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On the Possible Role of Montmorillonites in Prebiotic Peptide Formation

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Summary. The ability of montmorillonite clay minerals for catalyzing peptide formation from amino acids in aqueous solution has been investigated using glycine and Cu^{2+} and Ca^{2+} containing montmorillonites as reaction systems. Evaporation cycle experiments showed that minor amounts of di- and tripeptide are formed from the amino acid. Further polymerization of dipeptide, however, seems to be more favoured by this reaction system than the initial step of dipeptide formation, and a possible role of clays in prebiotic peptide evolution could be seen therefore in the prolongation of peptide chains. Cu^{2+} ions in the clay matrix did not show any advantage over the usual Ca^{2+} ions embedded in natural montmorillonite.

Keywords. Prebiotic peptide formation; Evolution; Clay catalysis.

Über die mögliche Rolle von Montmorilloniten bei der präbiotischen Peptidbildung

Zusammenfassung. Die Fähigkeit von Tonmineralien der Montmorillonitklasse zur Katalyse von Peptidbildungsreaktionen aus Aminosäuren in wäßriger Lösung wurde am Beispiel von Glyzin und Kupfer sowie Kalzium und Morillonit untersucht. Experimente mit Verdampfungszyklen haben gezeigt, daß kleinere Mengen von Di- und Tripeptiden aus der Aminosäure gebildet werden. Die weitere Polymerisation von Dipeptiden hingegen scheint wesentlich leichter in diesem Reaktionssystem zu verlaufen als der Anfangsschritt der Bildung des Dipeptides. Eine mögliche Rolle von Tonmineralien in der präbiotischen Peptidevolution kann daher in der Verlängerung von Peptidketten gesehen werden. Kupferionen in der Tonmatrix zeigen keinerlei Vorteile gegenüber den üblichen Kalziumionen, die in natürlichem Montmorillonit vorkommen.

Introduction

The possibility of peptide formation under primitive earth conditions with the help of clays has been proposed for a long time, and has been the subject of numerous experimental investigations [1–14]. Polymerization of amino acids on clay surfaces has been claimed, mostly on the basis of IR data [13–14], and formation of minor amounts of glycine oligomers could be achieved after numerous wetting/drying cycles [9] on clay surfaces. It has even been postulated that stereoselectivity can be observed in this process on kaolinite [10], however, many of these results could not be confirmed upon reinvestigation by other researchers [11, 12].

Recently, a very simple system producing peptides from amino acids in aqueous solution has been reported, being a solution of NaCl containing copper(II) ions [15–17]. Evaporation experiments as well as experiments with constant volume have produced up to 10% oligopeptides within a few cycles or days, respectively. In the meantime, the reaction has been applied successfully to a variety of amino acids, namely gly, ala, asp, val, pro, his and lys [18–20].

Within this context it seemed worthwhile to reinvestigate the peptide forming ability of clays, especially, since a sensitivity of this ability to the metal ions contained in montmorillonite clays has been reported [6]. The main targets of this reinvestigation were the following:

1. Which mechanism forms peptides faster and in higher yields?
2. Can the clay matrix shift the equilibrium to higher oligomers, due to the better adsorption of peptides compared to amino acids?
3. Can the clay matrix enhance peptide formation at low amino acids concentrations, due to enrichment via adsorption?
4. Is Cu^{2+} a catalytic factor as in the salt induced peptide formation reaction?
5. Can oxidation processes by Cu^{2+} be avoided by its inclusion in a clay matrix?

Methods

Montmorillonite was chosen as clay material, since some preliminary investigations showed promising results. For reasons mentioned above, interest was focused on the Cu^{2+} – doted montmorillonite in comparison to Ca^{2+} – containing montmorillonite (in the following referred to as CuMM and CaMM, respectively).

Montmorillonite was separated by sedimentation of water suspension from Jelšovy Potok bentonite (Central Slovakia). Cu and Ca monoionic forms were prepared using ion exchange reactions with copper and calcium chloride solutions, respectively. Excess of the salt was removed by washing with distilled water until chloride ions were undetectable with silver nitrate solution.

Evaporation Experiments

0.01 g montmorillonite was immersed in 1 ml amino acid (or dipeptide) solution. Then the mixture was evaporated in a heating box at constant 80 °C. After 24 h (1 cycle) all water had been evaporated and 1 ml distilled water (or amino acid solution, in another series) was added. Evaporation and adding of water was repeated several times. In the end of these reaction cycles 1 ml 0.1 *M* calcium chloride solution was added to the solid residue to release peptides. The liquid phase was analyzed every 24 h during the first 5 cycles, later at larger intervals. The concentrations of amino acid solutions were 0.01, 0.02, 0.05, 0.1 and 1 mol/l and the reaction monitoring times were 1, 2, 3, 4, 5 days, and 2, 3, 4 weeks.

