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Adsorbent Selection by Functional Group Interaction Screening for Peptide Recovery

RENZE WIJNTJE*, HANS BOSCH AND ANDRE B. DE HAAN
University of Twente, Faculty of Chemical Technology, Separation Technology Group P.O. Box 217, 7500 AE Enschede, The Netherlands
r.wijntje@utwente.nl

PAUL BUSSMANN
TNO-MEP, Department of Industrial Processes and Applications P.O. Box 342, 7300 AH Apeldoorn, The Netherlands

Abstract. In order to selectively adsorb small peptides from complex aqueous feeds, selective adsorbents are required. The goal is to first find adsorbents with capacity for triglycine, as triglycine contains all groups common to small peptides. Selectivity studies will follow. Adsorbent selection was based on the interactions available to the triglycine groups and four types of interaction seemed theoretically suitable to provide adsorption of triglycine. These interaction types are hydrogen bonding, electrostatic interaction, π interaction and metal complex formation. This resulted in testing 16 different adsorbent functional groups for adsorption of triglycine under different solution conditions (triglycine only, with 0.2 M NaCl and at pH 8.2). Adsorbents using electrostatic interaction, such as zeolites, anion and cation exchangers, exhibit the strongest interaction, although in the presence of NaCl the distribution coefficient of triglycine is significantly lowered. The adsorbents, when loaded in the H^+ form, interacting electrostatically with the peptide amino group show the highest selectivity. Regarding π interaction, several aromatic adsorbents show a weak interaction with triglycine, with low distribution coefficients. Transition metal complex adsorbents (copper(II) and vanadium(IV)oxide) show a weak interaction, limited by pH, or are stripped from the immobilizing ligand by triglycine. The hydrogen bonding adsorbents show no measurable adsorption of triglycine.

Keywords: peptide, separation, adsorption, interaction, triglycine

Introduction

Small peptides (three to ten amino acid sequences) are commercially interesting as they can be valuable food ingredients (low allergenic properties (Nielsen, 1996)) and are present in most aqueous process streams of organic origin. For instance in aqueous washing streams in potato starch, sugar, vegetable canning and other agro industries. For larger peptides, such as lactoferrin, separation methods are known, based on size exclusion, isoelectric point, charge or combinations thereof. Small peptides however are much harder to separate from the other components present in these process streams, like mono- and disaccharides, amino acids, organic acids and their salts. Therefore separation methods selective for these small peptides are needed.

The peptide concentrations are low and therefore adsorption has been chosen as separation method. With a proper choice of adsorbent, selectivity and capacity for small peptides should be achievable. The bottleneck however lies in this choice of adsorbent, as, due to the large number of different amino acid building blocks, peptides have a wide variety of chemical groups available for interaction. The groups peptides have in common are the amino and carboxylic terminals and the amide groups (Fig. 1).

*To whom correspondence should be addressed.

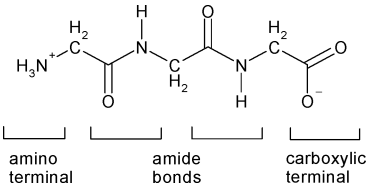


Figure 1. The functional groups of triglycine.

To achieve separation, an adsorbent must interact preferentially with the peptide groups over other similar groups. This requires study of the interaction of adsorbent functional groups with the peptide groups. Triglycine (Fig. 1) has all common peptide groups without disturbing side chains, therefore making it a very suitable choice as a model peptide to evaluate the interaction of adsorbent functional groups with the common peptide groups. To our knowledge the only study done with triglycine adsorption is with montmorillonite and modified montmorillonite, which were studied for triglycine adsorption capacity (Kalra et al., 2003), with K-values between 0.75 and 1.0. Therefore it was decided to study a range of adsorbents, including an alumina, a zeolite and several polymeric resins with differing functional groups.

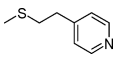
The objective of this paper is to determine the capacity of the adsorbents, differing in functional groups, for triglycine. A further study will focus on the selectivity of the adsorbents that show capacity for triglycine.

