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COMMUNICATION

Designing neutral metallophilic hydrogels from di- and tripeptides†

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Here we report the metallophilic attraction driven gel-forming capability of four cysteine-containing short peptides at neutral pH. Such peptides were designed to have an isoelectric point (pI) close to 7, aided by the introduction of an arginine unit with its highly basic guanidinium group.

Since the establishment of the concept of supramolecular chemistry[1] and inspired by biological systems, self-assembly of low molecular weight (LMW) compounds has been successfully applied for the creation of new materials and techniques. In recent years, self-assembly of short peptides[2] has become an especially powerful tool for the fabrication of supramolecular hydrogels. Very well known examples include the self-assembling peptides developed by the groups of Zhang,[3] Boden,[4] Stupp,[5] Schneider[6] or Xu,[7] among others.[8] The potential application of these type of systems in the biomedical field is almost unlimited.[9]

Despite all the recent advances achieved in the fabrication of supramolecular hydrogels,[10] the design of LMW gelators still remains a very difficult task. Why? The main reason is probably related to the fact that these systems are composed of small molecules that are held together by weak non-covalent interactions, and such interactions are affected dramatically by small structural changes. The challenge becomes even harder if the introduction of some extra functionality into the system is desired, because that involves structural modifications that will have an unpredictable effect in the gelling ability of the system. For example, such extra functionality could be needed in order to achieve responsiveness to determined external stimuli.

We have recently shown that cysteine-containing short peptides, or even single amino acid derivatives, can be converted into highly efficient hydrogelators.[11–13] This is achieved by just adding a coinage-metal salt to the amino acid or peptide solution. The resulting metal(I) thiolate undergoes instantaneous self-assembly to form a 3-dimensional network (Fig. 1a) that gels the water. The thiolate molecules in these systems are hold together by metallophilic attractions between the M^I centres,[14] and for this reason such systems have been designated as metallophilic hydrogels. Good examples are *N*-acetyl-L-cysteine (NAC) and glutathione, among others (Fig. 1b). Despite all the advances made in the field, it is worth noting that the hydrogel forming capability of these systems was discovered by the screening of many different thiols, rather than by design.

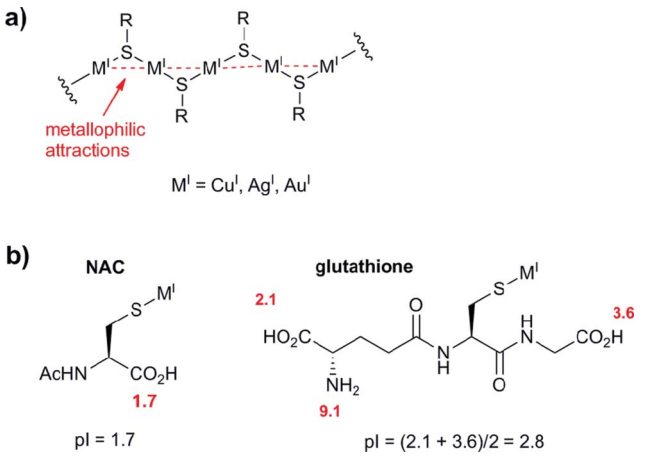

Fig. 1 Polymeric structure of metal(I) thiolates stabilized by metallophilic attractions (a), and chemical structure and calculated pI for NAC and glutathione thiolates (b).

The use of transition metals in medicine (metallodrugs) is not new.[15] Gold(I) has been used for the treatment of rheumatoid arthritis for more than 70 years,[16] and now this old drug is emerging as a new alternative for the treatment of other diseases such as cancer or pemphigus.[17] In this context, the design of stimuli-responsive gold(I)-based hydrogel systems will be a key issue for the development of new drug-delivery vehicles for *chrysotherapy*. The same applies for silver, whose derivatives are re-emerging as a viable treatment option for infections encountered in burns, open wounds, and chronic ulcers.[18] The use of coinage metals as new antitumor agents has been reviewed recently.[19]

Metallophilic hydrogels derived from NAC and glutathione (and some other examples that have not been published) present pH responsiveness, due to their free carboxylate groups. However, one of the major drawbacks of these hydrogels is the low pH ranges at which they aggregate to form gels, typically below 4. This is a disadvantage if the use of these systems in the biomedical arena is desired. Thus, our recent efforts have been focused on the design of peptide-based metallophilic hydrogels that are pH responsive, but do form hydrogels at neutral pH. Here we report a useful approach towards the design of such systems.

