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Binding of a Tryptophan-Containing Peptide (Lysyltryptophyllysine) to Deoxyribonucleic Acid Modified by 2-(*N*-Acetoxyacetylamino)fluorene†

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ABSTRACT: The binding of the tripeptide Lys-Trp-Lys to DNA modified by reaction with the chemical carcinogen 2-(*N*-acetoxyacetylamino)fluorene (AAAF) has been investigated by fluorescence spectroscopy. A quenching of tryptophan fluorescence was observed which increased when the degree of base substitution by AAAF increased. Similar results were obtained with the 7-iodo derivative of AAAF (AAAIF). Two hypotheses are discussed which could account for the experimental results: (1) stacking interactions of the tryptophyl residue of the peptide with nucleic acid bases in locally unpaired regions in the vicinity of modified bases; (2) energy transfer from the tryptophyl residue of the peptide to acetylaminofluorene bound to guanine bases without direct interaction of this residue with nucleic acid bases (outside binding). The results obtained with denatured DNA in the absence and the presence of chemical modifications by AAAF or AAAIF allow us to conclude that energy transfer contributes to fluorescence quenching in the case of AAIF but not in that of AAF. Stacking interactions are therefore responsible for fluorescence quenching of Lys-Trp-Lys when bound to AAF-modified DNA. In the case of DNA-AAIF, fluorescence quenching is due both to energy transfer and to stacking of the tryptophan ring with bases inside the helix. These results are discussed in relation to what is already known in terms of local structure and with respect to the role that could be played by aromatic residues of proteins in the recognition of chemically damaged DNA.

The binding of peptides containing tryptophan and basic residues (such as Lys-Trp-Lys) to nucleic acids has been shown to involve two types of complexes according to the scheme (Brun et al., 1975; Toulmé & Hélène, 1977; Maurizot et al., 1978)

$$\text{nucleic acid} + \text{peptide} \underset{K_1}{\rightleftharpoons} \text{complex I} \underset{K_2}{\rightleftharpoons} \text{complex II} \quad (1)$$

Electrostatic interactions between lysyl side chains and phosphate groups are involved in both complexes. In complex I the aromatic amino acid side chain does not interact with nucleic bases while stacking interactions take place in complex II (Dimicoli & Hélène, 1974a,b). The formation of complex II is strongly favored if the nucleic acid is single stranded (K_2 is much higher for single strands than for double strands) (Toulmé et al., 1974; Toulmé & Hélène, 1977).

If native DNA is submitted to UV irradiation, several damages occur, among which pyrimidine dimers have the more important biological role. The DNA region around such defects has an altered conformation. Hydrogen bonds are disrupted, leading to locally opened structures. It was previously shown that the tryptophyl residue of Lys-Trp-Lys was preferentially stacked with bases in these single-stranded regions (Toulmé et al., 1974; Toulmé & Hélène, 1977).

Chemical reaction of DNA bases with metabolites of strong carcinogenic compounds also leads to altered regions in the DNA double helix [for a review see Daune & Fuchs (1977)].

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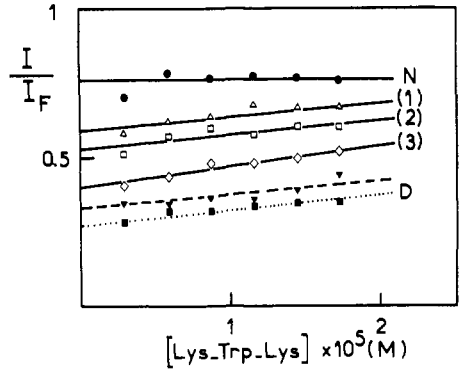

FIGURE 1: Relative fluorescence intensity of Lys-Trp-Lys vs. concentration of Lys-Trp-Lys in the presence of 5×10^{-4} M DNA: N is native DNA; D is heat-denatured DNA. Curves 1, 2, and 3 correspond to DNA modified by AAAF, the percentage of base modification being, respectively, 0.41, 1.05, and 2.9%. Filled triangles (broken line) correspond to DNA modified by AAAIF (4% of modified bases). All experimental points are corrected for the screening effect of DNA (I_F refers to the fluorescence intensity of the free peptide).

