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SHORT COMMUNICATION

Non-biological selectivity in amino acids polymerization on TiO_2 nanoparticles

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Abstract For the first, time a strong selectivity is evidenced in inorganic peptide synthesis. When an equimolar mixture of Ala and Arg monomers is added to the synthesis medium of TiO_2 nanoparticles from Ti(IV) isopropoxide in benzyl alcohol, the Ala-Arg dipeptide is observed by ^{13}C NMR in the resulting solid, at the exclusion of other dipeptides or higher peptides.

Keywords Alanine · Arginine · Ala-Arg dipeptide · Polymerization selectivity · TiO_2 nanoparticles · ^{13}C NMR

There are many reasons to study the mutual influences between inorganic oxide nanoparticles on the one hand and biomolecules on the other hand.

One incentive comes from biomineralization and bioinspired syntheses. Complex biomolecules such as proteins are able to precisely control the precipitation of TiO_2 from inorganic precursors (Kharlampieva et al. 2008), and even very simple biomolecules have significant effects: monomeric amino acids exert an influence on the sizes and shapes of precipitated TiO_2 NPs (Durupthy et al. 2007; Wu et al. 2012). Closely related to this field is the biomaterials application: titania surfaces can selectively adsorb some proteins by recognition of specific peptidic sequences and modify the conformation of the protein in so doing (Cacciafesta et al. 2000), a phenomenon important in the osteointegration of titanium implants (Martin et al. 1995).

Another, independent reason for studying the effect of oxide surfaces on small biomolecules is prebiotic chemistry. Inorganic oxide NPs may have helped the condensation of amino acids to oligopeptides before the biochemical machinery of cells existed (Zaia 2004; Lambert 2008). However, to insert this step in a coherent scenario for the origin of life (Cleaves et al. 2012), it should be demonstrated that peptide condensation can occur in a "useful" way. The question of reaction selectivity is then of fundamental importance, since random polymerization would result in the proliferation of a huge variety of useless peptides. Evidencing non-biological processes that result in efficient selection from a mixture of biomolecules is one of the key targets of origin of life studies and has not clearly been evidenced so far for peptides although intriguing results have been observed, e.g., in the (Gly + Glu) and (Gly + Gln) systems (Leyton et al. 2011, 2012).

In these hybrid systems, information transfer goes both ways, from the biomolecules to the inorganic components, and vice versa. The unifying question one may ask is whether there is a correspondence between the primary peptide sequence and the structure of the inorganic surface. In a program aimed at the synthesis of TiO_2 nanoparticles with original properties in a benzyl alcohol (BzOH) medium containing amino acids (Spadavecchia et al. 2011), we have serendipitously evidenced a selective structuration of biomolecules interacting with inorganic precursors and/or NPs, which we are reporting here.

Whether TiO_2 is synthesized with amino acids or without them, XRD patterns showed anatase as the only crystalline phase. The average particle size, as estimated from TEM micrographs, was about 20 nm for all samples. The morphology of the particles was not very well defined; they can be assimilated to small deformed and aggregated

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cubes. If the particles are considered as cubes of uniform size, the surface area should be about 70 m^2/g.

Thermogravimetric analyses, carried out in flowing air up to 800 °C with a temperature ramp of 5 °C/min, exhibited weight losses within the 20–500 °C temperature range. The derivative (DTG) traces show two main weight loss events in the ranges 100–220 °C (slightly endothermic) and 295–500 °C (strongly exothermic). They are all assigned to the elimination of organic molecules including BzOH, ethanol and amino acids (or molecules derived from them), since pure TiO_2 only shows the elimination of adsorbed water at $T \leq 200$ °C (e.g. Lambert et al. 2009). The total loss expressed on the basis of the weight remaining at 800 °C corresponds to 21.1, 14.5, 20.5 and 21.5 % for TiO_2 NP, Ala-TiO_2 NP, Arg-TiO_2 NP, and Ala + Arg TiO_2 NP, respectively. In the case of TiO_2 NP, if we suppose that the whole amount of organic matter consists of benzyl alcohol (cf. infra), it would correspond to a surface density of 16.5 molecules per nm^2, indicating multilayer adsorption. The close similarity of the DTG traces of all four samples (while NMR shows the presence of amino acids and/or peptides in two of them, cf. infra) means that the amount of amino acids is small as compared to that of BzOH, so that the signals corresponding to their elimination is lost in those of BzOH (the latter comprise a non-oxidative pyrolysis followed by combustion of the remaining organic matter). Note, however, that Ala-TiO_2 NP contains significantly less organic matter than the other samples.