Theoretical Background

Several types of adsorbent functional groups can interact with one or more peptide groups. The interaction between adsorbents and peptide groups fall into four categories: electrostatic interaction, hydrogen bonding, π interaction (cation-π and/or NH-π interaction) and reactive interaction.

The first group of adsorbents has functional groups capable of hydrogen bond formation (type I, see Table 1). The interaction of these adsorbents is between proton donors and peptide carboxyl groups and between proton acceptors and peptide N-H groups. However, water is a competitor for the hydrogen bonding sites of the adsorbent and is present in excess. Therefore adsorption of triglycine on these adsorbents will probably be low. However, the selectivity could be high. Therefore this group of adsorbents is also tested.

Table 1. Distribution coefficients for triglycine of different adsorbents.

			Experiment		
Adsorbent	Type	Func. group	Triglycine $K(-)$	0.2 M NaCl $K(-)$	pH 8.2 $K(-)$
Alumina (neutral)	I	Al-OH	Nil	Nil	Nil
Amberlite XAD 7HP	I	Aliphatic ester	Nil	Nil	Nil
Divergan RS	I	N-vinylpyrrolidone	Nil	Nil	Nil
Diaion WA21J	II	R-$(CH_2CH_2NH_2)_n$	17	Nil	7.3
Dowex 22	II	Type II quarternary amine	>100	3.2	>100
CBV-720 -H^+	II	Zeolite Y	84	40	75
Dowex MSC-1 -H^+	II	Phenylsulfonic acid	>100	30	>100
Dowex MSC-1 -Na^+	II	Na^+ loaded phenylsulfonate	1.6	0.4	Nil
Duolite GT 73	II	R-SH	8.5	Nil	5.5
Toyopearl Blue HC-650M	III	Cibacron Blue F3GA	Nil	1.0	0.3
Duolite XAD 761	III	Phenolic matrix	7.9	1.7	4.7
MEP HyperCel	III		Nil	Nil	0.7
Dowex M4195	III	Bis-picolylamine	0.2	Nil	1.0
Amberlite IRC-718 -Cu^{2+}	IV	Cu(II) complex	3.2 (pH = 3.7)	2.1 (pH = 3.7)	2.9 (pH = 3.7)
Dowex M4195 -Cu^{2+}	IV	Cu(II) complex	Nil (pH = 3.2)	Nil (pH = 3.8)	1.3 (pH = 4.4)
Dowex M4195 -VO^{2+}	IV	V(IV) complex	Nil (pH = 2.6)	Nil (pH = 2.7)	Nil (pH = 2.6)

Ion exchanging adsorbents (adsorbent type II) interact with either the protonated amino terminal or the deprotonated carboxylic terminal. The electrostatic interaction is strong and in industry it is used to separate larger peptides (Korhonen et al., 1998) such as lactoferrin and lactoperoxidase. Amino acids are also separated with ion exchange, as several side chain groups are basic or acidic. Therefore strong interaction between ion exchange resins and triglycine is expected, although selectivity between peptides and other amino, carboxylic and salt components could be low.

Aromatic functionalized adsorbents form the third group of adsorbents (type III). The amino group is capable of interaction with π-electron systems (like aromatic functionalized adsorbents) by means of NH-π (unprotonated amino group) and cation-π interaction (protonated amino group) (Duan et al., 2000; Hunter et al., 1990; Janiak, 2000; Ma et al., 1997), of which the cation-π interaction is stronger. In addition, the amide bond has a partial π-system characteristic due to mesomerism (Duan et al., 2000; Sigel et al., 1982; Sovago, 1990) and its dipole also allows for NH-π interaction (Duan et al., 2000). Interaction between an aromatic group and an amide bond is comparable to benzene-benzene interaction and present in protein structures, together with cation-π interaction (Duan et al., 2000). It may therefore be assumed that triglycine can interact with aromatic functional groups. Cation-π interaction is however weaker than the electrostatic interaction between ions and adsorption will probably not be as strong as with cation exchangers. In addition, cation-π interaction is available to other ions too. The amide bond is also capable of interaction with the aromatic groups, but is weaker and probably only occurs if there are other aromatic groups (or the aromatic group consists of multiple aromatic rings) available in the proximity of the aromatic group interacting with the amino terminal. Furthermore, the interaction has to compete with solvation of the amide group by water. Aromatic functionalized adsorbents will probably adsorb triglycine and possibly with reasonable selectivity if the adsorbent has a high density or large aromatic functional groups, preferably with polar side groups to enhance wetting and increase the electron density of the aromatic group (Duan et al., 2000).

