ASCORBOSILANE C
AND MELANOGENESIS
- T2 -

ASCORBOSILANE C

MELANOGENESIS INHIBITION TEST

INTRODUCTION

In order to prove the Ascorbosilane C effect (Ascorbate Methylsilanol) on melanin synthesis, we developed a colorimetric method to quantify it.

BIOLOGICAL DATAS

1) Melanogenesis biology

For mammals, the pigmentary system is the result of the secretary activity of highly specialized cells coming from neuro-ectodermic origin, i.e. Melanocytes. These cells are mainly localized in the dermic and epidermic junction, on the upper part of the hair bulb and in choroid. They are ejecting their numerous dendrites in the neighbouring cells. Formation of the pigment is only perfected in one cytoplasmic organoid called melanosome, which contains the needed enzymes (tyrosinase, dopa-oxydase and perhaps also peroxidase) to catalyse tyrosine oxidation into dopaquinone (Figure 1).

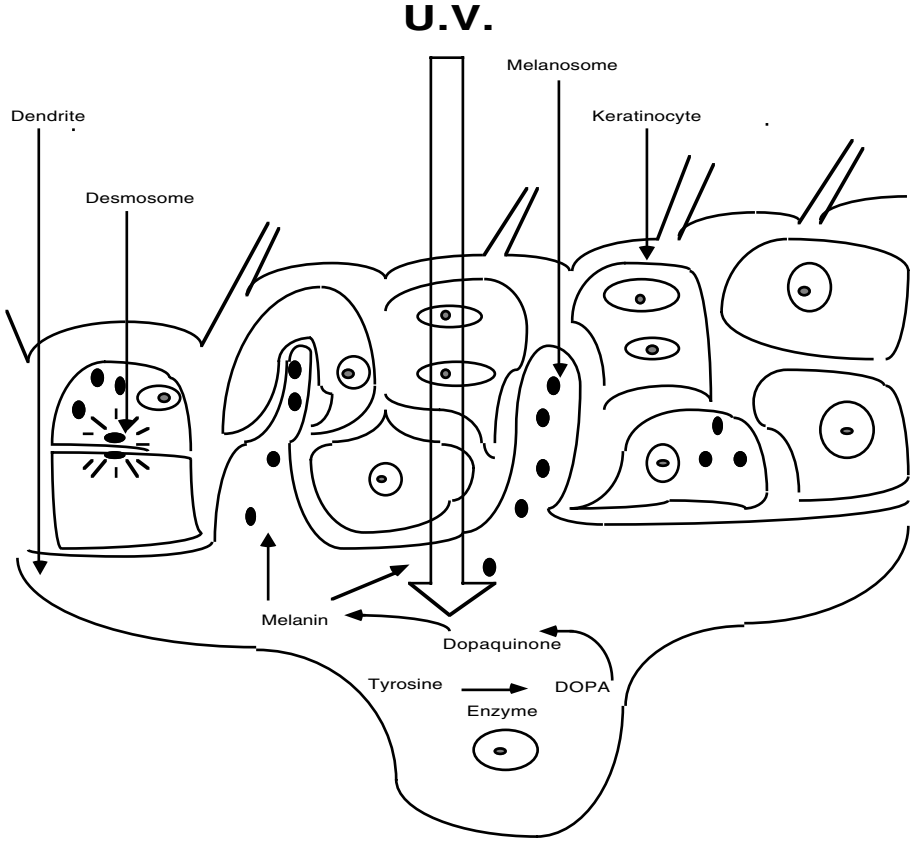

Figure 1 : Epidermic melanocyte unit

Whereas melanocytes are pigment synthesis organs, the surrounding keratinocytes are the means to get pigments in epiderm and in the hair.

After they are transferred to the externe part of the melanocyte dendrites by phagocytosis, melanosomes disperse in the keratinocyte's cytoplasm. The pigment which has been released in keratinocytes distributes in the environment of the nuclus to protect the genome from the bad effects of UV radiations;

2) Melanogenesis biochemistry

Studies of Raper (1928) and Mason (1948) have shown that tyrosinases catalyse the modification from tyrosine into dopa and into melanin according to the following reactions :

