DNA-binding of IQ, Me-IQ and Me-IQx, strong mutagens found in broiled foods

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Non-covalent DNA-binding has been studied of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Me-IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx), strong mutagens found in broiled foods. These mutagens are intercalated into DNA, as found by ultraviolet absorption and gel electrophoresis. The binding of IQ is stronger with GC pairs than AT pairs in DNA. The binding constants with calf thymus DNA are 1.6×10^6 (Me-IQ), 0.9×10^6 (IQ) and 0.7×10^6 M^{-1} (Me-IQx) at pH 6.0. This order of DNA affinity agrees with the order of mutagenicity towards *Salmonella typhimurium* TA98.

Mutagen *Carcinogen* *Broiled food* *DNA-binding* *Intercalation* *Binding constant*

1. INTRODUCTION

Strong mutagens have been isolated from pyrolysates of amino acids and some of them are found to be carcinogenic [1]. Strong mutagenicity has also been found in smoke condensates from broiled fish and beefsteak and also in charred surface of fish and beefsteak [1]. We have isolated two mutagens from the neutral fraction of broiled sardines and characterized their structures to be 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Me-IQ) (fig. 1) [2–5]. IQ has also been detected in heated beef extract [6] and in fried beef [7], suggesting that IQ is present in a variety of cooked foods. Furthermore, we have isolated another strong mutagen from fried beef and characterized its structure to be 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQx) (fig. 1) [8]. Mutagenic activities of these three mutagens towards *Salmonella typhimurium*

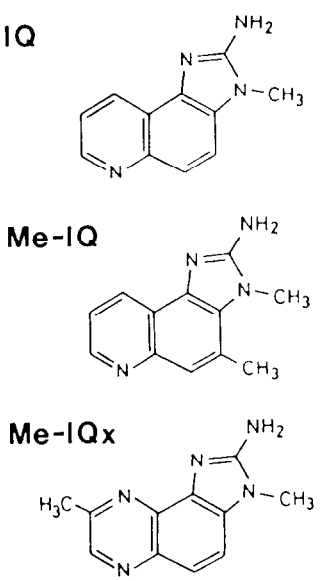

Fig. 1. The chemical structures of IQ, Me-IQ and Me-IQx.

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TA98 are higher than those of carcinogens from pyrolysates of tryptophan (IQ, 433000; Me-IQ, 661000; Me-IQx, 145000 revertants/μg) [8,9]. Accordingly, carcinogenic tests of these strong mutagens are now in progress. Here, we report the binding of IQ, Me-IQ and Me-IQx with DNAs with different GC contents.

1. MATERIALS AND METHODS

Samples of IQ, Me-IQ and Me-IQx were chemically synthesized [4,8,10]. Calf thymus DNA (type I) was purchased from Sigma Chemical Co. and used without further purification. *Streptomyces griseus* DNA was isolated and purified by Marmur's method [11] with some modifications. The sample solutions were prepared using phosphate buffer (10 mM) containing 1 mM EDTA. The concentrated DNA solution (7–9 mM) was added, in small increment, to the mutagen solution (15 μM). The mixed solution of mutagen and DNA was left at 23°C until the binding equilibrium was reached, and then the absorption spectrum was recorded with a Shimadzu UV-240 spectrophotometer using 10 cm cell.

Unwinding of double helical DNA was studied by the use of pBR 322 which was purified through ethidium bromide–CsCl equilibrium centrifugation. The DNA was incubated with superhelical DNA-relaxing enzyme (Bethesda Res. Lab. MD) in 20 mM Na-PIPES buffer (pH 6.8), 2 mM $MgCl_2$, 1 mM dithiothreitol and 5% ethanol at 20°C for 3 h, in the presence of Me-IQ. The relaxed DNA was analyzed as in [12].

3. RESULTS AND DISCUSSION

The absorption spectra of the mixed solutions of IQ and calf thymus DNA are shown in fig. 2. As [DNA] is increased, the $A_{\sim 330\,nm}$ of IQ is decreased, and such hypochromicity suggests that the IQ molecule is intercalated between the basepairs of the DNA molecule. There are isosbestic points at 300 nm and 455 nm, indicating that the IQ molecules are in the equilibrium between the free state and the DNA-bound state. Then, the Scatchard analyses [13] have been made with the equation:

$$r/c = K(n - r)$$

where:

