, S. Grunstels, Z. Shapira, M. Berant, O. S. Better, G. Boner, *Isr. J. Med. Sci.* **10**, 206 (1974); E. Sharon, B. Jones, H. Diamond, D. Kaplan, *Am. J. Obstet. Gynecol.* **118**, 25 (1974).
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Peptide Formation in the Prebiotic Era: Thermal Condensation of Glycine in Fluctuating Clay Environments

Abstract. *As geologically relevant models of prebiotic environments, systems consisting of clay, water, and amino acids were subjected to cyclic variations in temperature and water content. Fluctuations of both variables produced longer oligopeptides in higher yields than were produced by temperature fluctuations alone. The results suggest that fluctuating environments provided a favorable geological setting in which the rate and extent of chemical evolution would have been determined by the number and frequency of cycles.*

Recently we proposed (*1*) that environments where both water content and temperature fluctuate diurnally and seasonally presented the most favorable and geologically relevant settings for condensation reactions in the prebiotic era. Simulation of the main characteristics of such a system—that is, variable water content and temperature, high solute concentration during a dehydration process, the presence of a catalytically active solid surface, and the opportunity for redistribution of the organic molecular species during a hydration process—led to condensation of glycine to oligopeptides. For purposes of comparison, systems that were only subjected to temperature fluctuations were also examined (*2*).

In a typical experiment, 1 ml of 23 m*M* glycine was added to the clay mineral in the Na^+ form (kaolinite, 60 mg; bentonite, 20 mg). After hydration of the clay, the suspension was treated as follows for wetting-drying and temperature fluctuations (WDTF): (i) dehydration at 60°C for 1 to 2 days; (ii) heating at 94°C for 2 to 3 days; (iii) rehydration with 1 ml of water, and (iv) repetition of steps (i) to (iii) for *n* number of cycles. Suspensions tested for temperature fluctuations (TF) with dehydration were treated as in step (i) above, with subsequent intermittent heating at 94°C and cooling to 25°C; these TF treatments were performed in parallel with the wetting-drying series. Products were extracted from the clay with water and 1*N* NH_4OH (*3*). Identification of glycine oligopeptides was based primarily on their elution times as determined with an automatic amino acid analyzer (*4*).

In the absence of clay, trace amounts of diglycine formed occasionally after lengthy heating. The presence of clays, however, consistently brought about the synthesis of peptides, and diglycine was readily detected after 1 week at 94°C.

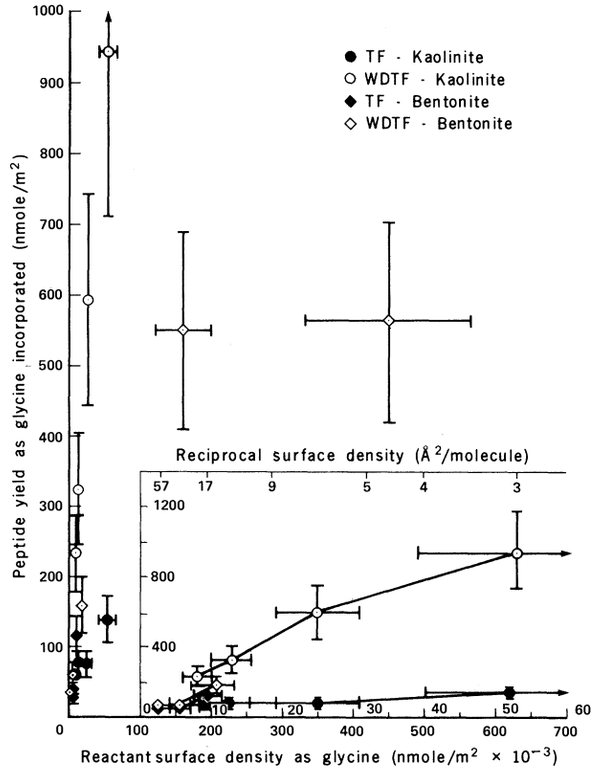

Fig. 1. Effect of initial glycine surface density on the total yield of peptide. In the inset the lower left-hand corner of the figure is redrawn for clarity. Values for the reciprocal surface density are provided on the upper scale of the inset for various reactant surface densities. Error bars represent the ± 25 percent uncertainty in the surface area estimates; replicate analyses from Table 1 typically exhibit much smaller uncertainties.

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Further heating increased the amount of diglycine and produced larger peptides. Diglycine was also formed at 80°C, but at a rate two and a half to four times slower than at 94°C. At 60°C, only trace amounts of diglycine were detected after 35 days (*5*).

