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PRODUCTION OF MUTAGENIC ARTIFACTS BY THE ACTION OF RESIDUAL CHLORINE ON XAD-4 RESIN

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SUMMARY

XAD resins are commonly used to recover and concentrate organics from chlorinated water. It was found that the action of residual chlorine on XAD-4 resin produced mutagenic artifacts in a dose dependent manner. The production of mutagenic artifacts could be suppressed at least ten-fold by converting free chlorine to monochloramine. Kinetic studies of the reaction between free chlorine and XAD-4 resin showed a reaction rate dependence upon pH and chloride ion concentration that suggests participation of species besides hypochlorous acid.

INTRODUCTION

Water chlorination produces mutagenic species from natural organic precursors[1–4]. These mutagens are formed *de novo* by electrophilic chlorine and are undoubtedly electrophiles. With some chlorinated waters the mutagens can be detected by testing aliquots in an assay such as the Ames assay[1–4], although usually mutagen levels are so low as to require concentration prior to bioassay[1,2].

Organic contaminants in water are frequently recovered and concentrated by chromatographic adsorption on Amberlite XAD resins followed by elution with organic solvents[5]. This method has the advantages of simplicity, good recovery of hydrophobic species, and the ability to handle large volumes of water[6]. One difficulty that is encountered with XAD isolation methods is that very polar or high-molecular-weight materials may be poorly recovered[6].

In general, lower-molecular-weight lipophilic species will cross cell membranes readily. Thus a proposal for prioritizing the study of organic contaminants assigns the more lipophilic, less than 500 molecular-weight compounds, the top priority while more polar or higher-molecular-weight materials would be of less concern[7]. Therefore, we routinely use XAD-4 resin to recover the mutagens found in chlorinated water[8,9]. We have observed, however, the possibility that the action of chlorine on the resin could give rise to mutagenic artifacts[2]. This paper describes some studies on mutagenic artifact formation by the action of residual chlorine on XAD-4 resin. The resin may be used with waters of many different pH, type of chlorine residual and chlorine concentration. Thus we also show how kinetic studies of chlorine–XAD-4

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interactions can indicate which conditions are likely to increase or decrease artifact formation.

EXPERIMENTAL

XAD-4 resin

XAD-4 resin was obtained commercially, and was prepared for use by modifying the usual procedure[5] as follows; the resin was washed repeatedly with 1 *M* hydrochloric acid, water, 1 *M* sodium hydroxide and water, until all visible color was removed. After treatment with 0.01 *M* sodium phosphate, pH 7.4, to assure near neutrality, and exhaustive washing with water, the resin was serially extracted in a Soxhlet apparatus (no thimble) with water, acetone, methylene chloride and methanol. Cleaned resin was stored under methanol until use[5,6].

Chlorinated water

Tap water was purified by passage through a reverse osmosis membrane followed by a Millipore MilliQ system (ion-exchange and activated carbon cartridges). Only purified water was used in these experiments.

Commercially available reagent-grade sodium hypochlorite was used to make solutions containing free chlorine residuals. Monochloramine was produced by adding a slight stoichiometric excess of ammonium chloride to volumes of buffered water containing free chlorine. Concentrations of free chlorine or monochloramine were determined with the N,N-diethyl-*p*-phenylene diamine (DPD) colorimetric assay[10].

All glassware was soaked with chlorinated water prior to use to eliminate any chlorine demand. pH values were maintained with phosphate buffer. No chlorine demand was exhibed by the purified water either before or after buffering.

Production of mutagenic artifacts

Water concentrates[2]. Volumes of 20 l of water containing various concentrations of free chlorine or monchloramine were passed through 100-ml bed volume (26 × 2.2 cm I.D.) columns of clean XAD-4 resin at a flow-rate of 1 l/h. The pH in these experiments was kept at 7.4 with 0.05 *M* sodium phosphate buffer. The resin was then washed with four column volumes of purified water to displace any material trapped but not adsorbed. The adsorbed organics were eluted with one column volume of acetone followed by eight column volumes of methylene chloride. The aqueous phase was discarded, while the organic solvent was evaporated in a rotary evaporator to yield a dry residue. Organics were redissolved in a volume of ethanol or DMSO equal to 1/10 000 the original volume of water, yielding the water concentrate solution. All concentrate volumes are expressed in terms of the original volume of water, thus 50 μl of concentrate corresponds to 0.5 l of original water.

