THE EFFECT OF UV LIGHT ON PIGMENTED IN BIOLOGICAL SYSTEMS AND THE ROLE OF PIGMENT

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The accumulation kinetics of free – Radicals (FRs) in white and pigmented keratin arc determined by UV light and arc found to obey the same law. The ESR signal in pigmented keratin has two components. These are the signals appearing in white wool due to pigment and UV light. For both $g=m$ 2.0054 and H_m = 0.6- 0.8mT for the central line.

We conclude that the events in melanin can be explained by the theory of Brillouin zones and pigments save the biological systems from the effect of IV light by behaving like a trap for FRs

1. Introduction

In previous publications the role of pigment epithelia was clearly studied (l)-(3) and it was found that the natural pigment in biological systems is more active hen being only a passive screen. However, these researches are not complete for the blowing reasons: 1) the suggested mechanism was not tested for other biological systems having pigment; 2) the role of pigment in FR processes was not it tidied, that is TR processes were not analyzed in systems having different degrees of pigmentation. The Lest system to lie used for these two purposes is the wool keratin which has a different pigment (t. e. different color). The FR processes funned by the effect, of UV radiation has been explained for white keratin [4]. However, we should make some more points clear and solve some other problems For example, in FR processes, the function of the sulphur atoms has not yet been understood (as is known, the amount of the amount of sulphur in keratin is 12% cystein + cystin). For this purpose although the method of recombination-kinetics, for some proteins, has been suggested (5)-(9) in this paper we prefer to me the accumulation and annihilation reactions of FR formed in wool keratin having various degrees of pigmentation due to the UV radiation effects.

2. Materials and methods

Keratin was taken up from the wool of white (albino) rabbits and sheep with van oust colors. The wool was cleaned by stirring it slowly in a chloroform-methilalcohol mixture (in a ratio 2 to 1) for 6 hours. Later the wool was dried at room temperature. Two different kinds of samples were used: wet and dry ones. We placed the wet samples in a parts ampoule and poured distilled water. The dry samples having a mass of 4-5gr were placed in a quartz ampoule having an inker diameter of 2mm. The air was taken out under a pressure of 1.3N/m during 30 minutes and the basin was closed.

The samples were irradiated through a glass ray filter UFS 2. a ray filter of thickness o cm and a high pressure Hg tube of power 500 Watt. First, at 77 K. the ESR spectrum of wool keratin subjected to UV was recorded and later the sample being left at different temperatures during 5 minutes intervals was radiated again and measured. The process was repealed 8 times.

3. Discussion

As in (4). a triplet signal for white wool keratin was observed in the dry samples and the extreme components belonged to FR. As the temperature increased, the integral intensity of the signal increased. This might be due to either the recombination of FR or the migration of free electrons. From the kinetics we saw that the total concentration of FR was proportional to temperature (fig. 1) and two kinds (fast and slow) of reactions occurred. The same phenomenon happened in silk fibroin and college (9)-(10). An Increase in temperature lowered the relative concentration of FR. (Fig.1): while in the fast and slow reactions it increased the concentration of FR respectively. In the interval between 333 and 393 K the concentration of paramagnetic centers increased and the concentration of FR in slow reactions remained constant (30 %).

