Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 4, pp. 413-416, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00023-1

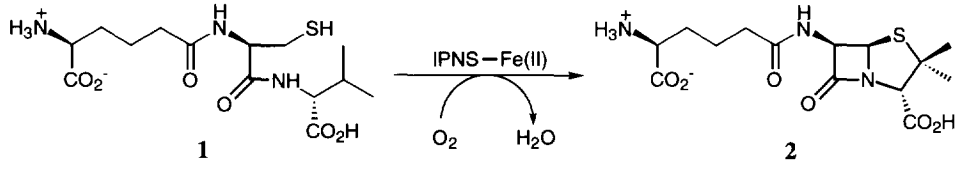
RESIN-BOUND PEPTIDE LIBRARIES SHOWING SPECIFIC METAL ION BINDING

Norio Shibata[a], Jack E. Baldwin[a] and Mark E. Wood*[,b]

[a]*The Dyson Perrins Laboratory and the Oxford Centre for Molecular Sciences, South Parks Road, Oxford, OX1 3QY, U.K.*, [b]*Department of Chemistry, University of Exeter, Stocker Road, Exeter, EX4 4QD, U.K.*

Abstract: A library of 7240 TentaGel® resin-bound peptides all containing the active site residues of isopenicillin N synthase was prepared. Several peptides showed a high binding affinity for Co^{2+}.

Recent studies in our group have revealed the X-ray crystal structure of recombinant isopenicillin N synthase (IPNS) from *Aspergillus nidulans*, the ferrous dependent enzyme responsible for the biosynthetic transformation of the Arnstein tripeptide L-δ-(α-aminoadipoyl)-L-cysteinyl-D-valine (ACV) **1** into isopenicillin N (IPN) **2** *via* desaturative ring closure with concomitant reduction of dioxygen to water[1] (Scheme 1).



Scheme 1

This work has suggested that under anaerobic conditions the active site iron is coordinated to two histidine residues (His 214, 270), a side-chain carboxylate from an aspartate (Asp 216), water and possibly the free-thiol group of the ACV tripeptide **1** (Figure 1).

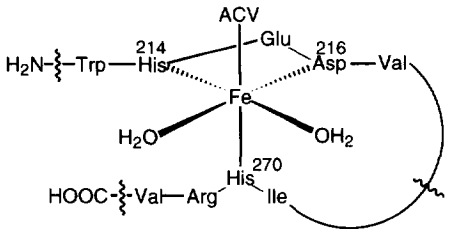


Figure 1

As a part of our studies to investigate the chemistry of this transformation, we wished to examine metal binding by these residues with a possible long-term view of producing shorter peptides with related catalytic activity. A logical approach to this problem was to generate combinatorial peptide libraries[2] and firstly probe for selective metal binding.

* E-mail: m.e.wood@exeter.ac.uk Fax: +44 1392 263434

The target peptides would have two conserved sequences, Ile-His-Arg and Trp-His-Glu-Asp-Val (both of which are found at the active site of IPNS) and have a variety of proteinogenic amino acids linking these moieties. To ensure no binding of the *N*-terminal amino group and to increase stability in solution, we also decided to terminally block the peptides with an acetyl group. This gave us target resin-bound peptides of general structure **3** (Figure 2).

AcTrp—His–Glu–Asp–Val–$(Xaa)_n$–Ile–His–Arg—**RESIN** **3**

Figure 2

The library of peptides generated would differ solely in the length and amino acid content of the linking peptide chain with n varying from 0 to 3 and being made up from 19 proteinogenic amino acids. (Only up to 3 combinatorial positions were included because of the practicalities of reaction scale and cysteine was omitted for ease of synthesis). The library thus produced would therefore consist of 7240 individual oligomers (Figure 3).

Peptide		**Combinations**
AcTrp—His–Glu–Asp–Val–Ile–His–Arg—**RESIN**	**3a**	1
AcTrp—His–Glu–Asp–Val—$(Xaa)_1$—Ile–His–Arg—**RESIN**	**3b**	19
AcTrp—His–Glu–Asp–Val—$(Xaa)_2$—Ile–His–Arg—**RESIN**	**3c**	19 x 19
AcTrp—His–Glu–Asp–Val—$(Xaa)_3$—Ile–His–Arg—**RESIN**	**3d**	19 x 19 x 19
	Total	7240

