The effect of reaction conditions on montmorillonite-catalysed peptide formation

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Oligomerization of glycine (gly) and diglycine (gly$_2$) on montmorillonite was performed as cyclic, drying–wetting process at temperatures below 100°C, under varying reaction conditions. The influence of substrate/clay ratio, temperature and pH was found to be different for amino acid (AA) dimerization, cyclic anhydride (CA) formation and peptide chain elongation. High temperatures and neutral pH favour CA formation over diglycine production. An AA/catalyst ratio of 0.2 mmol/g leads to optimal yields. This supports the assumption that amino acid dimerization and CA formation take place at the edges of clay particles. Peptide chain elongation, starting from gly$_2$, produces higher yields at higher temperatures and neutral pH.

Keywords: catalysis; chemical evolution; montmorillonite; prebiotic peptide formation

1. Introduction

Peptide bond formation catalysed by clay minerals has been observed in numerous experiments proceeding as cyclic heating/drying/wetting process, simulating assumed prebiotic conditions [1–9]. Several percents of glycine and alanine are converted to dipeptides and some other oligomers after several weeks of low temperature wetting and drying (45°C) and high temperature heating (94°C) cycles [1–4]. Oligomerization of gly at 80°C has been reinvestigated recently [5,6] and the reaction yields of gly$_2$ were found to be below 1% after 2 weeks. In reactions starting from short oligopeptides under the same conditions, better yields of higher oligopeptides were obtained [7]: about 10% of gly$_2$ converted to oligomers up to gly$_6$ after 3 weeks. Alanine itself does not produce any oligopeptides under these conditions, whereas oligomerization of dialanine was observed. The reaction mechanism could proceed via two types of activated compounds, or reaction intermediates [8,9]:

(1) Esters, formed between amino acid (oligopeptide) carboxyl groups and hydroxyl groups bound to Si and Al at the edges of clay particles.

(2) Cyclic anhydrides produced by intramolecular condensation of dipeptides.

The higher reactivity of short oligopeptides can be explained by both reaction mechanisms:

(1) Catalytic centers, where esters are formed, are probably too distant for the subsequent condensation reaction of two activated amino acid molecules. The oligopeptide chains can overcome this difficulty due to their increased length [7].

(2) The formation of CA proceeds via dimers and hence relatively high amounts of gly CA - diketopiperazine (DKP) are produced from gly on clay [3,4,9].

The objective of this work was to study the effect of reaction conditions on montmorillonite catalyzed peptide formation, starting from either gly or gly$_2$, in particular the influence of the ratio reactant/catalyst, reaction temperature and pH of the solution.

2. Experimental

Less than 2 μm fraction of Ca-saturated montmorillonite was isolated from bentonite (Jelšový Potok, Slovakia) by sedimentation [10]. X-ray diffraction confirmed no crystalline admixture in it. Amino acids and oligopeptides of analytical grade quality from Senn Chemical Co. were used as obtained.

Drying–wetting cycle experiments with gly/montmorillonite and gly$_2$/montmorillonite suspensions were performed. 0.01 g montmorillonite was immersed in 1 ml of organic compound solution. In experiments investigating the effect of reactant/catalyst ratio, 0.01 g montmorillonite was mixed with gly (gly$_2$) solutions of different concentrations: 10, 8, 5, 2 and 1 mM, in order to obtain the ratios 1.0, 0.8, 0.5, 0.2 and 0.1 mmol/g respectively. The concentration of the reactant in all other experiments was 10 mM with the exception of the reactions at 70°C, as evaporation at this temperature is slow and would not be complete within 24 h. For this reason, 20 mM solution was used, but using only half volume (0.5 ml). In order to study the pH influence some gly and gly$_2$ solutions were adjusted with hydrochloric acid to pH 5, 4 and 3. The original pH of gly and gly$_2$ solutions were 6.15 and 5.86, respectively. The suspensions prepared in 1 ml vials were heated in a heating box at 80°C for 24 h (1 cycle). Treatments at 70 and 95°C were applied in experiments to investigate the temperature effect. After 24 h heating 1 ml (0.5 ml in 70°C experiments) distilled water was added and the next cycle started. After the reaction the vials with the solid residues were kept in the refrigerator. One day before analysis, 1 ml 0.1 M calcium chloride solution was added to release the formed oligopeptides from the solid. After 24 h the liquid phase was analysed using a Hewlett-Packard HP-1090M HPLC apparatus for reversed phase ion pair chromatography [6,7,9]. Detection was performed with a diode array detector at 195 nm. The differences between determined reaction product concentrations of three parallel samples were below 10%, in cases of oligopeptides formed with very low yields (< 0.5%) this difference was lower than 25%. On the other hand the accuracy was higher (differences lower than 5%) for compounds formed with the highest yields. The reaction yields were determined as average percentage of reactant amount converted to the reaction product.