Mutagen bioassay. The Ames assay was used as a bioassay for mutagenic electrophiles. Testing was performed following the usual protocol[11] except that the bacteria were grown in 1 to 2 diluted Oxoid Broth No. 2 instead of Difco. Testing used strain TA100 because it gave the maximum response to drinking water mutagens, and S-9 was omitted because it only suppresses the mutagenic response[12].

Particular attention was paid to including positive controls for comparison of day-to-day reponses. Styrene oxide was used as the principal positive control, because

epoxides are among the mutagenic electrophiles likely to be found in drinking water[13]. Plating without added mutagen or water sample constituted the negative control. Ethanol or DMSO up to 50 μm per plate had little effect on the response. Since the volume of water concentrate added was kept below 50 μl, solvent controls were not needed. Slopes of the dose responses were calculated by least square fits. In general, the guidelines proposed by an expert group[14] were followed.

Kinetic studies

Volumes of 25 ml of packed clean XAD-4 resin were washed with ten volumes of purified water to remove methanol and were added to 900 ml buffered, temperature equilibrated solutions of free chlorine or monochloramine in 1 l Erlenmeyer flasks. Volumes were brought to 1 l with purified water. All concentrations below are expressed as final concentrations. The solutions were gently stirred (4 place synchronous magnetic stirrer) at a rate just enough to suspend the resin completely. Simultaneous blanks omitted resin. All reactions were shielded from light. The rate of reaction was followed by monitoring the disappearance of chlorine or monochloramine with the DPD colorimetric method[10]. Styrene and divinylbenzene residues in the XAD-4 resin were present at much higher concentration than the chlorine species so pseudo-first order $k_{observed}$ values were computed by $k_{obs.} = 0.693/t_{\frac{1}{2}}$, where $t_{\frac{1}{2}}$ = half life of chlorine.

RESULTS

Production of mutagenic artifacts

Table I lists the Ames assay results showing the generation of mutagens by chlorination of XAD-4 resin. The slopes of the dose responses are given in the fourth column. Day-to-day variation in the bioassay was anticipated, thus in each run a styrene oxide dose response was included. Each of the slopes of the dose responses due to chlorination artifacts could then be normalized by dividing by the styrene oxide dose response slope (in revertants per μg styrene oxide, fifth column) for that run. These normalized values are given in the rightmost column of Table I. Fig. 1 is a plot of the observed slopes and their normalized values from Table I. The x-axes are expressed as ppm of free chlorine or monochloramine. One ppm of either species means oxidizing power equivalent to one ppm of chlorine ($1.41 \cdot 10^{-4}$ M). The lines drawn on Fig. 1 are least squares fits of the points.

As can be seen, free chlorine gives rise to somewhat more than ten times the amount of mutagenic artifacts as does monochloramine, per molar equivalent.

Kinetics of the reaction between chlorine residuals and XAD-4 resin

Monochloramine. In a series of kinetic experiments, monochloramine reacted very slowly with XAD-4 resin. Over a pH range of 6 to 9, there was less than a 10% loss of the total chloramine residual by 4 h. Column effluents were collected in several of the mutagenic artifacts experiments just described. Where monochloramine was the chlorine species, only between 10% and 30% was removed by passage through the column, indicating limited reaction.