The last group of adsorbents react reversibly with triglycine (type IV). Currently our attention is focused on the complexation of triglycine by transition metal ions such as copper(II) (Sigel et al., 1982; Sovago, 1990; Wong et al., 1991) and vanadium(IV)oxide (Kiss et al., 1998, 2003; Rehder, 1999). These metal ions form chelates with peptides through coordination with one of the terminals and the neighboring amide oxygen or deprotonated amide nitrogen (Fig. 2). The deprotonated amide nitrogen complex is the most stable, but strongly pH dependent. Deprotonation is catalyzed by the type of metal ion and can be influenced by the other ligands of the metal ion (Kiss et al., 1998; Pearson, 1973, 1990). Copper(II) complexes with the amino terminal and neighboring amide nitrogen, vanadium(IV)oxide complexes with the carboxylic terminal and neighboring amide nitrogen. Adsorbents with transition metal complex functional groups should adsorb selectively, due to their interaction with both terminal and amide group of the peptide. However, a limited pH window is suitable for operation. This window is dependent on metal ion type and immobilizing ligands, due to the reaction equilibrium at lower pH's and stripping of the metal ion by the peptide at higher pH's (Hansen et al., 1992).

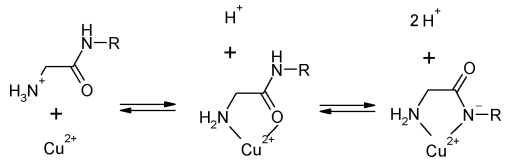


Figure 2. (N, O) and (N, N$^-$) complex formation of copper(II) with the amino terminal.

Experimental

To investigate the interaction of these four types of adsorbent functional groups with the triglycine groups, adsorbents from all groups are contacted with three different aqueous triglycine solutions: triglycine in water (1.0 g/l), triglycine (1.0 g/l) in 0.2 M NaCl solution and triglycine in water (1.0 g/l) brought to a pH of 8 with sodium hydroxide. The salty triglycine solution is used to study effects of salt competition and salting out on the interactions of the triglycine groups. The pH 8.2 solution is used to study the effects of pH on the interactions, as the $pK_{a,2}$ of triglycine is 7.96 (Hanaki et al., 1999). The triglycine in water solution has a pH of 5.4–5.6.

Prior to the experiments, the adsorbents are pretreated with milliQ water, pH 1 HCl-solution and/or pH 12 NaCl solution, until no contaminants are detected and are in the ionic form required. The tested

adsorbents and their functional groups are listed in Table 1. Before contacting the solutions with the adsorbents and at equilibrium the pH is measured. The vessels are agitated at 30°C for 16 hours.

As the adsorbent still contains residual water, an adsorbent sample is weighed, dried at 110°C for 16 to 18 hours and weighed again to determine the residual water fraction. A ratio F (dry adsorbent mass/wet adsorbent mass) is then calculated by dividing the dry adsorbent mass by the mass of the wet drying sample and used to calculate the adsorbed mass per kg dry adsorbent.

The equilibrated solutions are analysed by HPLC. The column is a LUNA-NH_2 with a 20 mM NaH_2PO_4 (pH = 6.05) eluent. The detector is a UV meter set at 190 nm. The triglycine samples are diluted with milliQ water before analysis.