Figure 1 presents the NMR spectra of all samples under study together with those of reference materials (Arg, Ala, and the two dipeptides Arg-Ala and Ala-Arg). Bulk alanine shows three main peaks assigned to the carboxylate (COO^- or A1 in Scheme 1), the α-carbon (A2) and the methyl group (A3), respectively. In Ala-TiO_2 NP too, only three sharp signals are visible, two of them being rather close to A2 and A3 while the third one is strongly shifted upfield from the position of A1.

Bulk arginine shows six signals. Their assignment, based on commercial software calculations, agrees well with that for Arg in D_2O solution (Surprenant et al. 1980). Arg-TiO_2 NP does not show any signal attributable to arginine: only the peaks of benzyl alcohol (128, 143 ppm) and ethanol from the reaction medium (20.5, 77 ppm) are observed.

The most interesting spectrum is that of (Ala + Arg)-TiO_2 NP (Fig. 1e), where nine peaks are observed in addition to those of BzOH and EtOH. Since one of these, at 171.2 ppm, lies in the region typical of an amide carbon (-CO–NH-), and since we had introduced two amino acids having respectively 3 and 6 non-equivalent carbons, it is logical to hypothesize that the new signals are due to the formation of a dipeptide. We recorded the spectra of the two heterodipeptides, H_2N-Ala-Arg-OH (Ala-Arg for short) and H_2N-Arg-Ala-OH (Arg-Ala). The similarity with the spectrum of Ala-Arg is striking. In contrast, we can rule out the presence of the other peptides (Arg-Ala) in significant amounts (we estimate that if it is present at all Arg-Ala represents <5 % of the amount of Ala-Arg).

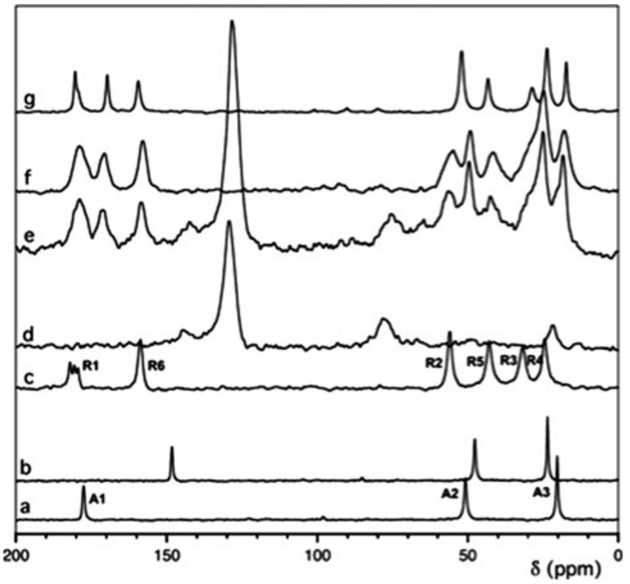
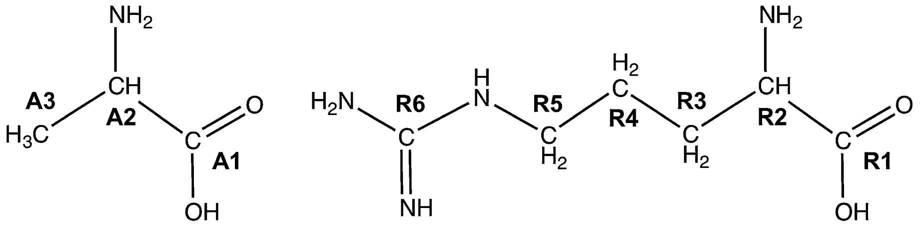