Reaction of 2-(*N*-acetoxyacetylamino)fluorene (AAAF) with DNA yields several adducts, the major product having the 2-(*N*-acetylamino)fluorene ring attached to the C(8) position of guanine (Kriek et al., 1967). Physicochemical studies have shown that such a modified DNA contains unpaired regions surrounding the chemically modified guanines (Fuchs & Daune, 1972). In order to determine whether these altered regions in DNA could allow preferential stacking interactions of aromatic amino acids, we have investigated the binding of the oligopeptide Lys-Trp-Lys to DNA which has been reacted with AAAF. Since fluorescence of tryptophan is quenched in stacked complexes with nucleic acid bases, this method was used to follow the binding of Lys-Trp-Lys to native and AAF-substituted DNAs (DNA-AAF). A model has been proposed in which the fluorene ring is inserted between bases in modified regions with the guanine moiety rotated outside (Fuchs & Daune, 1972; Fuchs et al., 1976; Weinstein & Grunberger, 1974). Substitution of the fluorene ring by an iodine atom on position 7 is believed to prevent such an insertion (Fuchs & Daune, 1973; Fuchs et al., 1976; Lefevre et al., 1978). A comparison was therefore made between DNA modified by AAAF and DNA modified by AAAIF.

Materials and Methods

Fluorescence was measured with a Jobin-Yvon spectrofluorometer equipped with a rhodamine B quantum counter to correct for lamp fluctuations. Corrections for the screening effect of DNA on Lys-Trp-Lys fluorescence were made as previously described (Brun et al., 1975). All fluorescence measurements were performed at 2 °C in 1 mM citrate buffer, pH 7.0.

Lys-Trp-Lys was purchased from Schwarz/Mann and used without further purification. Calf thymus DNA modified with AAAF and AAAIF was obtained as previously described (Fuchs et al., 1976).

Results and Discussion

(*1*) *Fluorescence Quenching.* The fluorescence of Lys-Trp-Lys is quenched in the presence of DNA which has been reacted with either AAAF or AAAIF. As shown in Figure 1, for a constant concentration of DNA, the extent of fluorescence quenching increases when the percentage of modified bases increases. In the case of unmodified DNA it has been previously shown that Lys-Trp-Lys fluorescence quenching is due to stacking interactions between the indole ring of the tryptophyl residue and the nucleic acid bases (Brun et al., 1975; Toulmé et al., 1974; Toulmé & Hélène, 1977). In the two-step model proposed earlier (eq 1), complex I is assumed to have the same fluorescence quantum yield (ϕ_F) as the free peptide. The fluorescence quantum yield of complex II is reduced to zero due to the stacking interaction of tryptophan with bases which has been shown to lead to a complete quenching of tryptophan fluorescence (Montenay-Garestier & Hélène, 1971). An analysis of fluorescence data at different DNA concentrations is required in order to determine the association constants K_1 and K_2 of eq 1. Equation 2 was derived under the assumption that the degree of saturation of DNA is very low; i.e., only a small percentage of the phosphates are covered by bound peptides (Brun et al., 1975). This was obtained by using low [peptide]/[DNA] ratios and by extrapolating the plots of I/I_F vs. peptide concentration to zero peptide concentration at constant DNA concentration (I_F and I are the fluorescence intensities of Lys-Trp-Lys in the absence and the presence of DNA; see Figure 1):

$$\frac{\phi_F}{\phi_F - \phi_L} = 1 + \frac{1}{K_2} + \frac{1}{K_1 K_2}\frac{1}{N_0} \qquad (2)$$

ϕ_F is the fluorescence quantum yield of the free peptide and ϕ_L is the overall fluorescence quantum yield of Lys-Trp-Lys in the presence of a given concentration of DNA, extrapolated to zero peptide concentration. N_0 is the initial DNA concentration expressed in moles of phosphate per liter.