Free chlorine–effect of pH and temperature. Free chlorine reacts much more rapidly with XAD-4 than does monochloramine. In the preceding experiments using

TABLE I

MUTAGEN PRODUCTION DUE TO REACTION BETWEEN CHLORINE AND XAD-4

Run No.	*Chlorine species*	*ppm chlorine (as Cl_2)*	*Ames assay slope of dose response (revertants/liter)*	*Styrene oxide response (revertants/μg)*	*Normalized Ames assay slope*
1	Free chlorine	0.5	39	0.84	46
		1.0	38	0.84	45
		2.0	188	0.84	224
2	Free chlorine	0.5	64	0.38	169
		1.0	108	0.38	284
		2.0	217	0.38	572
3	Free chlorine	0	−21	0.58	(−21)
		0.5	65	0.58	113
		1.0	141	0.58	245
		2.0	246	0.58	428
4	Monochloramine	2	19	0.68	79
5	Monochloramine	4	52	0.33	161
		8	63	0.33	195
		12	90	0.33	270
6	Monochloramine	0	−15	0.46	(−15)
		2	0	0.46	0
		4	30	0.46	65
7	Monochloramine	4	60	0.49	123
		8	86	0.49	176
		12	72	0.49	148

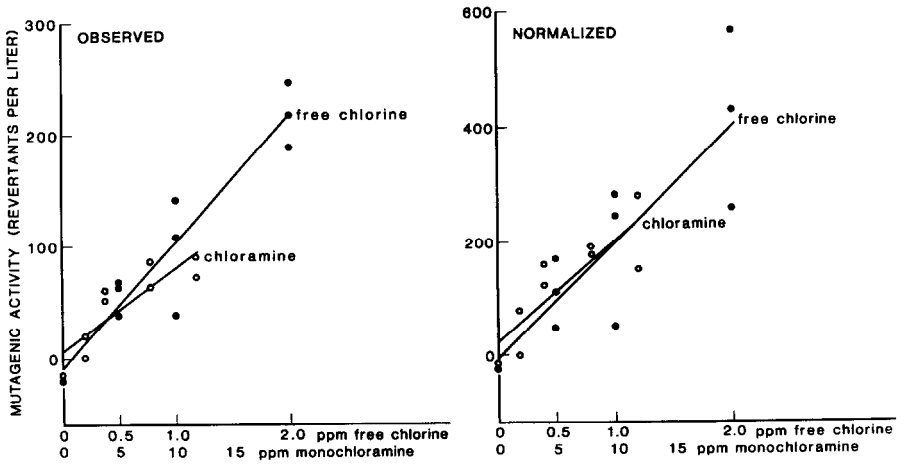

Fig. 1. Mutagenic activity resulting from the action of residual chlorine on XAD-4 resin. (Left) Observed values of mutagens per liter of chlorine containing water. (Right) Values normalized by dividing by the response with the positive control, styrene oxide.

columns at pH 7.4, free chorine residuals of all concentrations were totally consumed upon passage through the column. Rapid reaction was also observed in the kinetic experiments. Half-lives for the chlorine were measured in minutes rather than hours. As the pH increased, the rate of reaction decreased. Fig. 2 summarizes the effect of pH on chlorine consumption at 25°C. Based on the average rate of reaction at pH 7.0, a theoretical curve for the reaction rate at 25°C *versus* pH may be calculated knowing the pk_a of hypochlorous acid, 7.5 (ref. 15), and using the limiting assumption that reaction is due totally to hypochlorous acid, with no contribution from hypochlorite. The solid curve drawn in Fig. 2 shows this theoretical calculation. Interestingly, the reaction rates at low pH may be higher and at high pH lower than predicted using the extreme assumptions for participation by hypochlorous acid and hypochlorite.