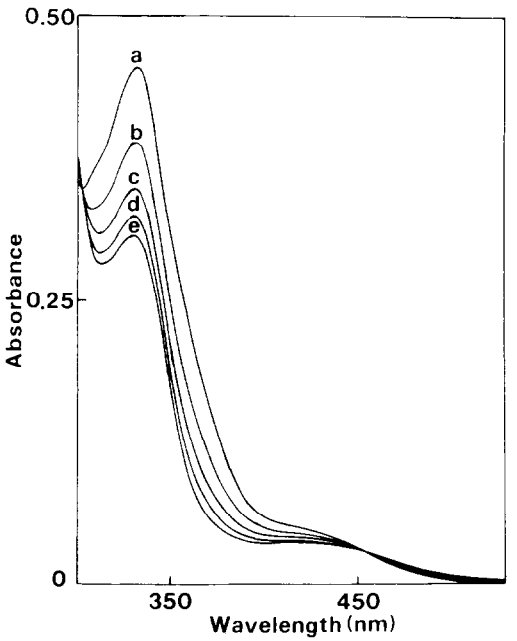

Fig. 2. The absorption spectra of the mixed solution (pH 6.0) of IQ (15 μM) and calf thymus DNA; [DNA] basepairs are: (a) 0; (b) 80; (c) 150; (d) 220; (e) 310 μM.

r = the ratio of the concentration of bound ligand to the total concentration of DNA basepairs;
K = the binding constant for IQ with DNA;
n = number of ligand-binding sites/basepair;
c = the concentration of free ligand.

The Scatchard plots for the binding of IQ to calf thymus DNA and to *Streptomyces griseus* DNA are shown in fig. 3. For low values of r (strong binding mode), the values of K and n are obtained to be 0.9×10^6 M^{-1} and 0.06, respectively, for calf thymus DNA, and 1.8×10^6 M^{-1} and 0.09, respectively, for *Streptomyces griseus* DNA. In addition to this strong binding mode, another weak binding mode with $K \sim 0.1 \times 10^6$ is found to exist by the Scatchard plots at higher values of r.

As for the strong binding mode, therefore, the IQ molecules bind more strongly to *Streptomyces griseus* DNA (GC content, 73%) than to calf thymus DNA (GC content, 42%). This result indicates that the binding of IQ is stronger with GC pairs than with AT pairs in DNA. According to the method in [14], the parameter N is defined as:

$$\ln\{[\lim_{r\to 0}(r/c)]_1/[\lim_{r\to 0}(r/c)]_2\}/\ln[(X_{GC})_1/X_{GC})_2]$$

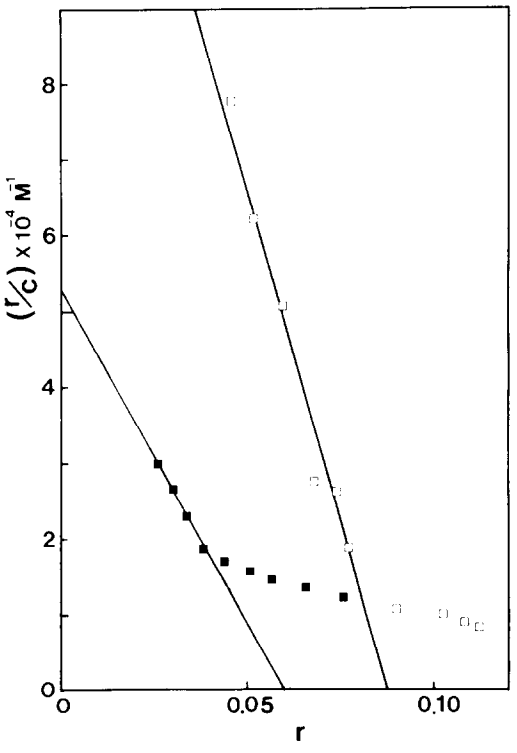

Fig. 3. The Scatchard plots for the binding of IQ with calf thymus DNA [GC content 42%] (—■—) and *Streptomyces griseus* DNA [GC content 73%] (—□—).

where X_{GC} is the GC content and suffices (1 or 2) are for the two different species of DNA. If the ligand binds to the sites between G–C pairs only of DNA, N will be equal to 2 whereas if the ligand has no specificity for the basepair sequence, N will be equal to zero. In the present case of the IQ molecule, this parameter N is obtained as 1.8, indicating that this molecule binds preferentially to the sites between G–C pairs in DNA.

The Scatchard plots as shown in fig. 3 are made for the experimental results on the mixed solutions of IQ and DNA at pH 6.0, where about 60% of IQ molecules are positively charged at the aminomethylimidazole moiety ($pK_a = 6.2$). To examine the effect of such a positive charge on DNA-binding, we have also made spectroscopic measurements on the mixed solution at pH 7.0, where only 15% of IQ molecules are protonated. Fig. 4 shows the relative hypochromicity of IQ, as a function of DNA concentration, at pH 6.0 and 7.0; the

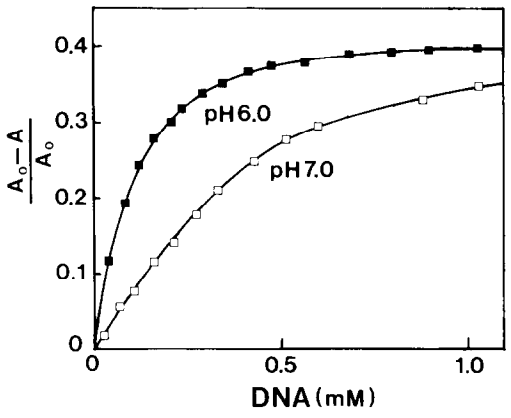

Fig. 4. The hypochromicity $[(A_o - A)/A_o]$ of IQ (15 μM) at pH 6.0 and 7.0, where A_o is the absorbance of free IQ and A is that of IQ in the presence of calf thymus DNA.