In the above model fluorescence quenching is assumed to be due exclusively to the stacking interactions which take place in complex II. If another mechanism leading to fluorescence quenching (e.g., energy transfer, see below) is superimposed on that due to stacking, then a higher apparent value of K_2 will be obtained since part of the peptides in complex I will be counted as complex II. Therefore, in a first analysis values of K_2 obtained according to eq 2 must be considered as reflecting only the ratio of the concentration of peptides which are bound to DNA and whose fluorescence is quenched to that of peptides which are bound to DNA but have the same fluorescence quantum yield as the free peptide. The analysis of K_2 in terms of complexes I and II requires the knowledge of the quenching mechanism(s) (see below).

Results obtained according to eq 2 with native, denatured, and modified DNAs are shown in Figure 2. The extrapolated value of $\phi_F/(\phi_F - \phi_L)$ when $N_0^{-1} \rightarrow 0$ decreases when the percentage of modified guanines increases. Values of K_1 and K_2 deduced from Figure 2 are given in Table I and Figure 3 as a function of the percentage of modified bases.

At the low ionic strength used in these experiments (1 mM sodium citrate, pH 7.0), the values of K_1 are subjected to an important error because they are deduced from the slopes of the plots of $\phi_F/(\phi_F - \phi_L)$ vs. N_0^{-1} which are quite small especially at high percentages of guanine modification. On the

Table I: Association Constants for the Binding of Lys-Trp-Lys to Calf Thymus DNA Modified by AAF or AAIF in 1 mM Sodium Citrate, pH 7.0, at 2 °C

	K_2	$K_1 \times 10^{-4}$ (M^{-1})	$K_1(1+K_2) \times 10^{-4}$ (M^{-1})	$K_2/(1+K_2)$
native DNA	0.33	1.87	2.5	0.25
DNA-AAF, 7%	3.12	1.5	6.3	0.76
DNA-AAIF, 4%	2.45	0.85	2.9	0.71
denatured DNA	5.4	0.35	2.25	0.84
denatured DNA-AAF (5%)	5.9	0.34	2.35	0.855
denatured DNA-AAIF (5%)	7.4	0.47	3.95	0.88

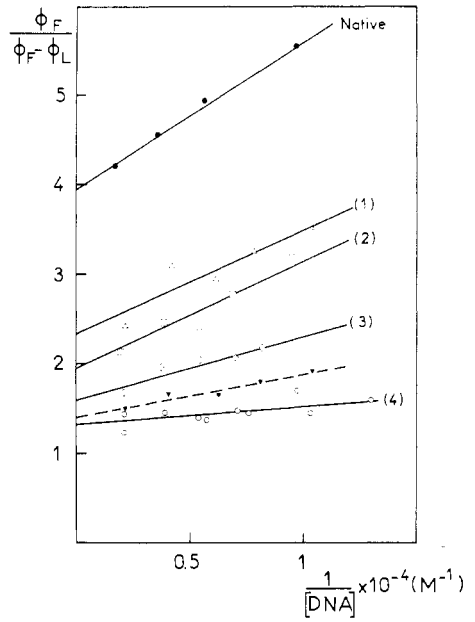

FIGURE 2: Analysis of fluorescence data according to eq 2 for the binding of Lys-Trp-Lys to different DNAs. Curves 1, 2, 3, and 4 correspond to DNA modified by AAAF with percentages of modified bases of 0.41, 1.05, 2.9, and 7%, respectively. Filled triangles correspond to DNA modified by AAAIF (4% modified bases). (For denatured DNAs see Figure 5.)

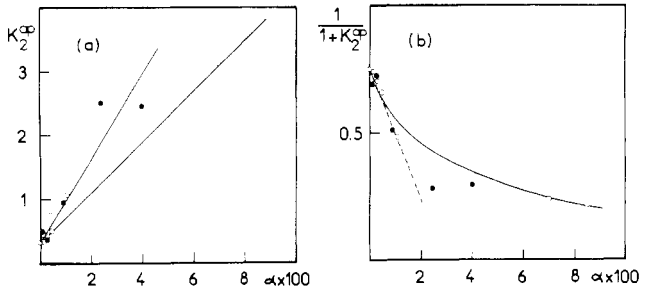

FIGURE 3: Variation of K_2 (a) and $1/(1 + K_2)$ (b) with the fraction α of modified bases. DNA was modified by AAAF (○) or AAAIF (●).

contrary, the values of K_2 are obtained with a better precision since they are calculated from the extrapolation of these plots when $N_0^{-1} \rightarrow 0$. As seen in Figure 3, K_2 increases with the extent of AAF substitution.