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In order to design neutral metallophilic hydrogels we started from the hypothesis that such hydrogels are formed at pH values close to the pI of the peptide, without taking into account the contribution of the thiol group, which is bound to the metal centre. This was concluded from the pH-induced behaviour found for previously studied systems. Thus, calculated pI values for NAC–M[1] and GS–M[1] are 1.7 and 2.8, respectively,‡ and such systems form hydrogels at that pH range. The former dissolves at higher pH values[11,13] and the latter dissolves at both higher and lower pH values,[12] due to the presence of an additional free amino group.

In order to design a cysteine-containing di- or tripeptide with a pI ~7, we envisaged a peptide having the structure depicted in Fig. 2, *i.e.* a cysteine residue for the formation of the metal thiolate, a protected terminal amine, a free terminal carboxylic group and an arginine (Arg) residue. The unusually high basicity of the guanidinium group of Arg (pK_a 12.5) increases the final pI of the molecule to nearly neutral values, taking into account that a terminal carboxylic group has a pK_a of ~2 (pI = 12.5 + 2)/2 = 7.3. Thus, such peptides would form hydrogels at physiological pH, and would dissolve at higher or lower pH values.

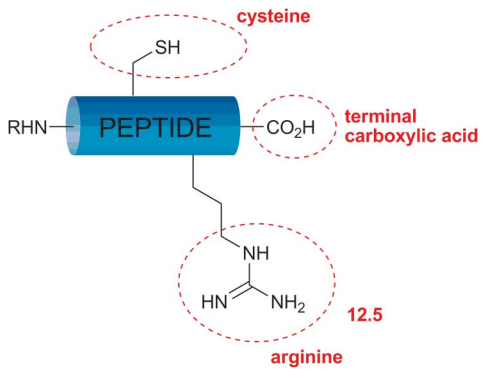

Fig. 2 Schematic structure of a cysteine-containing peptide designed to possess a neutral pI.

To confirm our hypothesis we designed two dipeptides (**CR** and **RC**) and two tripeptides (**RGC** and **RCG**) by combining Cys, Arg and Gly (Fig. 3). With the protection of the terminal amine (acetylation), these combinations always give peptides with just two free acid/base functionalities: one guanidinium and one carboxylate, which makes their corresponding pI around 7.

The formation of the hydrogels was carried out by adding a solution of Au(I) or Ag(I) to an aqueous solution of the peptide, followed by neutralisation with NaOH 1 N until neutral pH was reached.[20] The final peptide concentration in the hydrogels was ~3 wt%.

The gel-forming ability of each of the peptides was tested using both Au(I) and Ag(I). The results obtained were quite surprising; from the 8 possible combinations, 4 different hydrogels were obtained: **CR–Ag**, **RC–Au**, **RCG–Ag** and **RGC–Au**. Unexpectedly, none of the peptides led to the formation of hydrogels with both gold and silver, but rather two of them gave just aurophilic hydrogels and the two others just formed argentophilic gels (Fig. 4). Opaque hydrogels were obtained in all cases.

Characterisation of the four hydrogels was performed by FTIR, ^{1}H NMR, and EDX (see ESI†). FTIR spectra were consistent with the formation of the metal(I) thiolate, the main feature being the disappearance of the S–H stretching bands from the original

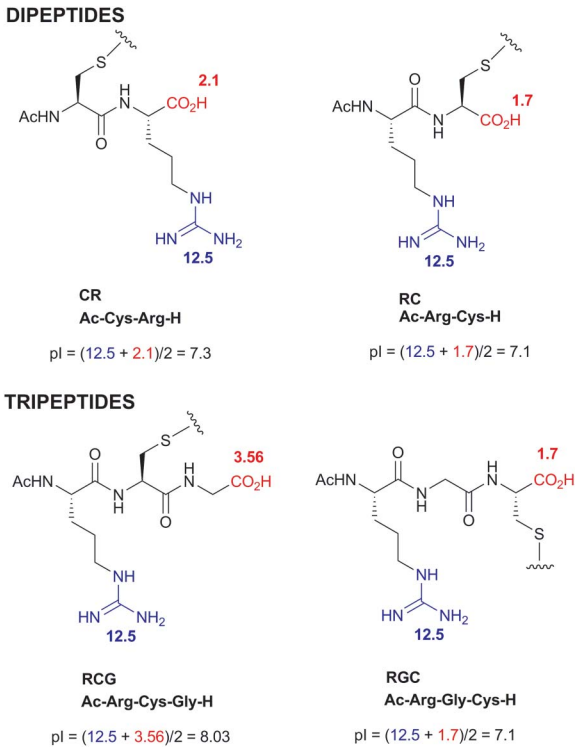

Fig. 3 Cysteine- and arginine-containing peptides selected for the present study, and their calculated pI values.