Fig. 1 ^{13}C CP-MAS NMR spectra of: **a** bulk alanine (A), **b** Ala-TiO_2 NP, **c** bulk arginine (R), **d** Arg-TiO_2 NP, **e** (Ala + Arg)-TiO_2 NP, **f** bulk H–Ala-Arg–OH dipeptide (or Ala-Arg), **g** bulk H–Arg-Ala–OH dipeptide (or Arg-Ala). For the two amino acids A and R, peak assignments are provided according to the numbering in Scheme 1

In summary, our data suggest the following:

1. Arginine by itself has little affinity for the titania NPs and when introduced in the synthesis medium cannot compete with the majority BzOH for retention in the solid phase.
2. Alanine in contrast is retained in significant amounts in Ala-TiO_2 NPs. The strong shift of the A1 carbon as compared to free Ala suggests adsorption through the carboxylate end, possibly by coordination of the carboxylate to surface Ti^{4+} centers. Coordinative binding of amino acids on titania surfaces has been evidenced many times, on flat surfaces (Qiu and Barteau 2007) as well as on NPs (Giacomelli et al. 1995; Roddick-Lanzilotta and McQuillan 2000; Sverjensky et al. 2008; Paszti and Guczi 2009; Jonsson et al. 2010). In the case of alanine, (Martra et al. 2002) have reported adsorption on titania through the –COO^-. There seems to be a discrepancy as regards to Ala-TiO_2, between NMR (which observes no signal of BzOH) and TG (which suggests that BzOH remains largely present, although less than for other samples). One has to remember that the cross-polarization (CP) technique used for ^{13}C detection is not innocuous;

Scheme 1 Numbering of carbon atoms in Arginine (Arg, R), and Alanine (Ala, A)



highly mobile molecules may be completely undetectable through CP. Both sets of data may be reconciled if we suppose that competition for strong adsorption sites favors Ala over BzOH, so that the amount of BzOH retained by Ala-TiO_2 NP is lesser than for other samples, and this molecule is only present in a highly mobile state.

3. When both amino acids are present together, peptidic condensation occurs to yield the Ala-Arg dipeptide along:

   H_2N–Ala–COOH + H_2N–Arg–COOH = H_2N–Ala–CO–NH–Arg–COOH (= Ala-Arg) + H_2O.

In water solution, peptide condensation is thermodynamically unfavorable (Brack 2007), even in the presence of inorganic NPs. (Marshall-Bowman et al. 2010) On the other hand, in conditions of low water activity, condensation is favored by Le Châtelier's principle, as the corresponding reaction produces one water molecule. Therefore, peptide bond formation becomes thermodynamically possible upon drying, and it should also be the case in an anhydrous organic solvent, as in the present study. Thus, the peptide formation in itself is not surprising; its strong selectivity is, however. Why is Ala-Arg almost exclusively formed, and not Arg-Ala, Ala-Ala, Arg-Arg … or higher polypeptides? It is plausible that there is some kind of interactional complementarity between Ala-Arg and the sites exposed on the anatase surface. This may be the subject of future studies by molecular modeling techniques, which have proved their efficiency for many comparable systems including dipeptides/TiO_2 (Monti et al. 2007), and also for the understanding of surface-directed peptide formation (Rimola et al. 2007). At any rate, such a pronounced selectivity in inorganic peptide synthesis has not been evidenced before to the best of our knowledge.

The previously reported study of TiO_2 NP formation by the BzOH route (Spadavecchia et al. 2011) already evidenced two of the phenomena discussed here: facilitation of condensation reactions involving water elimination (in that case, between carboxylic acid and benzyl alcohol to give an ester) and coordination of carboxylate groups to a surface Ti^{4+} (the additive was a dicarboxylic acid-terminated PEG).

The present study goes further in evidencing a strong selectivity in the condensation, and in this respect, it is encouraging for prebiotic scenarii involving mineral surfaces, although the particular system for which this selectivity was established is not prebiotic of course. This is because it indicates that abiotic systems can direct "anabolic" syntheses to a particular outcome with a high selectivity.

We have undertaken a more detailed study to determine if the observed polymerization selectivity extends to other amino acid combinations, including of course the problem of chiral selectivity within mixtures of L- and D-forms of the amino acids.

Conflict of interest The authors declare that they have no conflict of interest.

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