(*2*) *Analysis of Fluorescence Data.* Fluorescence data can be analyzed according to two limit models: (a) fluorescence quenching is only due to stacking interactions between the tryptophyl residue of Lys-Trp-Lys and nucleic acid bases; (b) fluorescence quenching is due to energy transfer from tryptophan to modified bases.

(*a*) *Fluorescence Quenching Is Due to Stacking Interactions.* Since K_2 increases upon guanine modification, this means that the fraction of stacked complexes calculated as $K_2/(1 + K_2)$ increases (Table I). This is what is expected if the peptide Lys-Trp-Lys binds preferentially to locally unpaired regions around modified guanines due to the much higher probability of stacking in these regions. The K_2 value obtained with the highest level of base substitution (7%) is not very far from that for heat-denatured DNA. This would indicate that the extent of unpairing arising from guanine modification is high.

It should be noted that association constants K_1 and K_2 obtained with modified DNAs represent average values over native and altered regions. The apparent value of K_2 (K_2^{app}) can be expressed as a function of the fraction y of peptide bound to unpaired regions (K_2^u) and that of $1 - y$ bound to double-stranded native regions (K_2^n)

$$\frac{1}{1 + K_2^{app}} = \frac{1}{1 + K_2^u} y + \frac{1}{1 + K_2^n}(1 - y) \qquad (3)$$

Equation 3 can be derived in a simple way by considering the average fluorescence quantum yield that Lys-Trp-Lys should have if it was bound to either unpaired regions or double-stranded native DNA

$$\phi_{app}^u = \frac{1}{1 + K_2^u}\phi_F \qquad (4)$$

$$\phi_{app}^n = \frac{1}{1 + K_2^n}\phi_F \qquad (5)$$

where ϕ_F is the fluorescence quantum yield of the free peptide and of complex I. It must be recalled that complex II does not emit fluorescence at all. The average fluorescence quantum yield of the peptide bound to a mixture of unpaired and double-stranded nucleic acids will then be

$$\phi_{app} = \phi_{app}^u y + \phi_{app}^n(1 - y) \qquad (6)$$

which leads to eq 3.

The ratio of the concentrations of peptide bound to unpaired and native regions is given by

$$\frac{y}{1 - y} = \frac{K^u x}{K^n(1 - x)} \qquad (7)$$

where x is the fraction of unpaired regions in modified DNA and K^u and K^n represent the overall association constants for the binding of peptide to modified and double-stranded regions. $K^u = K_1^u(1 + K_2^u)$ and $K^n = K_1^n(1 + K_2^n)$.

Different assumptions can be made regarding the values of the different association constants. Electrostatic interactions determine the value of K_1. If it is assumed that the electrostatic potential in the neighborhood of modified guanines is the same as that of the native double helix, then K_1 should not markedly depend on the extent of AAF reaction with guanine bases. Under this assumption ($K_1^n = K_1^u$) eq 7 becomes

$$\frac{y}{1 - y} = \frac{1 + K_2^u}{1 + K_2^n}\frac{x}{1 - x} \qquad (8)$$

If n is the number of base pairs which are disrupted around each modified guanine, $x = 2\alpha n$ where α is the fraction of modified bases. Combining eq 8 and 3 leads to

$$K_2^{app} = K_2^n + 2\alpha n(K_2^u - K_2^n) \qquad (9)$$

Equation 9 can also be obtained if K_2^{app} is defined as the ratio of the concentration of the nonfluorescent complexes (II^n and II^u) to the concentration of the fluorescent complexes (I^n and I^u):

$$K_2^{app} = \frac{K_1^n K_2^n(1 - x) + K_1^u K_2^u x}{K_1^n(1 - x) + K_1^u x} \qquad (10)$$