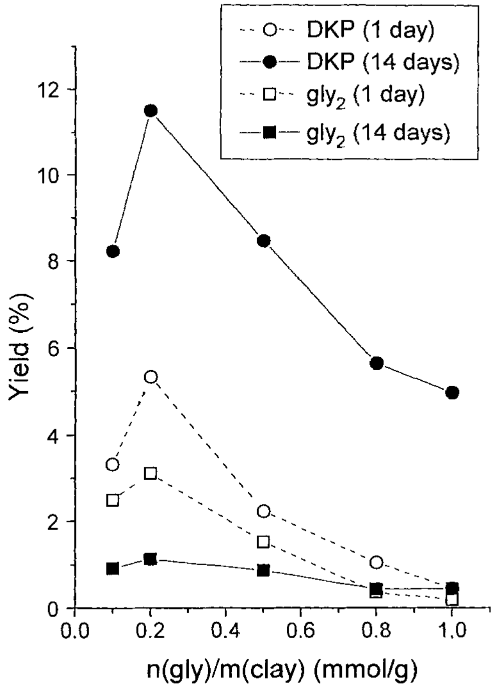

Fig. 1. The effect of the ratio gly/montmorillonite on the relative reaction yields of DKP and gly$_2$ at 80°C.

3. Results and discussion

3.1. The reactant/catalyst ratio

Previous studies have indicated that the edges of clay particles are the catalytic sites for peptide formation [9]. In order to confirm that oligomerization of gly and gly$_2$ takes place on these edges, experiments with different ratios gly (gly$_2$)/montmorillonite were carried out. The reaction proceeds most effectively if all catalytic sites on the clay surface are saturated, and yields should drop with any excess of reactant. The dependencies of DKP and gly$_2$ yields on the ratio gly/clay are shown in fig. 1. Less than 1% of gly converts to gly$_2$ or DKP at the highest ratio (1 mmol/g) after one day. The maximal yields were found for the ratio 0.2 mmol/g, which approximately corresponds to the capacity of ion exchange for the external surface of clay particle aggregates [9]. Further decrease of gly concentration leads to a decline of the yields. More than 10% of gly is converted to DKP after 14 days in experiment starting with ratio 0.2 mmol/g. This is about twice as much as after one day. On the other hand, the amount of gly$_2$ formed after one day is higher than after 14 days. Lower content of gly$_2$ after longer reaction is probably caused by rapid conversion of gly$_2$ to DKP. This was not observed in experiments starting from high-concentrated gly solutions (8 and 10 mM), where mainly gly is activated, which oversaturates catalytic sites. Under such conditions conversion of gly$_2$ to DKP proceeds to a lower extent.

These results support the assumption that the reaction proceeds at clay particle edges. Moreover, the sorption of amino acid zwitterions onto clay surfaces was reported to be not homogeneous, but dominantly taking place at these edges [11]. Negative charge of montmorillonite layers seems to disable sorption in the interlayer spaces, whereas clay particles exhibit suitable sorption properties on the edges. This is due to broken bonds and structural defects providing centers for sorption of both cations and anions [12].

