FLUORESCENCE OF ACRIDINE ORANGE COMPLEXES WITH BASES AND NUCLEOTIDES

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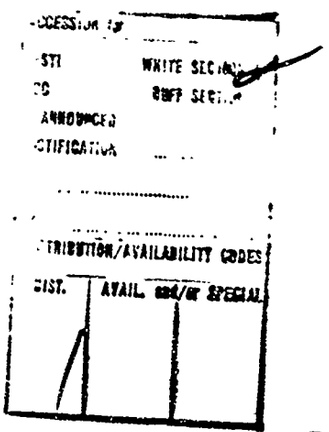

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FLUORESCENCE OF ACRIDINE ORANGE COMPLEXES WITH BASES AND NUCLEOTIDES

[Following is the translation of an article by L. Ye. Minchenkova and L. A. Tumerman, Institute of Radiation and Physico-Chemical Biology, AN, USSR, published in the Russian-language periodical Biofizika (Biophysics), Vol X, 1965, No 4, pages 696--698. It was submitted on 4 Jan 1965, Translation performed by Sp/7 Charles T. Ostertag, Jr.]

It was shown in the work /1/ that the fluorescence of complexes of acridine orange dye (AO) with a double strand molecule of DNA is different from the luminescence of solutions of this dye in the following relationships.

1. The quantum output of fluorescence (η) and its duration (τ) for the complexes is 2.5 times greater than for free molecules in a solution.

2. The spectral position of the band of fluorescence does not change (in both cases the maximum is at 530 mμ), but the band of fluorescence of the complexes is somewhat narrowed, mainly due to a steeper drop in intensity in the long-wave section of the spectrum.

3. The maximum of the absorption band, located for solutions at 492 mμ, is shifted for the complexes to 502 mμ; simultaneously in the absorption spectrum for complexes the shoulder disappears at 464 mμ, which is characteristic for dimers of AO which are formed in the solution.

The last two points testify to the fact that during combination with native DNA the dye preserves a monomeric form, in spite of the exceedingly high local concentration of it in the space occupied by the DNA. This may be explained by the fact that, as Lerman demonstrated /2/, following combination with the double strand molecule of DNA, molecules of AO are encountered between the "bridges" of complementary pairs of bases, being oriented parallel to them.

The parallelism between the changes in the values of η and τ, taking place with the formation of complexes of AO with DNA, testifies to the fact that during combination there is a lessening of the probability of heat dissipation of energy from the electronic excitation of the AO molecule during the time of its stay in the excited condition. Apparently this is the result of some specific interaction between the electronic systems of the dye and of the bases of DNA. It is possible that such an interaction plays a role in the specific bacteriostatic and mutagenic action of AO. Therefore, there is interest in clearing up whether this interaction takes place with all four nitrogen bases of DNA or just with some of them. →

For this purpose an investigation was made of the fluorescence of solutions of AO, to which we added adenine, dimethyladenine, adenosine, adenosine triphosphate, thymine, uracil and cytosine, and also a purine compound -- caffeine. The concentration of these substances in solution ranged within the limits of 10^{-5} up to 10^{-1} M with a constant concentration of AO (10^{-5} M).

It turned out that with a sufficiently high concentration of adenine, dimethyladenine, adenosine and ATP the same changes of fluorescence are observed as during the combining of AO with native DNA, that is, an increase by 2.5 times in the duration of fluorescence (figure 1) and its quantum output, and also a narrowing of the spectral band of luminescence without a change of its spectral position, a shift of the maximum of absorption to 502 mμ and the disappearance of the shoulder at 464 mμ in the absorption spectrum. On the other hand the addition of pyrimidine bases had practically no influence on the absorption and fluorescence of AO. Thus, it can be considered that the specific interaction which we spoke about above takes place between AO and adenine and does not take place with pyrimidine bases. We were not able to set up direct tests with guanine due to its poor solubility, but the fact that precisely such an interaction takes place between AO and caffeine (figure 1, curve 3) makes it possible to think that during combination with DNA the molecules of AO interact with both purine bases.