Without exception in the kaolinite system, WDTF cycles enhanced peptide synthesis as compared to TF cycles alone yielding peptides up to pentaglycine (Table 1). In similar experiments with bentonite, no oligomers higher than triglycine were detected, with the exception of trace amounts of tetraglycine after 27 cycles (experiment 17). Although the effect of WDTF cycles on the total yield in the presence of bentonite was less pronounced than in the case of kaolinite, the yields of triglycine were always higher than with the TF cycles alone. Within the uncertainty of the data, there appears to be relatively little systematic change in either peptide yield or composition on increasing the number of cycles from 11 to 33. From cycle 1 to 11 in the bentonite system with high initial glycine-to-clay ratio (experiments 21 to 23), however, enhancement of oligomer production occurs just as on kaolinite, and synthesis from dimer to higher oligomers is quite clear.

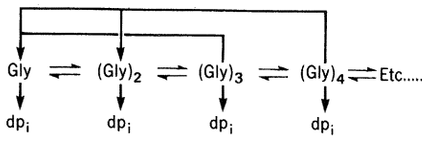

Fig. 2. Glycine oligomerization scheme. The letters dp_i represents unspecified decomposition products. Oligomers grow by addition of single glycine units. Partial hydrolysis of an oligomer, however, can yield glycine or other shorter oligomers (or both).

Four sets of reactions show the influence of initial glycine-to-clay ratio on oligomerization. Increases in the ratio within each set by a factor of 3 (experiments 26 to 28), by a factor of 6 (experiments 10, 12 to 14, 3, and 5 to 7), and by a factor of 58 (experiments 17 to 20) produced significant and systematic enhancements in oligomer length and total yield. Addition of experiment 23 to the WDTF-bentonite set, however, shows that increasing the ratio by another factor of 3 (from 58 to 168) did not produce any additional product enhancement. To permit comparison of data between clays, initial glycine-to-clay ratios and total oligomer yields (Table 1) were normalized to clay surface area and plotted in Fig. 1. Although the data points acquire uncertainties as large as ±25 percent as a result of this normalization (*6*), their trend is as follows. Oligomer yield increases as the reactant surface density is raised from less than 5×10^3 to 50×10^3 nmole/m^2; after this point a "saturation" effect prevails.

The saturation effect occurs at a reciprocal surface density less than 4 Å^2 per reactant molecule (*7*). By comparison, 25 Å^2 is the approximate area one glycine molecule would occupy were it adsorbed flat on the basal plane of the clay surface. The results indicate that efficient condensation requires either direct participation of nonadsorbed amino acid molecules in conjunction with those adsorbed as a monolayer on the clay, or an excess population of reactants (not in the monolayer) to provide facile solid state diffusion to and from the catalytic sites on the clay surface, or both. At low reactant surface densities, where comparative data are available, there appears to be little difference in the total productivity in the peptide yield with either kaolinite or bentonite.

In kaolinite experiments, the higher oligopeptide yield achieved with WDTF cycling as compared to that with TF cycling results from redistribution of organic molecules during the former. Each wetting redistributes the molecules by

Table 1. Oligomerization of glycine on clays: reactants, conditions, products, and yields.

