
Ion-pairing separation of bioactive peptides using an aqueous/octan-1-ol micro-extraction system from bovine haemoglobin complex hydrolysates

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RÉSUMÉ

Hydrophobic ion pair has been often used for non-aqueous enzyme reactions or enhanced protein stability in organic solvent. Major advantage of hydrophobic ion pairing is to increase the lipophilicity of hydrophilic molecules. An other strategy has been also developed to the separation of peptides or proteins by ionic pairing with amphipathic molecules. Hydrophobically modified peptides or proteins allowed the increase of their extraction in organic solvent. From mixtures containing more than hundred different peptides, this strategy appears also a good means to separate peptides by modifying their hydrophobicity thanks to ion pair agent – peptide complex formation and in using liquid/liquid medium to selectively extract some bioactive peptides.

The ion-pair concept was applied on complex haemoglobin hydrolysates to extract two opioid peptides, LVV-haemorphin-7 (LVVh-7) and VV-haemorphin-7 (VVh-7), in an aqueous/octan-1-ol microextraction system in presence of alkyl-sulfonic acid as surfactant agent and in relation to the haemorphin physico-chemical properties (charge, hydrophobicity). LVVh-7 and VVh-7 correspond respectively to residues 31-40 (Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe) and 32-40 (Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe) of bovine haemoglobin β -chain.

The effect of combined alkyl chain length/aqueous phase pH and the haem behaviour during the extraction, on the haemorphin recovery yield and enrichment have been determined. It has proved that transport over the organic phase is mediated by the alkyl-sulfonic acids, whatever the aqueous phase pH. However, increasing both the alkyl chain length and the pH in the aqueous

phase shows an haemorphin enrichment ratio increase but a recovery decrease of the extracted opioid peptides in the organic phase. Therefore, best conditions to extract LVVh-7 and VVh-7 are respectively the use of the octanesulfonic acid at aqueous phase pH of 5 or 7 and the octane or the heptane-sulfonic acid with an aqueous phase pH of 5 or 7. In these conditions, a partition coefficient of 1.64 and 1.60 for respectively LVVh-7 and VVh-7 are obtained and represents about 40 times that acquired without agent.

MOTS-CLÉS : Ion-pair micro-extraction, Liquid/liquid biphasic system, Haemoglobin, Peptides, Haemorphins