The effect of the reactant/catalyst ratio has been investigated previously for gly–kaolinite (bentonite) fluctuating temperature reaction systems [1]. Only the effect of reactant/catalyst ratio on the concentration of reaction products has been discussed, but relative yields can be derived. The most effective ratio for kaolinite was the lowest used: 0.123 mmol/g. In reactions proceeding on bentonite, the reaction yields increased only slightly with the amount of gly up to 2.141 mmol/g. Probably the high temperature (94°C) affected conversion of most gly to DKP in these experiments. Unfortunately, DKP yields, as well as the source of materials and the data on composition and isolation of the clays are not reported in this work.

The effect of the ratio gly$_2$/montmorillonite on the reaction yields is shown in fig. 2. Formation of small amounts of gly$_3$ and gly$_4$ is observed after one cycle, and gly$_6$ is detectable in samples produced from more concentrated solutions (>0.8 mmol/g). The decreasing amount of gly$_2$ is paralleled by a slight increase of gly$_4$ and DKP. The relationship becomes quite different after 14 cycles: the highest amount of gly$_4$ is formed from the

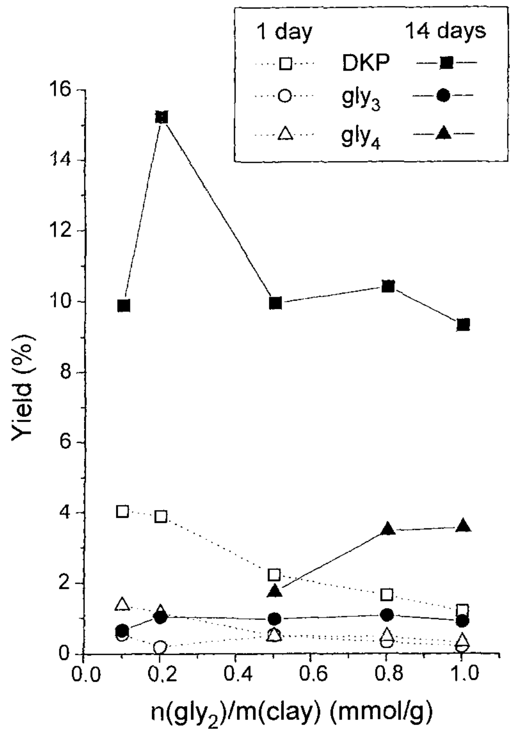

Fig. 2. The effect of the ratio gly_2/montmorillonite on the relative reaction yields of DKP and gly oligomers at 80°C.

highest concentrated solution, whereas the yield of gly_3 seems to be independent of the ratio reactant/clay. This can be explained by the way of gly_3 formation: gly_3 can be formed by hydrolysis of gly_4, or by the reaction of gly_2 (or DKP) with gly produced by gly_2 hydrolysis. Hydrolysis proceeds more readily in more dilute solutions and causes a decrease of gly_4 content in favour of gly_3. Gly, as a hydrolysis product, was not detectable, because of the lower sensitivity of analytical method for this compound. The amount of DKP formed from gly_2 is much higher than that of gly oligomers. DKP formation proceeds via an intramolecular mechanism and after 14 days its dependence is similar to that observed in the gly experiments (maximum yield 15.2% at 0.2 mmol/g ratio, while yields at other ratios are about 10%).

3.2. *Reaction temperature*

In this work the reaction temperature influence on gly and gly_2 oligomerization was investigated at three different temperatures. The yields of gly_2 and DKP produced from gly at 70, 80 and 95°C are summarized in table 1. DKP yields and the sum of DKP + gly_2 yields increase with the reaction temperature. After one day the highest temperature gives the highest yield of gly_2 (0.82%), further cycles reduce its amount (0.59% after 14 days). After 1 day at the temperatures 70 and 80°C less than 0.2% of gly_2 are present. The reaction yields at these temperatures exhibit a maximum after 7 days and are reduced then in a similar way as at 95°C. After 14 days the highest yield of gly_2 is found in experiments at 70°C, where gly_2 transformation to DKP formation is slower. This process also explains the decrease of gly_2 yields at 95°C.

