Molecular Modeling of Peptidomimetics: An Approach For The Design of Innovative Skin Care Products

Jean-Francois Nicolay, Ph.D., and Patrick Lafitte

Exsymol S.A.M., 4 Avenue Albert II, MC-98000, Monaco

Introduction

Nowadays, active ingredient suppliers are requested to display comprehensive data about the aftermath of their compounds following application on the surface of the skin. Percutaneous absorption, for instance, must be compatible with the claimed activity. It is necessary to determine the "bioavailability" of the active ingredient, e.g. to provide evidence that the compound can reach its target within cutaneous tissue. The design of innovative *in vivo*-effective skin care products must now take into account a number of parameters that dictate bioavailability (Figure 1). These parameters also bring some insight into possible systemic exposure, useful for risk assessment. :

- The ability to cross the stratum corneum, and to reach the inner layers of the epidermis (penetration)
- The capacity to permeate, e.g. to leave the epidermal compartment, and reach the dermis
- The sensitivity to intra or extra cellular cutaneous enzymes (metabolization)
- The possible uptake of the substance into the vascular system (resorption)

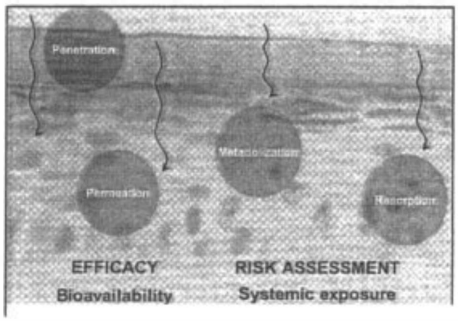

Figure 1: parameters modulating percutaneous absorption and systemic distribution of skin care products

The design of peptide-based bioactive ingredients for skin care is difficult, since peptides generally have a very low bioavailability. This is due to their high hydrophilicity that opposes penetration, and also to their sensitivity to cutaneous proteolytic enzymes, which rapidly hydrolyze them (metabolization) into inactive fragments. Structural modification of peptides (synthesis of peptidomimetics structurally related to the parent peptide), is needed to limit the sensitivity to proteolytic enzymes and to enhance lipophilicity. The design of suitable chemical modification, e.g. the molecular modeling of peptidomimetics, requires specific approaches.

Methods for molecular modeling of peptidomimetics

Molecular modeling for optimization of the bioavailability of peptidomimetics was achieved using several tools:

Molecular modeling software, widely used in medicinal chemistry, can predict a number of physicochemical properties on the basis of the molecular formula. For instance, they display ionization constants, water-solubility estimation, and a predictive partition coefficient. Log P, the logarithm of the partition coefficient, or Log D the pH-dependent coefficient for ionizable compounds, are important tools for estimation of percutaneous absorption since it is generally accepted that the stratum corneum, considered as a globally lipophilic layer, predominantly controls penetration.

Standardized human reconstituted epidermis, an easy-to-handle "3-D" *in vitro* system, is suitable for the rapid screening of cutaneous penetration. Reconstituted epidermis obtained from normal keratinocytes grown at the air-liquid interface have a well characterized barrier function, close to normal skin [1]. They can be mounted on diffusion systems for the determination of the total mass balance (non-diffusible material/trans-epidermal diffusible material/intra-epidermal material). Study of the distribution within the thickness of the epidermis is also possible by immunohistolocalization.

Multi-enzymatic mixtures enable the "modelization" of biotransformations that may occur upon percutaneous transport. The peptidomimetics may be exposed to microsomial fractions obtained from cutaneous cells, or to standardized liver homogenates (S9 fractions). Metabolites resulting from biotransformation can be identified by HPLC monitoring of the reaction mixtures. In order to better identify the benefits obtained from the structural modification, we have also carried out a substrate competition test [2].

Examples of molecular design

The methodology was applied to the synthesis of a bioactive structural analogue of L-carnosine, a natural dipeptide (figure 2) capable of preventing lipid peroxydation *in vitro* [3]. The bioavailability of L-carnosine is very low, due to its high polarity (Log D at physiological pH is –5,8), and sensitivity to cutaneous hydrolases including the specific ubiquitous dipeptidase carnosinase [4]. We chose to remove the alpha-carboxylic function (figure 2, in red) as a minimum structural modification (original biological properties must remain), resulting in increased lipophilicity, molecular weight decrease (favorable to percutaneous adsorption), and anticipated high resistance to proteolytic enzymes. In addition, decarboxylated carnosine has been identified *in vivo* as a minor metabolite of carnosine (named carcinine) [5]; this was regarded as a positive feature for tolerance.

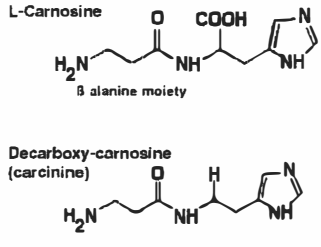

Figure 2 : chemical structures of the natural peptide L-carnosine, and its peptidomimetics

Predicted Log D (–3,3 at physiological pH), outlined that withdrawal of the negatively charged carboxylic acid moiety may result in a 100 fold improvement of the penetration. Still, the compound remains strongly hydrophilic (negative Log D). The "substrate competition test" has shown that unmodified dipeptides, even with a ß-aminoacid moiety, have a short half-life when applied to the skin. As expected, decarboxylation results in a strong resistance to cutaneous hydrolytic enzymes. Finally, a diffusion study on reconstituted epidermis indicated that the peptidomimetic can cross the stratum corneum (approx. 35% after 24 hours). These predictive experiments were confirmed later with an *ex vivo* permeation study.

Another application was the design of chemically modified arginine-containing dipeptides (Figure 3). Chemical modifications were made in an attempt to limit their enzymatic hydrolysis in upper layers of the epidermis. N-acetylation of the naturally occurring dipeptide citrullyl-arginine isolated from the red alga chondrus crispus (figure 3, compound A) resulted in unexpectedly high resistance to enzymatic hydrolysis. This chemical modification has provided some limited benefits for polarity, as predicted by our modeling software.

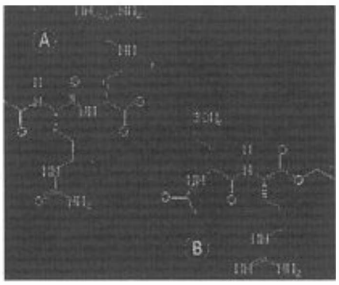

Figure 3 : chemical structures of chemically modified arginine-containing dipeptides.

The antioxidant dipeptide N-acetyl-(D,L)-methionyl-L-arginine ethylester (figure 3, compound B) was designed for the sustained release of L-arginine within the deep layers of the epidermis. Chemical modification of two of the three ionizable functions of the molecule enables to improve the lipophilicity by 100 fold compared to the unmodified peptide. We have shown that the modifications also delay the hydrolysis of the peptide via a mixture of enzymes. HPLC monitoring of the enzymatic mixture revealed that L-arginine was released at a measurable rate.

References

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