Materials

All solutions make use of Millipore milliQ water. Other chemicals used are: triglycine (Sigma), cupric chloride dihydrate (>99.99%, Sigma), sodium chloride (ACS reagent, Merck), vanadylsulfate hydrate (99.99+%, Aldrich), sodium hydroxide (97%, 20–40 mesh beads, Aldrich). Toyopearl Blue HC-650 M was obtained from Tosoh Bioscience Europe. MEP HyperCel was purchased from Ciphergen Biosepra. Zeolyst International and BASF thankfully provided samples of CBV 720 and Divergan FS. Aluminium oxide 90 (Alumina) was obtained from Merck. The other adsorbents were obtained from Supelco.

Results

The batch equilibrium adsorption experiments give the equilibrium triglycine concentration (c_{eq}, in g/l) in the solution. The feed triglycine concentration is known and corrected for the residual water in the adsorbent. The adsorbed triglycine concentration (q, g triglycine/kg dry adsorbent) and a distribution ratio (K, l/kg) can then be calculated. The distribution ratio K is calculated according to Eq. (1).

$$K = \frac{q}{c_{eq}} \tag{1}$$

The K-values of the adsorbents for triglycine in the different experiments are listed in Table 1. For the metal-complex adsorbents, the solution pH at equilibrium is noted between brackets besides the K-values. This as the copper(II) and vanadium(IV) loaded Dowex M4195 show coloring of the solution at equilibrium in all three triglycine solutions and the copper(II) loaded IRC-718 has the same equilibrium pH for all experiments.

Discussion

Of the four interaction types, the results clearly show that interaction based on hydrogen bonding shows no adsorption of triglycine. This is probably due to competition with water for the functional adsorbent groups in combination with the good hydration of triglycine by water. Thus these types of adsorbents have no potential of adsorbing small and highly soluble peptides from aqueous solutions. For small peptides possessing bulky hydrophobic side chain groups they might still be selective, as these hydrophobic side chain groups lower solubility of the peptide in water.

Contrary to the hydrogen bond interaction, electrostatic interaction based adsorbents show very good adsorption of triglycine, resulting in high K-values. However, in presence of salt (NaCl), K-values are significantly lower due to the relatively high concentration of competing ions (37 times higher molar concentration than triglycine in the feed). Remarkably the K-values of the cation exchangers CBV-720 (zeolite Y, H^+-form) and Dowex MSC-1 (H^+-form) remain relatively high under the influence of the 0.2 M Na^+ concentration, where the K-value of Dowex 22 drops to 3.2 under the same conditions. Thus strong cation exchangers, even in the presence of high Na^+ concentrations, such as saline solutions, adsorb triglycine. For Dowex MSC-1 part of the explanation can be found from the experiments with the Na^+ loaded form. The experiments with the Na^+ loaded MSC-1 indicate interaction is only with the charged triglycine form. The experiment with the pH 8.2 feed solution gives no measurable adsorption with the Na^+ loaded MSC-1, as triglycine is present in the negatively charged form and a bit in the zwitterionic form. The H^+ loaded MSC-1 however, is capable of protonating the triglycine and thus adsorbing triglycine (equilibrium pH = 2.4 for the pH 8.2 experiment), with $K > 100$. That triglycine is strongly adsorbed even in presence of competing Na^+ (0.2 M) can be attributed to a preference of R-NH_3^+ over Na^+ and a π-type interaction between triglycine and the sodium phenylsulfonate functional group. The zeolite

gives significantly higher K-values than the literature results for the natural zeolite, montmorillonite (max. $K = 1.0$). The results for Duolite GT 73 suggest that it interacts with triglycine similar to a weak cation exchanger.