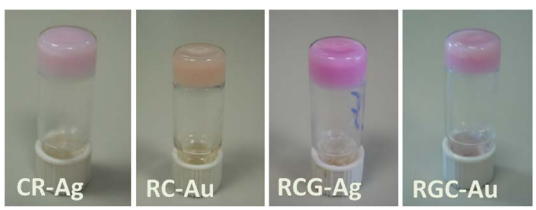

Fig. 4 Photographs of hydrogels **CR–Ag**, **RC–Au**, **RCG–Ag** and **RGC–Au**. Pink coloration is due to the presence of phenol red as pH indicator.

peptides. ^{1}H NMR spectra showed a general broadening of the signals, consistent with the formation of oligomeric species.[13] Moreover, a strong unshielding effect of the CH_2–S methylene signal was observed in all cases, which is caused by metal attachment to the sulfur. EDX analysis showed a 1 : 1 sulfur : metal ratio, in good accordance with a (RS-M)$_n$ molecular formula.[21]

SEM micrographs of hydrogels **CR–Ag**, **RC–Au**, **RCG–Ag** and **RGC–Au** are shown in Fig. 5. They all show a microporous structure, in the form of flakes rather than fibres, in good accordance with our previous results obtained for peptide-based metallophilic hydrogels.[12]

As expected, such metallophilic hydrogels resulted in being pH reversible. Moreover, all of them behaved as hydrogels only at neutral pH values (pH ~5–9), exactly in the way they were designed. At higher and lower pH the systems behaved as clear solutions.

All the hydrogels appeared to be thermoreversible. The phase transition temperatures for 3 wt% hydrogels were found to be around 75–85 °C.

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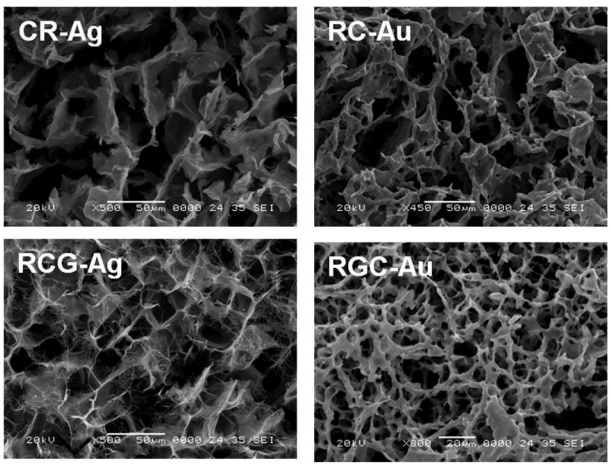

Fig. 5 SEM micrographs of hydrogels **CR–Ag**, **RC–Au**, **RCG–Ag** and **RGC–Au**.

In summary, the present study reveals that the possibility of making hydrogels from cysteine-containing short peptides and Au(I) and Ag(I) salts is not an isolated case, but is rather a quite general and straightforward strategy for the obtaining of pH-responsive gels. This work shows that cysteine-containing peptides can be easily designed to have a determined pI, and that the pI will determine the pH range at which such peptides will form a metallophilic hydrogel. Even if the gelling ability of a determined peptide with Au^{I} and Ag^{I} cannot be fully predicted *a priori*, the results suggest that many different gelator peptides can be possibly designed applying the same principle. These systems represent an advancement towards the development of responsive soft materials, which could have potential application in the field of coinage-metal-based metallodrugs.[19]

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Notes and references

‡ For simple amino acids, the pI is an average of the pK_a's of the carboxyl and ammonium groups. If additional acidic or basic groups are present as side-chain functions, the pI is the average of the pK_a's of the two most similar acids. To assist in determining similarity, two classes of acids are defined. The first consists of acids that are neutral in their protonated form (*e.g.* $-CO_2H$ and –SH). The second includes acids that are positively charged in their protonated state (*e.g.* $-NH_3^+$). In the case of glutathione, the similar acids are the two α-carboxyl functions (pK_a = 2.1 and 3.6), so pI = (2.1 + 3.6)/2 = 2.8.

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