Under the assumption $K_1^n = K_1^u$, eq 10 leads to eq 9. A plot of the experimentally determined K_2^{app} vs. α should give a straight line if the above assumption ($K_1^u = K_1^n$) is correct. As shown in Figure 3a, a straight line is obtained whose slope is about 40. One may assume as a first approximation that the value of K_2 for unpaired regions around modified guanines is identical with that obtained for denatured DNA (~5.4). The calculated value of n is then ~4. This value is in agreement with previous results using formaldehyde as a probe for single-stranded regions in DNA-AAF (Fuchs & Daune, 1973, 1974).

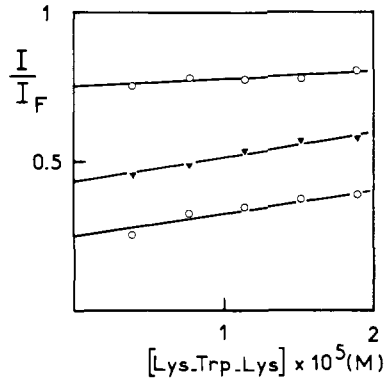

FIGURE 4: Relative fluorescence intensity of Lys-Trp-Lys vs. Lys-Trp-Lys concentration in the presence of 5×10^{-4} M native DNA (O, upper curve), 5×10^{-4} M DNA-AAF with 7% of base modification (O, lower curve), and the mixture of these two DNAs (5×10^{-4} M total concentration).

In the case of DNA-AAIF a similar analysis leads to a value of $n = 6.6$ (Figure 3a). However, it is known that the destabilization of DNA-AAIF is less than that of DNA-AAF (Fuchs & Daune, 1974; Fuchs, 1975). This suggests that other mechanisms than stacking might be responsible for tryptophan fluorescence quenching when Lys-Trp-Lys binds to DNA-AAIF (see below).

Instead of assuming $K_1^u = K_1^n$, one could make the hypothesis that the overall association constants for unpaired and double-stranded regions are the same, i.e., $K^u = K^n$. Then eq 7 will give $x = y$. Equation 3 or 10 can thus be written as

$$\frac{1}{1 + K_2^{app}} = \frac{1}{1 + K_2^n} + 2\alpha n\left(\frac{1}{1 + K_2^u} - \frac{1}{1 + K_2^n}\right) \quad (11)$$

A plot of $1/(1 + K_2^{app})$ vs. α should be a straight line. As shown in Figure 3b, this behavior is not observed experimentally. From the initial slope one may calculate a value of n (number of disrupted base pairs around each modified guanine) equal to 21. This value is in contradiction with the results of unwinding experiments using formaldehyde as a probe (Fuchs & Daune, 1974). Therefore, the hypothesis leading to eq 11 ($K^u = K^n$) can be rejected.

In order to test this conclusion, we have performed competition experiments to determine whether the overall association constant $[K_1(1 + K_2)]$ was higher for DNA-AAF as compared to native DNA. The apparent fluorescence quantum yield of the peptide Lys-Trp-Lys was measured in the presence of native DNA, DNA-AAF (7% of modified bases), and an equimolar mixture of these two DNAs with a total DNA concentration equal to that of the two separated samples. Let K^n and K^m be the overall association constants for native and AAF-substituted DNAs. The total concentration of bound peptide on each DNA in the mixture is

$$C^n = K^n[N][p]$$

$$C^m = K^m[M][p] \quad (12)$$

where [p] is the free peptide concentration and [N] and [M] are the concentrations of free binding sites on native and modified DNAs, respectively. Under our experimental conditions the concentration of bound peptides was low as compared to the concentration of binding sites and the fluorescence intensity was extrapolated to zero peptide concentration (see Figure 4). Therefore, one can make the assumption that $[N] \simeq [N]_0$ and $[M] \simeq [M]_0$ with $[N]_0 = [M]_0$. Then from eq 12 one can write