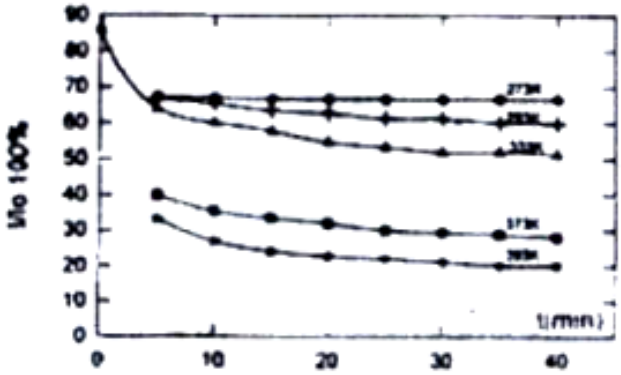
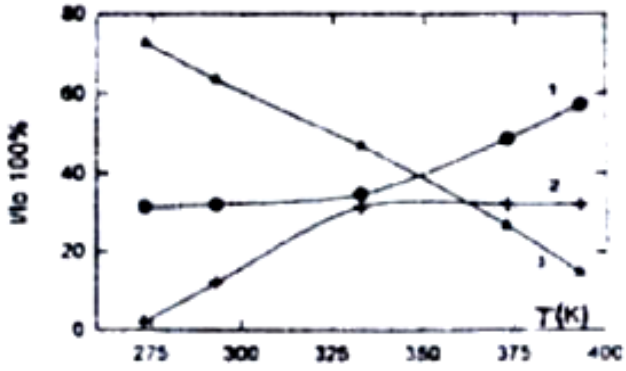


Fig.1. The recombination kinetic corves of FR, formed by the effect of UV radiation, in while wool keratin. 1 and 1 are the integral intensities of ESR spectrum in the standard and experimental samples, respectively.

In the wet samples, the change of the thermal reactions of FR, were the same as in the dry samples but 20% more FRs were added in the reactions. However, the recombination rate of FRs became lower and the reactions stopped at lower temperatures. Wetting might increase the portion of regular regions in proteins.

Using the kinetic proportionality in fig 1 all the basic beat treatment curves are plotted in fig.2. The treatment was initiated at temperatures below 273K and completed at 423K. In the dry samples, the slow reaction was initiated at 273K and completed at 333K (fig.3 (b)). In these samples the amount of the slow reactions increased up to 333K with a percentage of 30±3 then it stayed constant as the temperature increased. The fast reaction increased starting from 273K and was completed at 393K. Thus in the wool keratin the thermal treatment of FRs formed by the effect of UV, occurred at temperatures in a wide range (fig.2). Comparison of our

experimental results with those for globular and other fibroin proteins, yields: 1) The FRs in keratin, subjected to the effect of UV, have two recombination reactions. 2) This can be explained by the existence of regular and irregular regions, as well, including the a-spiral. The FR reactions in irregular regions completed at 393K in the dry samples whereas in the wet samples it completed at 243K. The total reactions of FR forming in the regular regions occur at 393K in the dry samples and at 273K in the wet samples. In the slow recombination, the amount of FR is 30%. The amount of the peptide chains in the wool keratin not belonging to the *a*-structure is 30%. The remaining 70% expresses the amount of the peptide chains belonging to the Q - structure. A highly intense singlet signal, with g=2.0054 and H_m = 0.6-0.8mT, is observed for wool keratin having various degrees of pigmentation in the absence of UV effect. The amount of spins (unpaired electrons) increases as the degree of pigmentation increases. The ratio of the high spin concentration showed a 10 fold increase, from white to black wool.



Fig,.2. At 77 K, in white wool keratin, the initial amount of FR formed by UV radiation is proportional to temperature:: 1 - stable FR, 2 - FR in slow reaction, 3 - fast reaction.

This ratio might be different for different wool. In our previous investigations, we stated two mechanisms for the formation of the signal in the biological systems: 1) "Developed Zone Theory", restricted for amorphous semiconductors, might be applied to melanines. For the unpaired electrons near the valance and conduction band boundaries, the theory of zone to zone transition is valid. 2) It is assumed that as a result of the absorption of light quanta, FRs form in monomer unit of keratins. Thus, melanin (pigment) having both too many FRs and electron traps protects the biological systems against photochemical destruction. In addition, melanin plays the role of deactivator for the chemical protector. Although the FR concentration belonging to melanin increases with the amount of pigment in wool keratin, the amount of FR emerging due to the UV - radiation and its speed of formation decrease efficiently. In fig. 3, the accumulation kinetics of FRs by the effect of UV in both white (1) and black (2) wool keratin and the increasing curve belonging to the pigment, are shown. From this we end up with the following two conclusions: a) The signal of the pigment and the kinetics of FR accumulation with UV in keratin form through a two-stage process in all proteins, that is, each of the paramagnetic centers is spread throughout the crystal - like and amorphous regimes of the biopolymer. In this case, it is possible, using recombination- kinetic method, to determine the amounts of the polymer chains both belonging and not belonging to spiral, h) As seen from fig. 3. the accumulation speed of FR in the pigment keratin is as low as it can be. According to the calculations, passing from the low pigment keratin to the high pigment one decreases this speed to its half. This event proves the protective role of pigment for electrons in biological systems.