Regarding the aromatic adsorbents and the π-type interaction (cation-π and/or NH-π interaction) with triglycine the experiments show this interaction to be very weak, resulting in low K-values. The Toyopearl Blue HC-650M adsorbent shows lower K-values than expected, as the functional group contains sulfonic and amine groups attached to the large aromatic system. These should strengthen the aromatic system and be able to interact electrostatically with triglycine. Looking at the Na^+ loaded MSC-1 experiments the lack of interaction between the sulfonic groups and the amino terminal can be explained, as these groups are present in the sodium form. The amines lack interaction with the carboxylic terminal, probably due to the aromatic character of the amine. This leaves only cation-π and NH-π interaction. Of these two cation-π interaction seems dominant, as the K-value for the pH 8.2 experiment is lower than the K-value for the 0.2 M NaCl experiment (less protonated amino terminals at pH 8.2). However, for Toyopearl Blue HC-650 M and MEP HyperCel it must be noted that, due to the hydrophilic nature of the matrix and thus possible bonded water, adsorption could be somewhat higher as the dry weight factor would then be too low. MEP HyperCel and Dowex M4195 only show significant adsorption in the pH 8.2 experiments, which can be explained by the fact that in the other experiments (triglycine only and 0.2 M NaCl) adsorption of triglycine is less feasible due to protonation of or sodium adsorption by the functional groups, as the pH lies between 5.5 and 6. On the other hand Duolite XAD 761 shows exceptionally high K-values considering the other aromatic adsorbent results. These results imply that another type of interaction is also in play. A possibility could be the weak acidic character of the phenol hydroxyl group. This would also explain the decrease in adsorption in the 0.2 M NaCl experiment, where a slight increase was expected due to salting out.

The transition metal functionalized adsorbents have interaction with triglycine, as shown by the K-values of Cu^{2+} loaded IRC-718 and the coloring of the equilibrium solution by a copper or vanadiumoxide complex. It is assumed that the complex is a metal-triglycine complex, as coloring does not happen without triglycine present. According to literature (Sigel et al., 1982; Sovago, 1990), a copper(II) iminodiacetic acid complex is capable of complexing with a peptide until the pH drops to around 4. At this pH, H^+ competes effectively with Cu^{2+} for the amino terminal (Fig. 2). Between pH 4 and 6 the (N, O) complex is dominant and at higher pH the (N, N^-) complex is dominant (Hansen et al., 1992) (Fig. 2). Our copper iminodiacetic acid complex (Cu^{2+} loaded IRC-718) has equilibrium pH's of 3.7, implying (N,O) complexation of triglycine. This (N, O) coordinated triglycine complex is relatively weak, which is confirmed by the drop in K-value with 0.2 M Cl^- (competing ion) present. Dowex M4195 has bis-picolylamine as an immobilizing ligand, which should favor the equilibrium towards complexation. Unfortunately the lack of an anion in the immobilizing ligand makes the Cu^{2+} susceptible to stripping by the coordinated triglycine. The pH 8.2 experiment shows adsorption, coloring and a higher equilibrium pH, which points to protonation of bis-picolylamine before stripping of copper(II) by triglycine. Dowex M4195-VO^{2+} shows no adsorption at all, however the equilibrium pH is exceptionally low (2.6–2.7) for all experiments and all equilibrium solutions show coloring. The equilibrium pH's are lower than the Cu^{2+} loaded IRC-718, thus more protons have been released, and compared to copper(II) more triglycine has formed a complex with vanadium(IV). This indicates that vanadium(IV)oxide has a very strong interaction with triglycine. However to adsorb the triglycine a stronger interaction between vanadium and the adsorbent ligand is needed, without disrupting the deprotonation.

Conclusion

Electrostatic type adsorbents have the highest capacity for triglycine (see Table 1, type II). Of these electrostatic type adsorbents, the adsorbents interacting with the amino terminal (cation exchangers) adsorb triglycine significantly, even with Na^+ ions present in higher concentrations. Metal complex based adsorbents (Table 1, type IV) have a low capacity, which could be higher as capacity is currently negatively influenced by the pH (reaction equilibrium) or stripping of the metal ion. Thus pH control and/or different immobilizing ligands could provide to be beneficial to triglycine adsorption capacity. Adsorbents with π interaction (aromatic adsorbents, Table 1, type III) also show low K-values, with XAD-761 (phenolic functional groups) as the exception, probably due to a

combination of π interaction with electrostatic interaction. Adsorbents with hydrogen bonding interaction (Table 1, type I) show no measurable adsorption.

To provide an understanding of the influence of the amide group on the adsorption interactions, adsorption experiments with glycine and triglycine/glycine mixtures will be performed.

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