In connection with this it is interesting to note that Weber /3/ observed the quenching of the fluorescence of riboflavine following the addition to the solution of caffeine, while this quenching was not accompanied by a change of τ (extinguishing of the first type according to Vavilov). We confirmed these observations and showed that the fluorescence of riboflavine is also quenched by adenine. Thus, the influence of the combination of various dyes with purines on the characteristics of luminescence of these dyes may be very diverse. It is possible that this is connected with differences in the donor-acceptor electronic relationships between the components of the pair.

The fact that following the addition of pyrimidine bases to solutions of AO changes are not observed in the fluorescence and absorption of the dye may be explained by the fact that in general the formation of complexes of these compounds does not take place, or by the fact that though complexes are formed, that specific interaction between the electronic systems of the dye and bases is lacking. The described changes are the result of this. Measurements of the polarization of fluorescence of AO in solutions (all polarization measurements were carried out in 50% saccharose) with the addition of adenine and pyrimidine bases show that the first explanation is correct. In actuality, as is seen from figure 2, the degree of polarization of fluorescence of AO and the time of relaxation of Brownian rotational movement ρ, calculated from these measurements according to the known formula of Perrin-Vavilov

$$1/\rho - 1/3 = (1/\rho_0 - 1/3)(1 + 3\tau/\rho)$$

does not change following the addition of even very high concentrations of pyrimidine bases, whereas the addition of adenine leads to a significant (3 times) increase of p.

It is important to note that following the combination of AO with native DNA the complete binding of the dye takes place already under conditions when one molecule of dye falls on several nucleotides, whereas in our case, a noticeable shift of equilibrium between the free and bound molecules to the side of the latter takes place only with such a high concentration of purines that on one molecule of AO several hundreds or thousands of molecules of purine fall. This is probably explained by the fact that the energy of the bond of AO with bases which are included in the composition of DNA is significantly greater than with free bases in solution. However, the possibility is not excluded that in solution molecules of AO combine only with "stacks" made up of several molecules of adenine and caffeine, which, according to the data of Helmkamp /4/, are formed with a sufficiently high concentration of these substances.

Literature

1. Borisova, O. F., Tumerman, L. A., Biofizika, 9, 537, 1964.

2. Lerman, L.S., J. Mol. Biol., 3, 18, 1961.

3. Weber, G., Biochem.J., 145, 51, 1952.

4. Helmkamp, G., Ts'o P., Symposium physikalische Chemie biogener Makromolekule. Jena, 1963.

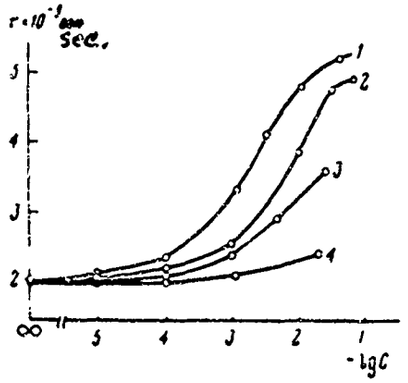

Figure 1. Dependency of the duration of fluorescence (τ) of acridine orange on the concentration of additive substances. 1 - dimethyladenine; 2 - adenine; 3 - caffeine; 4 - thymine.

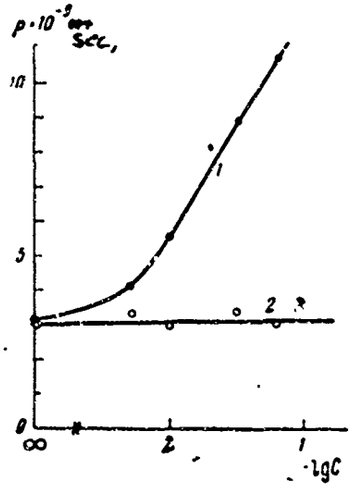

Figure 2. Relaxation time (ρ) for acridine orange depending on the concentration. 1 - adenosine triphosphate; 2 - thymine.