Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.130*

Total Bound Nitrogen Analysis for the Quantification of Immobilized Peptides on Dynabeads

Peter Boelens[1], Sylvia Schöne[2], Stephan Weiss[2], and Franziska Lederer[1]
[1]Helmholtz-Zentrum Dresden-Rossendorf, Helmholtz Institute Freiberg for Resource Technology, D-09599 Freiberg, Germany; [2]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, D-01328 Dresden, Germany

Introduction

According to the most recent listing reported by the European Commission, rare-earth elements (REEs) are the critical raw materials with the highest supply risk, whereas their recycling rates remain very low in the European Union [1]. End-of-life fluorescent lamps are a promising secondary source of REEs, but their recycling requires innovative separation processes [2,3]. By using phage surface display, Lederer and co-workers identified selectively surface-binding peptides that specifically bind to fluorescent lamp phosphors [4]. In a following study, Schrader et al. immobilized these peptides on coated well plates to investigate their binding to various REE phosphors [5]. The immobilization was facilitated by an activation with benzotriazole-1-yl-oxytripyrrolidinophosphonium-hexafluoro-phosphate (PyBOP) in the aprotic solvent *N*-Methyl-2-pyrrolidone (NMP) in the presence of the sterically hindered base diisopropylethylamine (DiPEA), a coupling reaction commonly used for chemical peptide synthesis. Recently, we investigated the immobilization method presented by Schrader et al. for the functionalization of Dynabeads [6]. Dynabeads are highly spherical and monodisperse composite magnetic beads, consisting of superparamagnetic iron oxide nanoparticles dispersed in a polystyrene matrix. They are commercially available with various surface coatings. The functionalization of amine coated Dynabeads with phosphor binding peptides, immobilized with the coupling reaction described above, did not change the Dynabeads' zeta potential and had no significant effect on the interaction with REE phosphors [6]. On the other hand, we found that the immobilization onto carboxylic acid coated Dynabeads changed the Dynabeads' zeta potential and isoelectric point. We also observed that this immobilization had a detrimental effect on the interaction of the beads with the targeted phosphor particles and suggested that this may be an indication of polymerization of the peptides on the Dynabeads' surfaces. In this work, we present a quantitative analysis of the total bound nitrogen (TN_b) for the quantification of the immobilized peptides on the Dynabeads.

Materials and Methods

A peptide with the amino acid sequence RCQYPLCS (FL464) was obtained from DGpeptides, Co., Ltd., whereas PyBOP, DiPEA and NMP were purchased from Carl Roth GmbH + Co. KG. The functionalization of M-270 amine and carboxylic acid coated Dynabeads (ThermoFisher Scientific Inc.) with diameters of 2.8 µm was conducted as described by Boelens et al. [6]. Briefly, 1.8 mg amine and carboxylic acid coated Dynabeads were each mixed with 1.0 mg of the peptide, 10 molar equivalents of PyBOP and 20 molar equivalents of DiPEA, as compared to the peptide concentration, in NMP with a total volume of 1 mL. Additionally, we prepared control samples in which the Dynabeads were mixed with the reagents without the presence of the peptide. All samples were prepared in triplicate. The samples were incubated in an overhead shaker for 120 minutes. Subsequently we washed the samples twice with NMP and four times with deionized water by collecting the Dynabeads with a permanent magnet and refreshing the supernatant. Next, we lyophilized the samples overnight to completely remove all traces of NMP and finally, we dissolved the Dynabeads in 5 mL of a 2 M HCl solution prior to TN_b analysis with a multi N/C 2100S (Analytik Jena GmbH).

The multi N/C 2100S was originally developed for analysis of environmental samples, such as soils and (waste)waters. The working principle of this setup for determination of the samples' TN_b content is based on a sample injection into a combustion tube, where the sample is digested at high temperature and a platinum catalyst facilitates the total conversion of nitrogen to NO-gas, which is detected by a chemoluminescence detector.

Results and Discussion

The mechanisms of the peptide immobilization onto the amine and carboxylic acid coated Dynabeads are depicted in Figure 1A and Figure 1B, respectively, as intended by Boelens et al. [6]. As PyBOP activates carboxylic acid groups for a coupling reaction with nucleophilic amines, based on the structure of the peptide FL464, we assumed that the peptides' *C*-terminus would bind to the surface of amine coated Dynabeads, whereas both the *N*-terminus and the guanidino group in the arginine sidechain could bind to the surface of carboxylic acid coated Dynabeads. Figure 1C shows the TN_b concentration in the samples of the amine coated Dynabeads, incubated without peptide (NH2 DB control) and with peptide (FL464@NH2 DB), and in the samples of the carboxylic acid coated Dynabeads, incubated without peptide (COOH DB control) and with peptide (FL464@COOH DB). The incubation with the peptide did not significantly change the TN_b concentration of the samples with amine coated Dynabeads ($p = 0.25$), indicating that the peptide immobilization on these beads may not have been successful, which is in line with findings of the beads' zeta potential and interaction with REE phosphors [6]. On the other hand, the immobilization of FL464 onto carboxylic acid coated Dynabeads significantly increased the TN_b concentration from 4.10 ± 0.06 mg.L^{-1} to 5.94 ± 0.30 mg.L^{-1} ($p = 0.0033$).