Constant Volume Experiments

The same conditions as for the evaporation experiments were also used for constant volume experiments. The vials containing the reaction mixture were sealed with caps for the prevention of water evaporation. The reaction monitoring times, where solution over the solid was analyzed, were 1, 2, 4, 7, 10 days, and 2, 3, 4 weeks. In the end of the reaction a small amount of concentrated calcium chloride solution was added (to form 0.1 *M* Ca^{2+} solution in the reaction mixture) to liberate peptides from solid phase.

Analytical Method

The samples were analyzed directly by a Hewlett-Packard HP-1090M HPLC apparatus. For separation 2 µl were injected, using a Shannon Hypersil ODS 5 µm/200* 2.1 mm column. The mobile phase consisted of 50 m*M* KH_2PO_4/7.2 m*M* $C_6H_{13}SO_3Na$, $pH = 2.5$ adjusted by H_3PO_4, with a flow of 0.35 ml/min. Detection was performed by diode array detector at 200 nm. All amino acids and peptides were identified by retention time of analytical grade reference substances (Sigma); details of the method are given in Ref. [16].

Results and Discussion

CuMM/Glycine

Without continuous amino acid supply

The results of the evaporation experiments with 0.01 g CuMM plus 1 ml glycine solution (0.01, 0.02, 0.05, 0.1 and 1 *M*) are collected in Table 1. They show that the best percentual yield of diglycine is achieved with an initial concentration of 0.1 *M*, for triglycine formation 0.02 – 0.05 *M* glycine concentration seems favourable. In

Table 1

a) concentration of digly/trigly (nmol/l), in evaporation experiments with CuMM and glycine

Time	*c*(gly)/mmol 10	20	50	100	1000
1 day	+/–	–/–	+/–	+/–	0.005/–
2	+/–	+/–	0.008/–	0.006/–	0.028/+
3	+/–	+/–	0.014/–	0.014/–	0.048/+
4	+/–	+/–	0.016/–	0.018/+	0.045/0.006
5	+/–	0.008/–	0.032/–	0.029/+	0.082/0.010
2 wks.	0.003/–	0.015/0.002	0.034/0.003	0.094/+	0.849/+
3	0.008/–	0.023/0.002	0.081/0.007	0.159/+	1.499/+
4	0.009/–	0.022/0.002	0.040/0.004	0.212/0.009	1.507/+

b) yield of digly/trigly in % of initial amino acid concentration for the same experiments

Time	*c*(gly)/mmol 10	20	50	100	1000
1 day	+/–	–/–	+/–	+/–	0.001/–
2	+/–	+/–	0.032/–	0.013/–	0.006/+
3	+/–	+/–	0.054/–	0.028/–	0.010/+
4	+/–	+/–	0.062/–	0.036/+	0.010/0.001
5	+/–	0.076/–	0.130/–	0.057/+	0.017/0.002
2 wks.	0.050/–	0.153/0.032	0.134/0.020	0.188/+	0.170/+
3	0.160/–	0.234/0.026	0.323/0.040	0.318/+	0.300/+
4	0.180/–	0.219/0.029	0.161/0.023	0.425/0.025	0.301/+

+ qualitatively detectable
– undetectable

general, yields are much smaller than those observed in the salt-induced peptide formation reaction [16, 17, 20]. After longer periods of evaporation cycles (> 1 week) hydrolysis and/or oxidation processes even reduce the amount of formed peptides.

Addition of $CuCl_2$ in the same molar amount as amino acid to the solutions leads apparently to complete oxidation of the amino acids so that no peptides could be observed.

With continuous amino acid supply

In Table 2 the results for evaporation experiments are shown, where after each cycle glycine solution was added instead of pure water, resembling a continuous supply of "raw material". Apparently such a supply does not enhance the peptide formation, on the contrary, we observed lower yields of dipeptide and no tripeptides. Since the best percentual yields were obtained for the lowest concentration, we believe that the peptide forming capacity of the CuMM is very limited and that larger concentration of amino acid may even be disadvantageous. This is also in accordance with the results without continuous amino acid supply, where lower concentration also gave better results.