$$\frac{C^n}{C^m} = \frac{K^n}{K^m} \quad (13)$$

The overall fluorescence quantum yield of the peptide bound to the two DNA samples can then be expressed as described above (see eq 3–6). From an equation analogous to eq 3, the fraction of peptide bound to native DNA in the mixture can be calculated. Substitution in eq 13 gives the ratio K^n/K^m. From the results shown in Figure 4, one can calculate that the overall binding constant to DNA-AAF containing 7% of modified bases is about 1.8 times higher than that corresponding to native DNA. This value is quite close to that (2.5) calculated from Table I where the association constants were derived from the slopes of plots according to eq 2. Therefore it can be concluded that AAF substitution does not affect K_1 very much and that the increase of K_2 is mostly responsible for the increase of the overall association constant $K_1(1 + K_2)$.

(b) Energy Transfer from Tryptophan to DNA-AAF and DNA-AAIF. The conformation of DNA-AAIF was shown to differ from that of DNA-AAF (Fuchs & Daune, 1973; Fuchs, 1975; Fuchs et al., 1976; Lefèvre et al., 1978). A so-called outside binding model (Fuchs et al., 1976) was coined in order to fit the different experimental data. However, the data presented in Figure 3 show that the binding of Lys-Trp-Lys to DNA-AAIF is not markedly different from that to DNA-AAF at the same level of modification. The K_2 values are even higher for DNA-AAIF.

These results suggest that either the structure of modified DNA does not depend strongly on whether or not the fluorene ring is substituted by iodine or the quenching of tryptophan fluorescence is due to another mechanism than stacking with bases in unpaired regions around the modified bases. One such mechanism could be energy transfer from the tryptophyl residue to aminofluorene. As a matter of fact, there is a good overlap of the fluorescence spectrum of the tryptophyl residue in Lys-Trp-Lys with the absorption spectrum of AAF or AAIF bound to C(8) of guanosine (Guo-AAF; Guo-AAIF) which is the major adduct in DNA modified with AAAF or AAAIF (Kriek et al., 1967; Lefèvre et al., 1978). The Forster critical distance (R_0)—that is, the distance at which the probability of energy transfer is equal to the probability of all other modes of deactivation of the excited donor—has been calculated from eq 14:

$$R_0^6 = (8.78 \times 10^{-25})K^2\phi n^{-4}\frac{\int F(\bar{\nu})\epsilon(\bar{\nu})\, d\bar{\nu}/\bar{\nu}^4}{\int F(\bar{\nu})\, d\bar{\nu}} \quad (14)$$

where K^2 is an orientation factor which was taken as 2/3, ϕ is the fluorescence quantum yield of the donor, n is the refractive index, $F(\bar{\nu})$ is the fluorescence spectrum of the donor on a wavenumber scale, and $\epsilon(\bar{\nu})$ is the extinction coefficient of the acceptor.

The fluorescence quantum yield of Lys-Trp-Lys was calculated to be 0.09 at pH 6.0 assuming a value of 0.14 for tryptophan (Chen, 1967). The refractive index was taken as 1.33 (water). The overlap integral was calculated from the spectra of Guo-AAF and Guo-AAIF. The calculated values of R_0 are 1.22 and 1.57 nm for energy transfer from Lys-Trp-Lys to Guo-AAF and Guo-AAIF, respectively.

If the peptide Lys-Trp-Lys is bound to modified DNA without stacking interaction of the tryptophan residue with nucleic acid bases, then the fluorescence of this tryptophan can be quenched by energy transfer to AAF or AAIF. Although the relative orientations of donor and acceptor molecules are not known, the unstacked tryptophyl ring has a rotational mobility high enough for its fluorescence polarization

to be very low (0.07) (Toulmé & Hélène, 1977). Since it is not possible to estimate the relative orientation of each donor (tryptophan) and each acceptor (modified base), one must use a model to determine an average quenching distance. One can make the following assumption: every tryptophan which is located in a sphere of radius r around one modified base will be completely quenched whereas a tryptophan outside this sphere will not be quenched. The efficiency of energy transfer from a donor to an acceptor located at a distance R depends on $1/(1 + x^6)$ where $x = R/R_0$. One may then calculate the radius r of the "quenching sphere" as