Figure 3

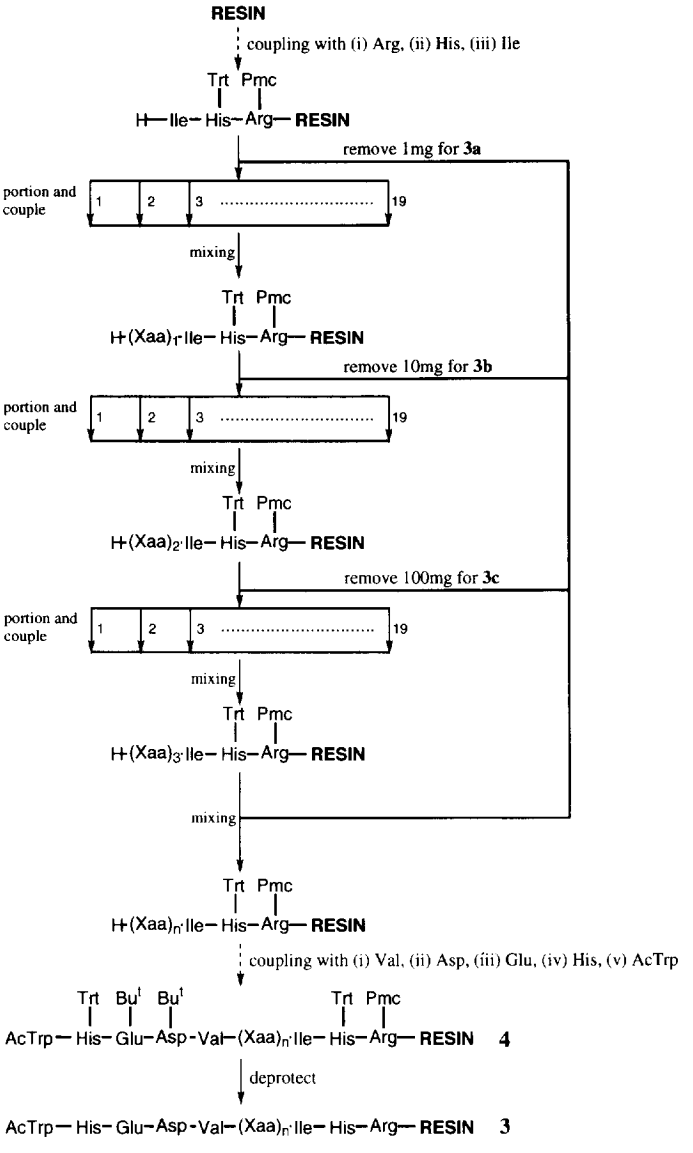
The peptides were to be kept in a resin-bound state to look for selective metal ion binding and we chose TentaGel® resin[3] because of its excellent swelling properties in water and its suitability to standard Fmoc solid phase synthesis methodology[4]. The well-established "portioning and mixing" technique[2] was used to generate the required libraries. Details of the solid phase synthesis conditions are summarised in Figure 4[5].

Carrier	TentaGel® resin
N-(α)-protection	Fmoc
N-(α)-deprotection	50% piperidine in DMF (15min)
Side-chain protection	Bu^t for Asp, Glu, Ser, Thr and Tyr; Pmc for Arg; Trt for His; Boc for Lys
Coupling	Fmoc-Xaa-OPf in DMF with HOBT or Fmoc-Xaa-OH in DMF (and AcTrp-OH) with DIC / HOBT except for Ser and Thr which were introduced in DMF *via* Dhbt esters with HOBT (2h)
Side-chain deprotection	TFA / thioanisole / phenol / water / ethanedithiol (82.5 : 5 : 5 : 2.5 : 1 v/v) (5h)

Figure 4

The first three amino acids (Arg, His and Ile) were coupled in sequence to the solid support (TentaGel® resin, 1.0g, 0.27mmol) using standard Fmoc chemistry[4] and the combinatorial positions were added using a "divide, couple and recombine" process. An appropriate quantity of the resin beads was removed for syntheses of the different peptide chain lengths before accurately weighing the resin into 19 equal portions for coupling to the next L-amino acid. After coupling, the 19 portions were recombined and mixed thoroughly before repeating the procedure twice more to generate the peptide library **3** with n = 0 to 3 (*ie.* **3a** to **3d**). The resin for **3d** was combined with that for **3a**, **3b** and **3c** and the next five conserved amino acids (Val, Asp, Glu, His and AcTrp)

were coupled to give fully side-chain protected library **4**. Side-chain deprotection was then accomplished using a mixture of trifluoroacetic acid : thioanisole : water : phenol : ethanedithiol (Scheme 2). The overall library thus obtained contained 7240 individual resin-bound peptides, each representing approximately 3.37 x 10^{-5}mmol.



Scheme 2

The metal coordinating properties of the peptides were assayed with aqueous solutions of Cu^{2+}, Co^{2+} and Fe^{2+} by stirring the resin beads with the appropriate metal ion solution. All solutions were prepared using 18MQ deionised water with metals present at a concentration of 1.0mM and 0.1mM. Significant metal binding was accompanied by a change in colour of the beads from their initial pale yellow. This was found to be

particularly marked in the case of Co^{2+} where only a small number of the beads took up a strong pink / purple colour, easily visualised by the naked eye or using an optical microscope (Figure 5).

Figure 5

Unfortunately, *N*-terminal blocking with the acetyl group prevented sequencing of the resin-bound peptides and mass spectrometric analysis was also not possible.

In summary, we have demonstrated specific Co^{2+} binding by individual resin-bound peptides in a "partially combinatorial" library. Preparation of such libraries without *N*-terminal blocking should allow rapid construction, assay and sequence determination of peptides showing a high affinity for specific metals. Keeping the peptides resin-bound allows trivial identification of binding peptides by visual inspection. Jacobsen and co-workers have also recently reported a similar combinatorial approach to the formation of metal complexes, without in this instance, incorporating predefined metal binding sites.[6]

Acknowledgements:

The authors wish to thank the Japan Society for the Promotion of Sciences for Research Abroad for funding to N. S., Glaxo-Wellcome for a fellowship to M. E. W. and Dr. Peter Roach (Oxford) for providing information regarding the IPNS crystal structure.

References and Notes:

1. Roach, P. L.; Clifton, I. J.; Fülöp, V.; Harlos, K.; Barton, G. J.; Hajdu, J.; Andersson, I.; Schofield, C. J.; Baldwin, J. E. *Nature (London)* **1995**, *375*, 700.
2. Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233.
3. Bayer, E.; Rapp, W. *Chem. Pept. Prot.* **1986**, *3*, 3.
4. Atherton, E.; Sheppard, R. C. In *Solid Phase Peptide Synthesis, a Practical Approach*, IRL Press at Oxford University Press, **1989**.
5. Abbreviations: DIC, diisopropylcarbodiimide; Dhbt, *N*-hydroxy-oxo-dihydrobenzotriazine; Pf, pentafluorophenyl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl.
6. Francis, M. B.; Finney, N. S.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1996**, *118*, 8983.

(*Received in Belgium* 4 *November* 1996; *accepted* 8 *January* 1997)