IQ–DNA interaction is clearly much stronger at pH 6.0 than at pH 7.0. Therefore, the electrostatic interaction between the positively charged IQ and negatively charged phosphate groups of DNA is considered to be important for IQ–DNA binding. In fact, the concentration ratio (r) of bound IQ molecules to total base pairs (350 μM) is decreased ($0.048 \rightarrow 0.031 \rightarrow 0.023 \rightarrow 0.018$) as [NaCl] is increased ($0 \rightarrow 50 \rightarrow 100 \rightarrow 150$ mM) in the buffer solution of IQ (17 μM) at pH 6.0.

Scatchard analyses have also been made for the binding of Me-IQ and Me-IQx with calf thymus DNA at pH 6.0 (fig. 5). The binding constants (K for the strong binding mode) are obtained to be 1.6×10^6 (Me-IQ), 0.9×10^6 (IQ) and 0.7×10^6 M^{-1} (Me-IQx), and the number (n) of binding sites/base-pair to be 0.09 (Me-IQ), 0.06 (IQ) and 0.06 (Me-IQx). These data show that the DNA-binding constants are in the order of Me-IQ > IQ > Me-IQx, in agreement with the order of mutagenic activities towards *Salmonella typhimurium* TA98 [7,8].

Intercalation of Me-IQ was also suggested because this ligand caused unwinding of the double helix of DNA. This unwinding was examined by treating superhelical DNA with superhelix relaxing enzyme at various [Me-IQ]. Mg^{2+} is known to cause overwinding of the double helix of DNA. Therefore, closed circular DNA relaxed in the presence of Mg^{2+} showed bands of positively superhelical components when it was analyzed in gel electrophoresis in the absence of Mg^{2+}. The

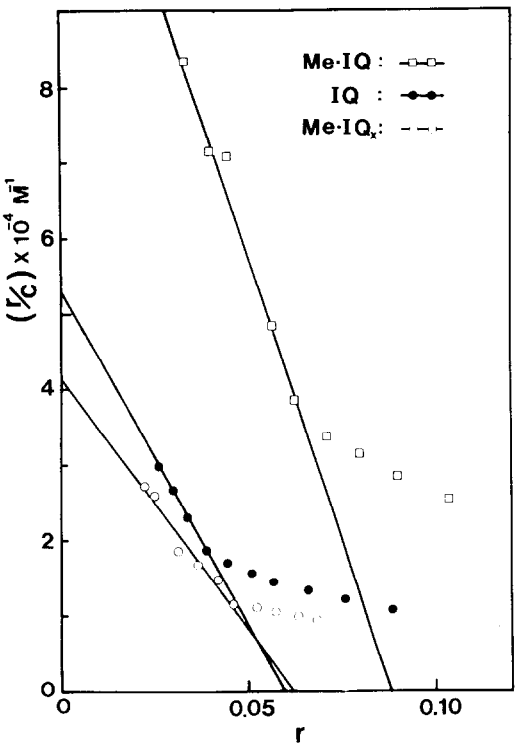

Fig. 5. The Scatchard plots for the binding of IQ, Me-IQ and Me-IQx with calf thymus DNA at pH 6.0.

presence of 34 μM Me-IQ at relaxation compensated for the effects of Mg^{2+} and showed no superhelical components. A further increase of Me-IQ to 340 μM yielded superhelical DNA of 4–9 negative turns, indicating that the binding of Me-IQ to DNA caused unwinding of the double helix.

The three mutagens (fig. 1) all have the 2-amino-3-methylimidazole moiety in common. IQ is metabolically activated by microsome enzymes; an active metabolite has been identified as 2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline [15]. Thus, Me-IQ and Me-IQx are probably activated by *N*-hydroxylation. On the other hand, the 6–6 membered rings of Me-IQ and Me-IQx are partly different from that of IQ (fig. 1). The order of mutagenic activities of the 3 mutagens [7,8] is found here to correlate with the order of DNA-binding constants (strong binding mode) among the IQ family. Such a correlation between the DNA affinity and mutagenicity has been found among carcinogens from pyrolysates of tryptophan [16] and of glutamic acid [17]. In the hope of elucidating the mode of DNA-binding, the NMR analyses of conformations of oligodeoxynucleotide–IQ complexes are now in progress.

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