Some of the effects of the reaction temperature on gly reactivity in experiments simulating wetting/drying/temperature fluctuating clay environments have been reported previously [1]. The highest yields of gly oligomers were detected after long reaction time at 94°C. Gly_2 was also formed at 80°C, however, with about four times slower reaction rate. Only trace amounts of gly_2 were detected after 35 days, if the reaction temperature was reduced to 60°C. The temperature effect on gly_2 formation found in this work (table 1), is quite different from that reported previously, probably due to the differences in reaction conditions in fluctuating temperature experiments (low temperature evaporation and drying) [1].

The effect of the reaction temperature on gly_2 oligomerization is demonstrated by figs. 3a–3c. Comparison of the reaction yields clearly shows that the reaction proceeds at 70°C only slowly, whereas there is not a large difference between the reactivity of gly_2 at 80 and 95°C. Higher temperatures also give a better relation for high oligomer yields compared to DKP formation.

3.3. *Starting pH of the reactant solution*

The effect of pH on peptide bond formation was also investigated in this work. pH is surely not constant during the reaction cycles, but the effect of starting pH in gly and gly_2 reactions is proven (table 2 and figs. 4a–4c). Clay minerals often take part as catalysts in natural and industrial processes [13], mostly playing a role of solid state Brønsted or Lewis acids. A similar role of montmorillonite has been suggested also in peptide bond formation [2], since the surface acidity of clay increases during the drying process. Increasing acidity converts

Table 1
The reaction yields (%) of DKP and gly_2 formed from gly at different reaction temperatures

T (°C)	DKP reaction time (days)			gly_2 reaction time (days)			$\sum$DKP/gly_2 reaction time (days)		
	1	7	14	1	7	14	1	7	14
70	0.12	1.52	3.52	0.15	1.72	1.13	0.27	3.24	4.65
80	0.45	3.36	4.96	0.18	0.57	0.44	0.63	3.93	5.40
95	0.51	3.08	5.12	0.82	0.70	0.59	1.33	3.78	5.71