$$\frac{r}{R_0} = \int_0^\infty \frac{1}{1 + x^6}\, dx$$

Using the calculated integral value, one gets $r \sim 1.25R_0$. Under this assumption, one modified base will quench a peptide whose tryptophyl residue is located within a sphere of radius 1.52 or 1.96 nm for AAF or AAIF, respectively. An overestimation of the quenching domain of one modified base would thus be 9 base pairs around one AAF and 11.5 base pairs around one AAIF (this would be an overestimation since conversion to a linear distance along the DNA axis includes peptides which will not be located within the quenching sphere). However, it may be seen that the length of the regions where tryptophan fluorescence quenching can be due to energy transfer to AAF- or AAIF-modified bases is of the same order of magnitude as that calculated above assuming that each modified base is the center of an unpaired region where the stacking probability of tryptophan with bases is the same as that of single-stranded (denatured) DNA. On the basis of these rough calculations, it would therefore be difficult to decide which of the two hypotheses (stacking or energy transfer) is correct. A study of the binding of Lys-Trp-Lys to denatured modified DNAs helped to solve this problem (see below).

(3) Binding of Lys-Trp-Lys to Denatured DNAs. In order to determine the possible contribution of energy transfer to fluorescence quenching, the binding of Lys-Trp-Lys to denatured DNAs was investigated. When DNA-AAF or DNA-AAIF is denatured, one should expect a higher quenching of Lys-Trp-Lys fluorescence than in unmodified denatured DNA. According to eq 1 and the K_2 value for heat-denatured DNA ($\sim$5.4), one can calculate that about 16% of the bound peptides have their tryptophyl ring free of interaction with bases (complex I) in the absence of base modification. These peptides have the same fluorescence quantum yield as the free peptide. When denatured DNA is modified by AAAF or AAAIF, these bound peptides of type I should be able to transfer their energy to guanosine-bound AAF or AAIF. As a result of this transfer one should observe a higher quenching for modified denatured DNA as compared to unmodified DNA. As reported in Figure 5, an analysis according to eq 2 shows that the behavior of denatured DNA-AAF with 5% base substitution is not very different from denatured DNA. However, with denatured DNA-AAIF it is clear that the extent of quenching is higher than with the other two DNAs. An "apparent" K_2 value of 7.4 is obtained as compared to 5.4 in the absence of modification. From these values one can calculate that the percentage of bound peptides which still emit fluorescence decreases from 16 to 12% when the DNA contains 5% of its bases substituted by AAAIF.

If this decrease is ascribed to energy transfer, the quenching domain of a modified base will be on the average five bases (this means that every bound peptide whose tryptophyl residue is located within 2.5 bases of a modified base will have its fluorescence quenched by energy transfer even if it is not stacked with bases). This experimental value is smaller than that calculated above (about 11.5 for AAAIF) in a very rough model. Nevertheless, it shows that energy transfer does contribute significantly to the observed fluorescence quenching when Lys-Trp-Lys binds to DNA-AAIF. These results also show that fluorescence quenching in the case of DNA-AAF is mainly due to stacking of the tryptophyl ring with bases in locally unpaired regions around AAF and not to energy transfer.