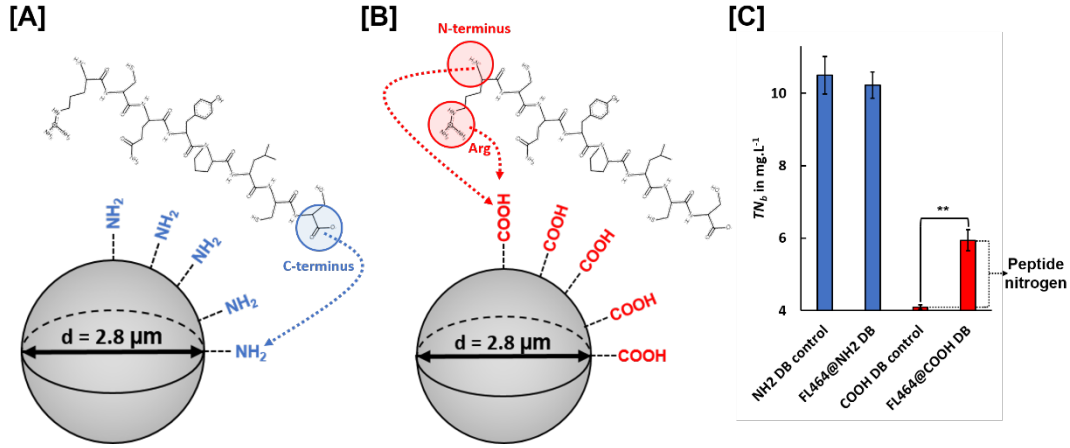

Fig. 1. Intended coupling reaction mechanism of the peptide FL464 to the surface of [A] amine coated and [B] carboxylic acid coated Dynabeads. The structures of the peptide were drawn with the online tool available under: http://www2.tulane.edu/~biochem/WW/PepDraw/.[C] TN_b concentration of the various samples, as determined with a multi N/C 2100S (Analytik Jena GmbH). The error bars depict one standard deviation.

From the obtained difference of the TN_b concentrations between the samples without and with peptides and the known size, shape, and amount of the Dynabeads, the surface density of the immobilized peptide, σ_{pept}, can be calculated according to the equation:

$$\sigma_{pept} = \frac{\Delta TN_b . N_A}{\Pi . M_N . n_N . d^2 . c_{particle}}$$

where ΔTN_b corresponds to the difference in the TN_b concentration in the samples with and without peptide, N_A stands for the Avogadro constant and M_N, n_N, d and $c_{particle}$ respectively represent the molar mass of nitrogen, the number of nitrogen atoms per peptide molecule, the bead diameter and the particle concentration of the beads.

Hence, we estimate a σ_{pept} on the carboxylic acid coated Dynabeads of 11.2 peptide equivalents per nm^2. This very high value may indicate, besides an immobilization, a polymerization of the peptide, which could confirm our previously suggested explanation.

Conclusions

In this work, we used a multi N/C 2100S (Analytik Jena GmbH) to investigate the chemical immobilization of the REE phosphor binding peptide FL464 onto M-270 amine and carboxylic acid coated Dynabeads by TN_b analysis. We found that the incubation with the peptide not significantly change the TN_b concentration of the samples with amine coated Dynabeads ($p = 0.25$), whereas it very significantly increased the TN_b concentration of carboxylic acid coated Dynabeads ($p = 0.0033$), from which we could estimate $\sigma_{pept} = 11.2$ peptide equivalents.nm^2. These results are well in line with our previous findings [6]. More generally, the high reproducibility, the quantification limit in the < µg.L^{-1} range and the sample volume in the mL range, could make the presented method an interesting tool for various peptide-related analyses.

References

1. European Commission, Study on the EU's list of Critical Raw Materials - Final Report (2020).
2. Patil, A.B., Paetzel, V., Struis, R.P.W.J., Ludwig, C. *Separations* **9** (2022), https://doi.org/10.3390/separations9030056
3. Binnemans, K., Jones, P. *Journal of Rare Earths* **32**, 195-200 (2014), https://doi.org/10.1016/S1002-0721(14)60051-X
4. Lederer, F., Curtis, S., Bachmann, S., Dunbar, S., MacGillivray, R. *Biotechnol. Bioeng.* **114**, (2016), https://doi.org/10.1002/bit.26240
5. Schrader, M., Bobeth, C., Lederer, F. *ACS Omega XXXX*, (2021), https://doi.org/10.1021/acsomega.1c04343
6. Boelens, P., Bobeth, C., Hinman, N., Weiss, S., Zhou, S., Vogel, M., Drobot, B., Azzam, S.S.A., Pollmann, K., Lederer, F. *J. Magn. Magn. Mater.* 169956 (2022), https://doi.org/10.1016/j.jmmm.2022.169956