Experiment	Glycine/clay (nmole/mg)	Cycles		Net heating period* (days)	Yields of oligomers (nmole/mg clay†)				Total yield‡ of glycine in oligomers (nmole/mg clay)
		Type	Number		Di	Tri	Tetra	Penta	
					Kaolinite				
1	374	WDTF	11	33.7	2.27 ± 0.37	0.45 ± 0.05			5.9 ± 0.9
2	374	WDTF	21	55.0	1.99 ± 0.15	0.79 ± 0.17	0.29	TR§	7.6 ± 0.8
3	374	WDTF	27	67.4	2.25 ± 0.13	1.01 ± 0.12	0.33	TR	8.9 ± 0.6
4	374	WDTF	33	77.3	2.21 ± 0.26	0.83 ± 0.16	0.32	TR	8.2 ± 1.0
5	123	WDTF	27	67.4	0.97 ± 0.12	0.38 ± 0.03	0.10	TR	3.5 ± 0.3
6	193	WDTF	27	67.4	1.31	0.55	0.15	TR	4.87
7	791	WDTF	27	67.4	3.50	1.58	0.60	TR	14.14
8	371	TF	11	33.7	0.87 ± 0.14	0.09			2.0 ± 0.3
9	371	TF	21	55.0	0.33 ± 0.08	0.07	TR		0.9 ± 0.2
10	371	TF	27	67.4	0.43	0.08	TR	—	1.10
11	371	TF	33	77.3	0.71 ± 0.20	0.19	TR	—	2.0 ± 0.4
12	129	TF	27	67.4	0.33	0.09	—	—	0.93
13	185	TF	27	67.4	0.44	0.09	—	—	1.15
14	780	TF	27	67.4	0.77	0.17	—	—	2.05
					Bentonite				
15	1,070	WDTF	11	32.8	6.37 ± 0.87	0.2	—	—	13.3 ± 1.7
16	1,070	WDTF	21	55.0	7.99 ± 2.48	0.6	—	—	17.8 ± 1.7
17	1,070	WDTF	27	67.4	4.92 ± 0.64	0.61 ± 0.19	TR	—	11.7 ± 1.7
18	554	WDTF	27	67.4	2.92 ± 0.72	0.2	—	—	6.4 ± 1.4
19	2,141	WDTF	27	67.4	12.7 ± 1.17	1.90 ± 0.13	TR	—	31.1 ± 2.7
20	32,000‖	WDTF	11	57.0	36.7 ± 8.5	8.2 ± 3.0		2.5 ± 0.3	111 ± 28
21	93,000‖	WDTF	1	10.6	11.9 ± 1.7	TR		—	24 ± 3
22	93,000‖	WDTF	5	25.4	26.9 ± 1.1	1.9 ± 0.9		—	60 ± 5
23	93,000‖	WDTF	11	57.0	40.1 ± 3.2	7.9 ± 0.5	1.2	0.8 ± 0.4	113 ± 10
24	1,054	TF	11	32.8	2.72 ± 1.65	TR	—	—	5.4 ± 3.3
25	1,054	TF	21	55.0	7.11 ± 0.46	TR	—	—	14.2 ± 0.9
26	1,054	TF	27	67.4	3.59 ± 0.37	TR	—	—	7.2 ± 0.7
27	580	TF	27	67.4	2.72 ± 0.32	TR	—	—	5.4 ± 0.6
28	1,894	TF	27	67.4	10.74 ± 1.80	0.47	—	—	21.5 ± 3.6

*At 94°C. †The ± indicates average and average deviation of three or four replicate analyses. ‡Given as sum of all glycine incorporated in oligomers. §TR, trace amounts. Dash, not detectable. ‖In these experiments, 10 mg of bentonite was used.

desorption; blocked active sites on the surface become free from blocking species; glycine in the solid phase becomes redissolved and available again for surface adsorption; and the surface concentration of glycine can be replenished during the next dehydration stage. The smaller, but real, enhancement associated with WDTF cycles in the presence of bentonite may result from a less efficient redistribution process for bentonite than for kaolinite (*8*).

On going from TF to WDTF cycles, total peptide yield increased, as did the proportion of large oligomers over small ones. In contrast, initial glycine-to-clay ratio had no apparent effect on the ultimate distribution of products among oligomers (experiments 3, 5 to 7, 17 to 20, and 23). Moreover, essentially no change occurs in oligomer composition after 11 cycles with either WDTF or TF cycles. With TF cycles, little or none is expected since there exists no redistribution mechanism other than slow surface diffusion. During WDTF cycles, however, redistribution is expected to occur. The fact that oligomer composition remains unchanged after many WDTF cycles is in accord with the attainment of a steady-state composition in which oligomer formation is balanced by oligomer decomposition (*9*). A plausible reaction scheme is shown in Fig. 2.

If the scheme that we propose is correct, and if reactions in the WDTF mode included a mixture of amino acids, then this model of a fluctuating system may produce nonrandom oligopeptides. The dynamic balance of peptide bond formation and destruction over a large number of cycles provides a mechanism for selective generation of oligomers with nonrandom sequences determined by factors characteristic of specific monomers and lower oligomers, such as strength of adsorption to clay, solubility, ease of condensation, stability to hydrolysis, and nearest neighbor interactions. Catalytic properties of such nonrandom oligomers would be of great interest.

We postulate that, in the prebiotic era when monomers were supplied at a reasonable rate by syntheses, the most favorable environment for condensation of amino acids, and perhaps for condensation reactions of other organic molecules, consisted of one in which diurnal fluctuations yielded a wet period during the night and a dry and hot period during the day. Prolonged dry periods would have inhibited further condensation, and incomplete dehydration would have led to low monomer surface concentrations and ineffective catalytic surfaces. The adsorptive and catalytic surface need not have been provided by clays or other inorganic substances (*10*); organic microstructures (*11*) may have served as well.

We have shown that condensation of glycine to oligomers can take place in a simple, geologic model system without implausible condensing agents, where changes in temperature and water content occur in cyclic fashion. Cycling is important because, in principle, the effective number of oligomer "generations" and, thus, the rate and extent of chemical evolution, can be increased simply by increasing the frequency and number of cycles (*12*).