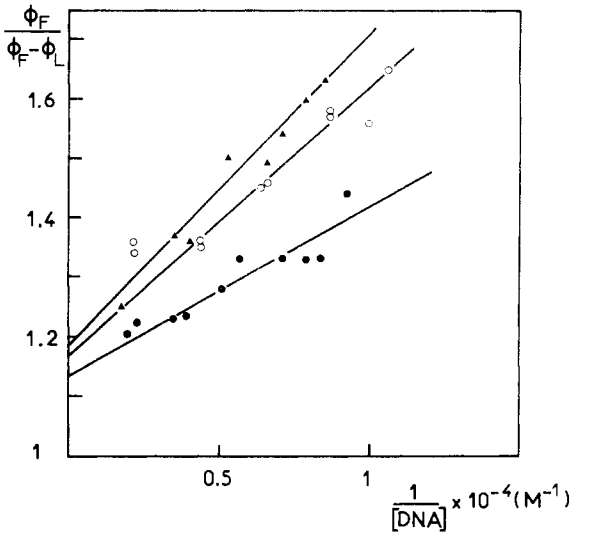

FIGURE 5: Plots of $\phi_F/(\phi_F - \phi_L)$ vs. $1/N_0$ according to eq 2 for the binding of Lys-Trp-Lys to denatured DNA, denatured DNA-AAF (5% base modification), and denatured DNA-AAIF (5% base modification). (▲) Denatured DNA; (○) denatured DNA-AAF; (●) denatured DNA-AAIF.

Conclusion

The results obtained for Lys-Trp-Lys binding to DNA modified by AAAF show that the enhanced fluorescence quenching observed with modified DNA as compared to native DNA is due to an enhanced stacking of the tryptophyl residue with nucleic acid bases. AAF-modified regions in native DNA behave like single-stranded regions, in agreement with both the insertion–denaturation model (Fuchs & Daune, 1972) and the base displacement model (Weinstein & Grunberger, 1974).

In the case of DNA-AAIF, we have shown that the observed tryptophan fluorescence quenching is due partially to energy transfer from the tryptophyl ring to the 7-iodofluorene residue. It is, however, likely that energy transfer does not account for the whole quenching. Therefore, one has to admit that an unknown fraction of the fluorescence inhibition in DNA-AAIF results from stacking of the tryptophyl ring similarly to what we observed in DNA-AAF. This conclusion seems in contradiction with the outside binding model, the features of which have been discussed earlier (Fuchs et al., 1976). This contradiction is, however, only apparent in view of the fact that by formaldehyde unwinding experiments (Fuchs & Daune, 1973) and by means of S_1 endonuclease hydrolysis kinetics (Fuchs, 1975) it was shown that from a dynamic point of view the binding of AAIF to native DNA gave rise locally to small opening units (one to two base pairs as seen by the formaldehyde unwinding). Therefore, one can assume that a molecule with greater affinity for single-stranded regions, such as the tripeptide, could displace the conformational equilibrium toward the extension of the preexisting small opening units. Similar observations were made with the protein coded by gene 32 of phage T4 which also binds to both AAF- and AAIF-

modified DNA (J. J. Toulmé and C. Hélène, unpublished results).

It should be noted that the specificity of stacking interactions of tryptophan with respect to single-stranded structures is even higher in the case of tyrosine. Stacking of tyrosine residues of oligopeptides with nucleic acid bases was observed exclusively when the nucleic acid was single stranded (Dimicoli & Hélène, 1974b; Mayer et al., 1979). Therefore, the recognition of locally unpaired regions in a DNA is not limited to tryptophyl residues but may involve tyrosyl residues as well. However, the fluorescence of tyrosine can be quenched by energy transfer to nucleic acid bases (Mayer et al., 1979; Montenay-Garestier, 1975) in the absence of stacking interactions. This precludes the use of fluorescence spectroscopy to provide evidence for tyrosine stacking interactions in complexes formed by damaged DNA with tyrosine-containing oligopeptides or proteins. On the contrary, fluorescence quenching is a good probe of stacking interactions in the case of tryptophyl-containing peptides (Hélène, 1977).

The results obtained with a small oligopeptide Lys-Trp-Lys show that chemical modification of DNA by a carcinogen such as AAAF induces a local perturbation of the double-helix structure which is large enough to permit the efficient stacking of an aromatic amino acid residue. This might be of importance in the recognition of chemically modified DNA by proteins and enzymes involved in the repair of these lesions. For example, one might put forward the hypothesis that the endonuclease responsible for the first step of the excision repair pathway could make use of its aromatic residues to anchor along the DNA at the site of the lesion. Single-strand-specific proteins which are certainly involved in the protection of single-stranded DNA against endonuclease action during the repair process could also make use of this property to recognize the locally unpaired region in damaged DNA.

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