CuMM/Diglycine

The experiments performed with diglycine as starting material were performed only with dilute solution (0.01 *M*), as such conditions had proven to lead to better results with the amino acid. The results are collected in Table 3, and they show that

Table 2. Evaporation experiment with CuMM/glycine, adding gly solution after each cycle: $c(gly_2)$/yield (nmol/l and % of total amino acid concentration added)

Time	*c*(gly)/mmol 10	20	50	100
1 day	–	–	0.002/0.010	0.003/0.006
2	+	–	0.015/0.031	0.008/0.008
3	0.004/0.027	0.009/0.031	0.020/0.026	0.019/0.026
4	0.045/0.227	0.015/0.038	0.021/0.021	0.004/0.004
5	0.040/0.159	0.022/0.045	0.024/0.020	0.005/0.004

Table 3. Evaporation experiments starting with digly and CuMM: (initial gly_2 concentration = 0.01 *M*)

Time	*c*(nmol/l)/yield(%) gly	gly_3	gly_4
1 day	0.224/1.1	–	–
2	1.809/9.0	+	+
3	1.849/9.2	+	+
4	2.670/13.4	0.022/0.33	+
5	2.034/10.2	0.022/0.34	0.011/0.21

polymerization occurs more readily in this case than in the case of glycine itself. After 4 cycles (=days), an equilibrium between gly, gly_2 and gly_3 seems to be established, in which about 10% of the originally used gly_2 is hydrolyzed to the amino acid. The amount of gly_3 formed after this short time is even higher than the amount of gly_2 formed from the amino acid under identical conditions after 4 weeks of continuous cycles. After 5 cycles, the amount of formed gly_4 becomes quantifiable and is higher than the amount of gly_2 formed from glycine at the same initial concentration (cf. Table 1). These results seem to indicate clearly that CuMM is much less suitable for the initial step of amino acid condensation to dipeptides, but rather suitable to enhance the formation of higher polymers.

Experiment with Constant Volume

In analogy to the investigations on the salt-induced peptide formation [16–19], another series of experiments was performed, where water evaporation was not allowed, thus keeping the reaction volume constant. Samples taken during the observation time of four weeks did not reveal any significant formation of peptides in dilute solutions (up to 0.05 *M*), diglycine could be found in very small amounts in 0.1 *M* and 1 *M* solutions (0.01 and 0.03%, respectively, after 4 weeks of reaction). Only traces of gly_3 are found after 3–4 weeks in these solutions. For comparison, the corresponding peptide concentrations obtained by the salt-induced peptide formation reaction are $\sim 10\%$ gly_2 and $\sim 1\%$ gly_3.

CaMM/Glycine

Finally it seemed of interest to see, whether the assumed advantage of CuMM over CaMM [13, 14] in inducing peptide formation really exists, especially over a longer period of reaction, or whether parallel oxidation reactions would exert a significant influence. Evaporation experiments with 0.01 *M* glycine solution were performed therefore, in parallel with both types of montmorillonite, and the data obtained after 2–4 weeks are summarized in Table 4. Already after 2 weeks, the concentration of glycine has dropped to 30% of the initial one in the presence of CuMM, whereas with CaMM still 70% of it are present. At the same time, the dipeptide yield is 10 times higher for CaMM as catalyst. This ratio drops to 2 after 4 weeks. It can be assumed therefore that CaMM may be a better or at least the same effective catalyst for dimerization of amino acids as CuMM, quite in contrast to the salt-induced peptide formation for which Cu^{2+} is essential due to its complex formation ability with amino acids [21]. This also indicates that the mechanism leading to the formation of peptides on the clay matrix is essentially different from that in solution.

Table 4. Comparison of Cu and Ca montmorillonites: peptides as nmol/l and % of initial amino acid concentration (concentration of gly = 0.01 mol/l, reaction times: 2, 3 and 4 weeks)

Time	Cu: *c*(gly)/%	*c*(gly 2)/%	Ca: *c*(gly)/%	*c*(gly 2)/%
2 weeks	3.011/30.1	0.003/0.05	7.077/70.8	0.031/0.62
3 weeks	2.025/20.3	0.008/0.16	7.146/71.5	0.016/0.33
4 weeks	1.859/18.6	0.009/0.18	6.485/64.8	0.018/0.35

Conclusions

1. Compared to the Salt-Induced Peptide Formation, neither Cu^{2+} – nor Ca^{2+} – containing montmorillonites seem to be a relevant alternative reaction system for the formation of peptides from amino acids in aqueous solution.
2. Montmorillonites may facilitate, however, the formation of higher oligomers, once peptides are present, and this polymerization process seems to take place best at low peptide concentrations. The reasons for this behaviour are seen in the better adsorption of peptides to the clay matrix, compared to amino acids, the optimal concentration seems to depend on the capacity of channels and surfaces on the clay for this adsorption process.
3. Copper montmorillonite does not display any advantage over the usual calcium montmorillonite in the peptide generating process, and the binding of Cu^{2+} in the clay matrix does not prevent redox reactions between amino acids/peptides and this ion.
4. Further investigations seem to be worthwhile concerning the formation of higher peptides from simple dipeptides of other amino acids and mixed dipeptides, so that eventual preferential sequencing could be investigated.
5. If clay materials should have assumed a role in prebiotic peptide evolution, it should have been rather in the prolongation of peptide chains than in the initial formation of peptides from amino acids.

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