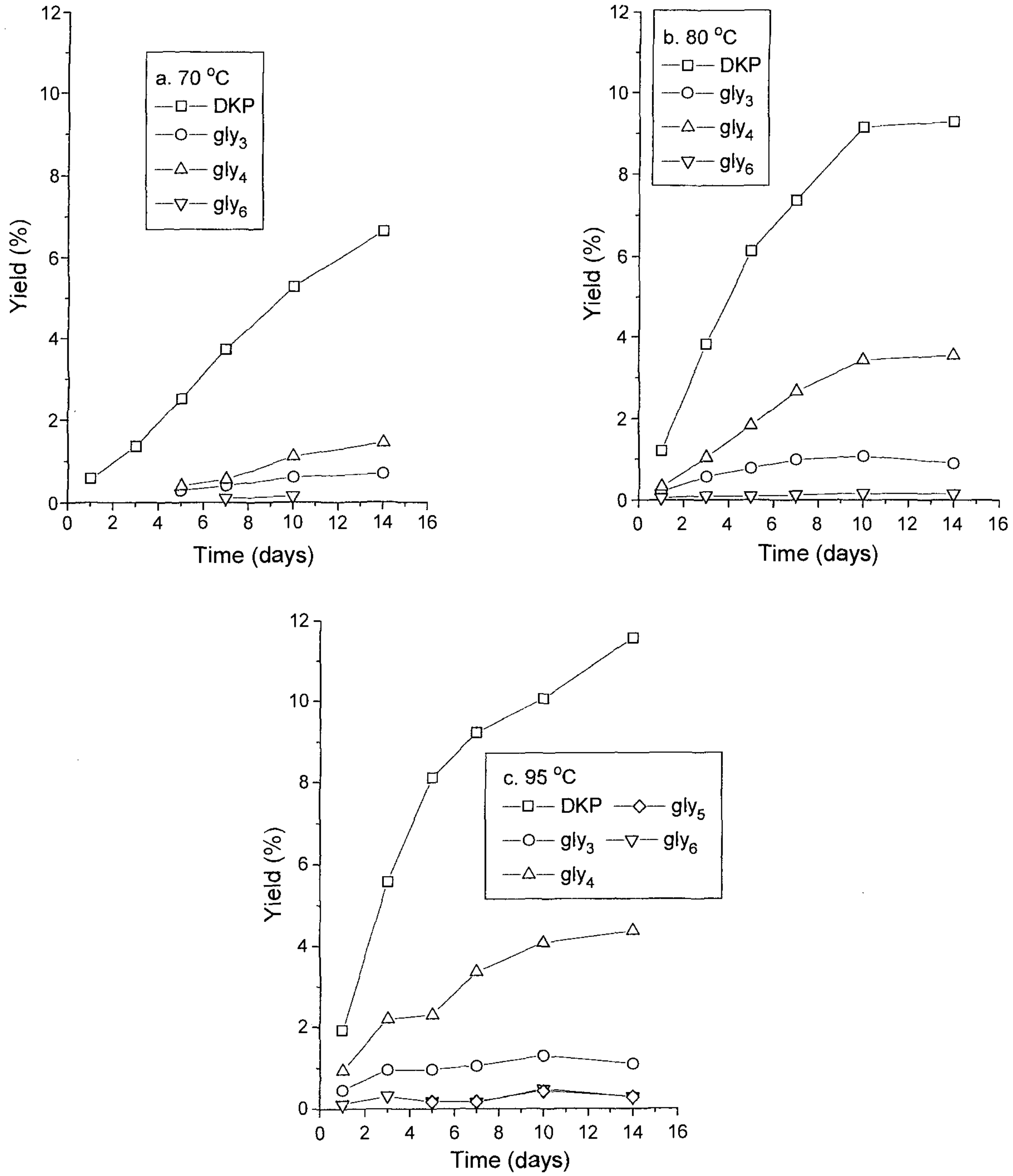

Fig. 3. Montmorillonite catalysed formation of DKP and gly oligomers from gly_2 at (a) 70, (b) 80, (c) 95°C.

Table 2
The reaction yields (%) of DKP and gly_2 formed from gly at 80°C, starting from gly solutions with different pH. Orig. = original pH of gly solution (6.15)

pH	DKP reaction time (days)			gly_2 reaction time (days)			$\sum$DKP/gly_2 reaction time (days)		
	1	7	14	1	7	14	1	7	14
orig.	0.45	3.36	4.96	0.18	0.57	0.44	0.63	3.93	5.40
5	0.88	2.28	4.28	0.71	1.11	0.89	1.59	3.39	5.17
4	0.68	1.71	2.94	0.55	0.48	0.52	1.23	2.19	3.46
3	+	0.04	0.09	0.21	0.26	0.48	0.21	0.30	0.57