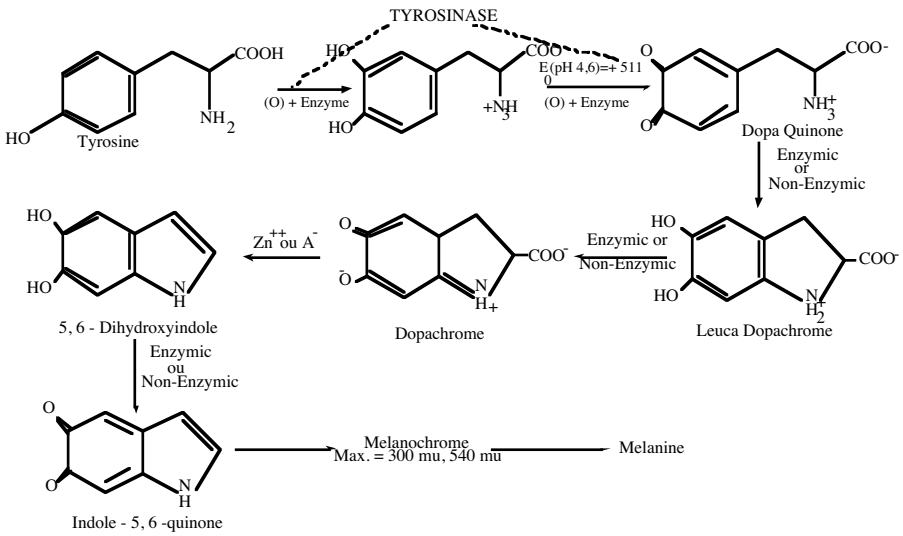

Figure 2 : Enzymatic oxidation of the tyrosine into melanin

Tyrosinase belongs to an important group family, the copper-dependent oxydases. It catalyses the oxidation of mono- and o-dihydroxyphenols into o-quinones. This enzyme is activated when the copper ion is in a +1 oxidation state ; when the ion is in a +2 oxidation state, it is not active. The first step considering tyrosinase activation is the reduction of the enzyme from +2 to +1 copper state;

Searching for a product with an inhibitory activity for melanogenesis lead us to establish in vitro the melanin synthesis. Ascorbic acid is widely used in cosmetology for its whitening activity. Taking as a basis this property, we tried to evaluate the inhibitory effect of a specific ascorbate : the ascorbate methysilanol, on melanin formation, reaction which is catalised by the tyrosinase.

EXPERIMENTATION

1) Chemical products

- "Soluene 100", 0,5 N solution of dimenthyl n-undecyl n-dodecyl ammonium in toluene (Packard Instrument Co).

- Tyrosinase, grade III, melanin, L-tyrosine, L-3, 4-dihydroxy-phenylalanine (dopa) (sigma).

- Ascorbosilane C : methysilanetriol ascorbate solution dosed at 0,63 % in ascorbic acid.

2) Principle :

47 mg of a mixture of 1-tyrosine and 1,25 ml of tyrosinase (25,000 U/ml) in 20 ml of 0,1 M (pH 6,8) phospate buffer was incubated during 4 hours at 37°C under shaking and one night at room temperature. The black pigment is precipitated with 1 ml of concentrated HC1, 6N, for 48 hours. The unsoluble pigment is washed, dried, then solubilized in " Soluene 100 ".

The preparations are suspended in an appropriate " Soluene 100 " volume then submitted to ultra-sounds for 2 minutes (Oikawa, 1973).

The absorption of the melanin solution is read at 400 nm, its concentration is defined compared to a standard reference.

The ascorbate methysilanol (Ascorbosilane C) is introduced in the incubation medium at different concentrations : 0,1 %, 0,5 %, 3 %, 5 % and 10 %, corresponding to the concentrations which are given in the following table :

	As 0,1 %	As 0,5 %	As 3 %	As 5 %	As 10 %
Ascorbic. Ac. mg/l	6,3	31,5	189	315	630
Silicon mg/l	1	5	30	50	100

RESULTS AND DISCUSSION

The proteine-free melanin which is synthesized in vitro, is solubilized in a tissue solubilizer called " Soluene 100 ", 0,5 N dimethyl n-undecyl n-dodecyl ammonium in toluene. Using this solubilization procedure, the melanin content is evaluated by spectrophotometry, its absorption is defined at 400 nm.

Figure 3-4, show that melanin synthesis in the presence of Ascorbosilane C is linked to its synthesis concentration. As a matter of fact, according to the Ascorbosilane C concentration, we observe a stimulation, then an inhibition of the *in vitro* melanogenesis.

Results on the free silanol form, which means not linked to ascorbic acid, show that the effect on melanogenesis are due to the ascorbic acid part of the molecule. As a matter of fact, the silanol itself does not contribute to an inhibition nor to an activation considering melanogenesis, at the tested concentrations.