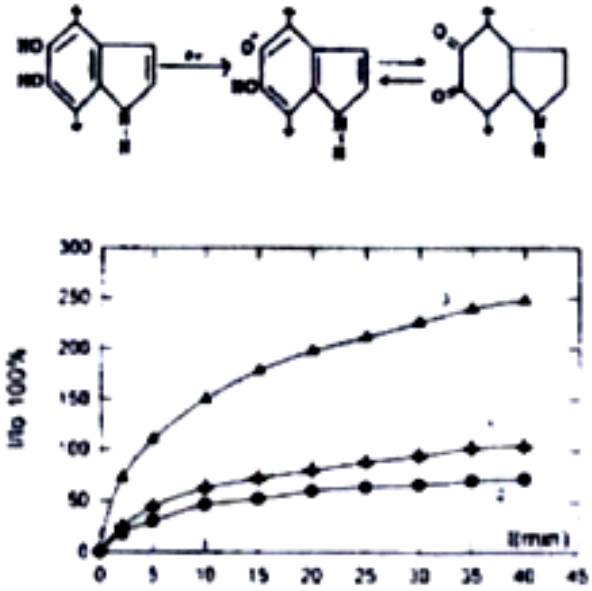


Fig.3. The accumulation kinetics of FR in pigmented wool keratin: 1 - while wool. 2-black wool formed by UV, 3 - paramagnetic renters of pigment.

The intensity of the ESR signal can determine the amount of the pigment in biological systems. But, in the wool samples containing man-made dyes, an ESR signal which is not proportional to the degree of pigmentation is observed. In this case, by the effect of UV we find out that the accumulation-recombination kinetics of FR is similar to that in white wool. Thus, we conclude that man-made dyes only play the role of screening the pigment but never play the biological protective role.

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BİOLOJİ SİSTEMLƏRDƏ UB ŞÜALARIN PİQMENTƏ TƏSİRİ VƏ PİQMENTİN ROLU

UB şüanın təsiri ilə ağ və rəngli yun keratinində sərbəst radikalların toplanma kinetikası eyni qanuna əsaslanır.

Rəngli keratində UB şüanın təsiri ilə alınan EPR siqnalı iki komponentli g=2.0054 və ΔH_m=0.6-0.8mT olan mərkəzi siqnaldan ibarətdir. Belə nəticə çıxarılır ki, melanində baş verilənlər Brillion zonalar nəzəriyyəsi ilə izah oluna bilər və bioloji sistemlərdə piqment UB şüalanmanın təsirini azaldır və köhnəlmədən qoruyur.

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ВОЗДЕЙСТВИЕ УФ СВЕТА НА ПИГМЕНТ В БИОЛОГИЧЕСКИХ СИСТЕМАХ И РОЛЬ ПИГМЕНТА

Накапливание кинетики свободных радикалов (СР) в белом и пигментном кератине шерсти определяется УФ светом и основывается на подчинении тому же закону. В пигментном кератине ЕПР сигнал имеет два компонента: g=2.0054 и ΔH=0.6-0.8mT для центральной линии. Мы сделали вывод, что происходящее в меланине может быть объяснено теорией зоны Брюллиэна и пигментов, спасающих биологические системы от воздействия УФ света, ведущие себя подобно ловушке для СР.

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