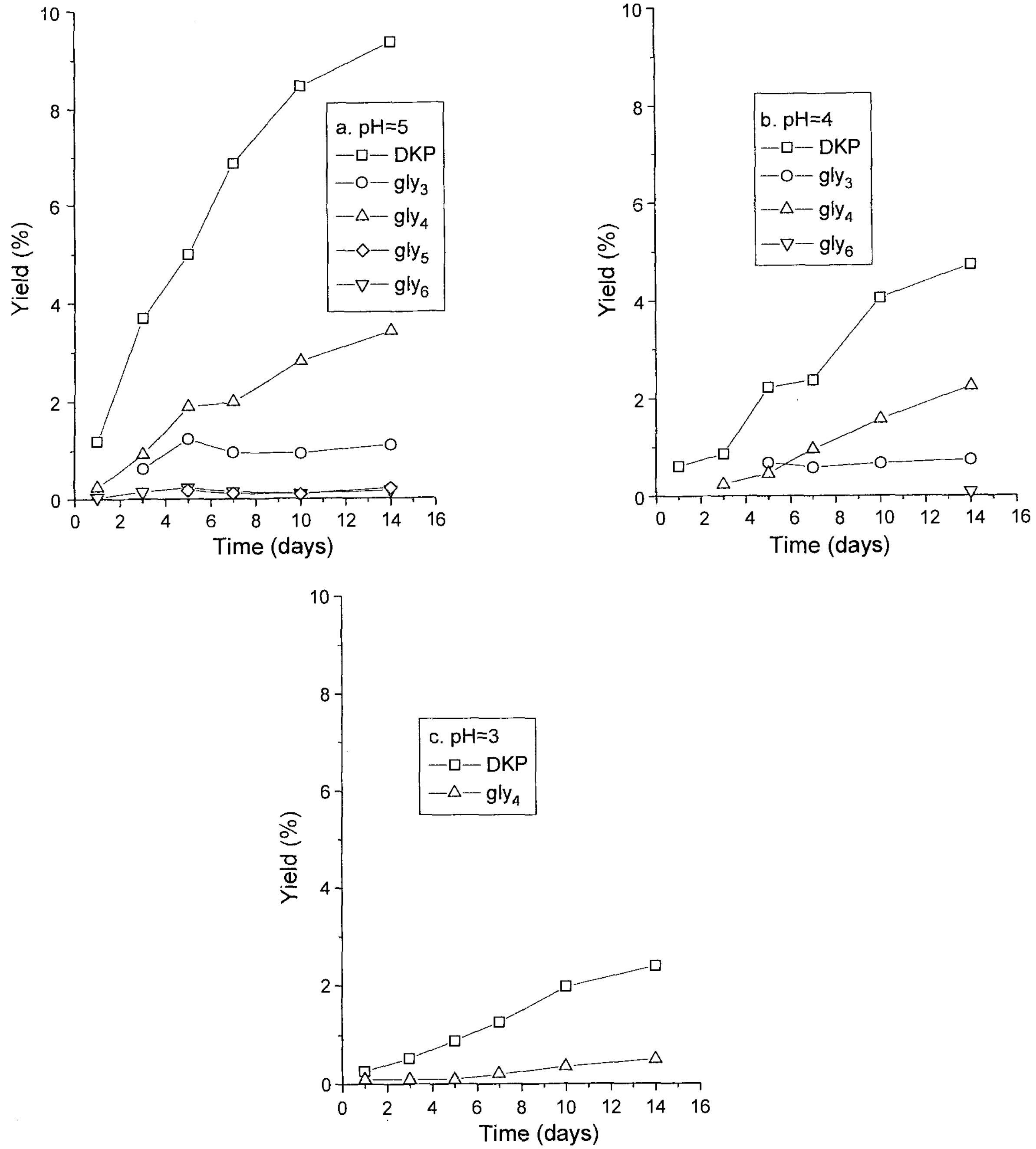

Fig. 4. Montmorillonite catalysed formation of DKP and gly oligomers from gly_2 solution adjusted to pH: (a) 5, (b) 4, (c) 3. (Reaction temperature 80°C.)

amino acid zwitterions to cationic form which would be accompanied by higher adsorption to negatively charged clay particles [14,15]. If clays played the role of acids in peptide bond formation catalysis, the lowering of pH would cause yield enhancement. Table 2 summarizes the DKP and gly_2 yields formed from gly solutions of different pH. In gly dimerization (table 2) the highest yields were obtained with pH = 5. Further lowering of the pH reduces yields. The yields of DKP are affected quite differently by pH. After one reaction day, the highest yield of DKP is formed at pH 5. After longer reaction times the highest amounts of DKP are obtained from unacidified gly solution. Any acidification of gly solutions leads to a considerable decline of DKP yields, probably due to accelerated hydrolysis of this product, proceeding faster than gly_2 hydrolysis. The interaction of protons with clay particles, leading to partial dissolution could also affect the catalytic efficiency of clay at pH < 5. Yield reduction with increasing pH was observed also in the case of higher oligopeptides in reactions starting with acidified gly_2 suspensions (figs. 4a–4c). The yield decrease of higher gly oligomers (gly_3–gly_6) in acidified suspensions (pH < 5) can be explained by their formation via DKP [9] and/or increasing hydrolysis. Apparently rather neutral pH provides the best conditions, and hence acidic catalysis should be irrelevant for these processes.

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