The melanin synthesis starts with a tyrosine and dopa oxidation ; these reactions need oxygen and the activity of an enzyme, the Tyrosinase. (Figure 1). The tyrosinase is copper dependent, which means that it is active when the copper ion is in a +1 oxidation state, and inactive in a +2 oxidation state.

At low Ascorbosilane C concentrations, like 0,1 % and 0,5 %, melanogesis is respectively stimulated from 180 % to 80 %. This stimulative activity on melanogenesis activity can be related to the ascorbic acid, which is a cofactor of the enzyme, helping copper to stay in a +1 oxidation state, the active form for tyrosinase. (Cort, 1982).

At high Ascorbosilane C concentrations, there is a melanogenesis inhibition. This activity can be due to the anti-oxidant part of the ascorbic acid. When Ascorbosilane C concentration, which means ascorbic acid concentration is high, the proportion of trapped oxygen is so important, that the tyrosine and dopa hydroxylation cannot be complete. This is compatible with the publications relating to a prooxidant effect of the ascorbic acid at very low concentrations (Symposium on oxidants and anti-oxidants) and was confirmed by comparable results on extemporaneous ascorbic acid, in order to avoid its oxidation. The Ascorbosilane C form is, considering the in vitro melanogenesis test, at the tested concentrations, a stabilized form of ascorbic acid.

As a conclusion, the ascorbic acid at low concentrations, react more easily with copper, thus stimulates melanogenesis. Whereas when there is some in excess, its reactivity with oxygen avoids the oxidative reaction needed for melanin synthesis. This inhibitory property on melanogenesis was proved with an Ascorbosilane, Concentrated in Ascorbic acid.

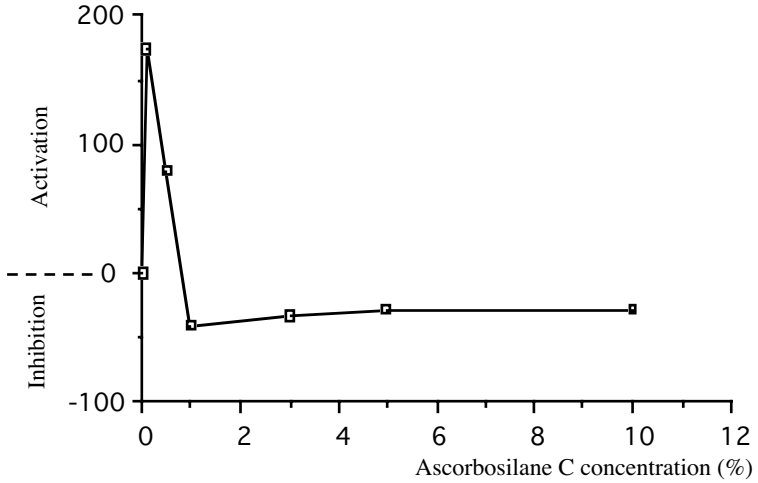

Figure 3 : Ascorbosilane C activity towards melanin synthesis, according to its concentration

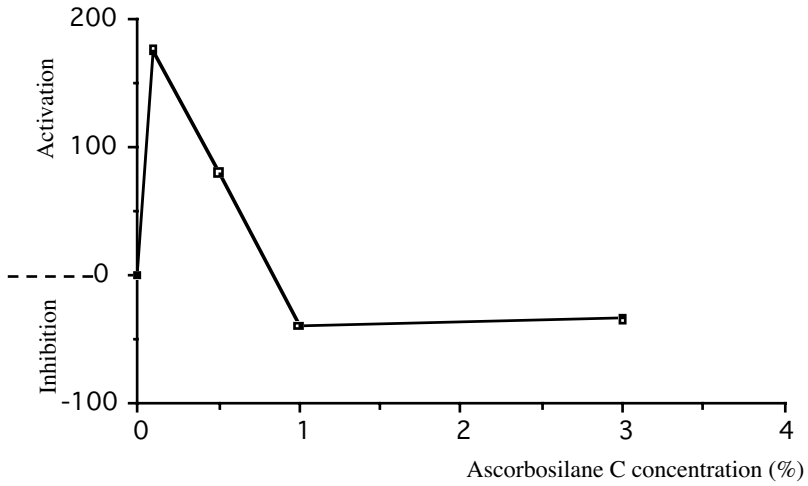

Figure 4 : Magnification of figure 3

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