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References and Notes

1. N. Lahav and S. Chang, *J. Mol. Evol.* **8**, 357 (1976).
2. See (*1*) for descriptions of the environments modeled in these experiments.
3. Consistent with glycine's low adsorbability [B. K. G. Theng, *The Chemistry of Clay-Organic Reactions* (Wiley, New York, 1973), p. 158] was our finding that more than 95 percent of ^{14}C-labeled glycine remained in the liquid phase of bentonite and kaolinite suspensions in the first wet period of the wetting-drying cycles. The recovery of diglycine from kaolinite was 93 percent.
4. The identity of diglycine was confirmed by combined gas chromatography–mass spectroscopy analysis of the *N*-trifluoroacetyl methyl ester [S. Chang, J. J. Flores, C. Ponnamperuma, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1011 (1969)]. The presence of glycine oligomers was verified by collecting the oligopeptide fraction eluted from the amino acid analyzer and comparing the chromatogram of a portion of the isolated sample with that of another portion that was hydrolyzed by acid. Di-, tri-, tetra-, and pentaglycine disappeared after the HCl treatment, and a concomitant release of glycine was detected.
5. For other examples of solid-state amino acid condensation at ≤100°C, see J. J. Flores and J. P. Leckie, *Nature (London)* **244**, 435 (1973); H. Sawai and L. E. Orgel, *J. Mol. Evol.* **6**, 185 (1975); H. Sawai, R. Lohrmann, L. E. Orgel, *ibid.*, p. 165; D. L. Rohlfing, *Science* **193**, 68 (1976).
6. We have assumed a surface area of 15 m²/g for kaolinite [R. E. Grim, *Clay Mineralogy* (McGraw-Hill, New York, 1968), p. 464]. The total external and interlayer surface area of bentonite ranges from 150 to 250 m²/g (unpublished measurements by N. Lahav) and is taken to be 200 ± 50 m²/g. This value is less than the theoretical value of 760 m²/g as a result of flocculation induced by aluminum hydroxide before bentonite was used in experiments.
7. A discussion of reciprocal surface density is found in (*1*).
8. Wetting of bentonite is a slow process [N. Lahav and A. Banin, *Isr. J. Chem.* **6**, 285 (1968)], and the hydration time may have been insufficient to complete wetting of the interlayer surfaces and to achieve effective redistribution.
9. Presumably, oligomers longer than pentapeptide were formed, but were not detectable with our analytical system.
10. For studies in which reactants or the reaction vessel provided the solid surface, see A. W. Schwartz, M. Veen, T. Bisseling, and G. J. F. Chittenden [*Biosystems* **5**, 119 (1973)] and E. Stephen-Sherwood, D. G. Odom, and J. Oro [*J. Mol. Evol.* **3**, 323 (1974)]; see also (*1*) and (*5*).
11. A. Nissenbaum, D. H. Kenyon, J. Oro, *J. Mol. Evol.* **6**, 253 (1976).
12. The effect of precipitate accumulation on clay-mediated condensation reactions and the possibility of selective solute adsorption and leaching with natural and metal ion-exchanged clays remain to be evaluated.
13. Supported in part by NASA Ames–University Consortium Interchange NCA2-OR685-702. This work was performed at Ames by N.L. and D.W. as National Academy of Sciences senior research associate and National Science Foundation faculty research fellow, respectively.
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Histamine H_1 Receptor-Mediated Guanosine 3′,5′-Monophosphate Formation by Cultured Mouse Neuroblastoma Cells

Abstract. *Incubation of cultured mouse neuroblastoma cells with histamine caused a rapid and marked increase in the formation of guanosine 3′,5′-monophosphate (cyclic GMP) by these cells. Receptor agonists for H_1, but not H_2, caused this effect which was reduced by H_1 but not by H_2 or muscarinic acetylcholine receptor antagonists. These results indicate that activation of H_1 receptors in these cultured nerve cells stimulated cyclic GMP formation.*

Histamine has many different effects on various cell types, and may serve as a neurotransmitter (*1*). This biogenic amine apparently brings about its effects by activation of two different receptors (H_1 and H_2) which are distinguished by their differential sensitivities to agonists (for example, 2-methylhistamine for H_1 and 4-methylhistamine for H_2 receptors) and to antagonists (for example, pyrilamine for H_1 and metiamide for H_2 receptors).

As do many neurotransmitters, histamine stimulates the formation of adenosine 3′,5′-monophosphate (cyclic AMP) in nervous and other tissues (*2*). This effect is mediated by both H_1 and H_2 receptors. Recently, tricyclic antidepressants were reported to be potent competitive inhibitors of the H_2 receptor-mediated response (*3*).

In the course of experiments on muscarinic receptor-mediated cyclic GMP (guanosine 3′,5′-monophosphate) formation in cultured mouse neuroblastoma cells (*4*), we found that histamine also

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