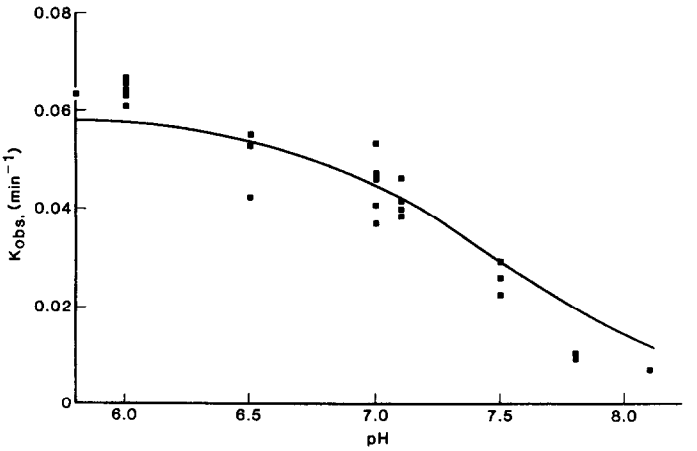

Fig. 2. Effect of pH on the reaction between free chlorine and XAD-4 resin at 25°C.

As expected, decreasing the temperature caused a decrease in reaction rate. Table II illustrates the effect of temperature on reaction rate at pH 7.0. Similar effects were seen at other pH.

TABLE II

EFFECT OF TEMPERATURE ON THE REACTION BETWEEN CHLORINE AND XAD-4 RESIN AT H 7.0

Temperature (0°C)	$k_{obs.}$ (min^{-1})	*Mean* $k_{obs.}$ (min^{-1})
0	0.010, 0.017, 0.011	0.013
15	0.034, 0.029, 0.033	0.032
25	0.046, 0.037, 0.041, 0.047, 0.053	0.045

Free chlorine–effect of chloride ion. The addition of sodium chloride caused the reaction between free chlorine and XAD-4 to increase markedly. Table III shows that the increase occurred over a wide pH range. The rates increased by 2.5 fold with 0.5 *M* added sodium chloride at pH 6 or 7.1. The increase appeared to be even greater

TABLE III

EFFECT OF CHLORIDE ION ON THE RATE OF REACTION BETWEEN FREE CHLORINE AND XAD-4 AT 25°C

pH	*Concentration of added chloride ion (M)*	$k_{obs.}$ (min^{-1})
6.0	0	0.064
6.1	0.15	0.077
6.1	0.3	0.107
6.0	0.5	0.17
7.1	0	0.042
7.2	0.15	0.050
7.1	0.3	0.066
7.1	0.5	0.107
7.8	0	0.010
7.9	0.15	0.029
7.8	0.30	0.039
7.8	0.5	0.049

at pH 7.8. The possibility that the increase in rate was due to an increase in sodium ion or ionic strength rather than chloride ion was eliminated by the observation that raising the ionic strength, μ, with various sodium phosphate concentrations yielding μ as high as 3.0 M had no effect on the rate.

DISCUSSION

The original observation of an artifactual production of mutagens by chlorine was made in a system where the artifacts could have arisen from sources other than the XAD-4 column[2]. This study shows that the action of chlorine on XAD-4 resin definitely is a source of mutagenic artifacts. The interaction between free chlorine and a styrene–divinyl benzene resin is not surprising in view of the report by Carlson and Caple[13] that dilute aqueous chlorine reacts slowly with toluene at pH 7.

As previously proposed, conversion of free chlorine residuals to chemically less reactive monochloramine sharply reduces the production of mutagenic artifacts. From this study the extent of reduction may be quantitated (Fig. 1). Mutagen production by free chlorine is calculated by linear regression to be, in revertants per liter water, 113 times ppm free chlorine, with a correlation coefficient of 0.94. With monochloramine, revertants per liter water equals 7.5 times ppm monochloramine with a correlation coefficient of 0.87. Thus the reduction factor is 15.

Normalization of these values by dividing each day's Ames assay response by the positive control, styrene oxide, response (Fig. 1) yielded values for revertants per liter *versus* ppm free chlorine or ppm monochloramine of 207 and 18 respectively. The correlation coefficients fell to 0.81 and 0.84 respectively, reflecting an increased data scatter after normalization. However, the ratio for artifact production by free chlorine *versus* monochloramine, 11.5, is still similar to the non-normalized value of 15. It should be noted that the normalized values have been transposed by dividing

by the styrene oxide slope and no longer represent actual mutagenic artifact levels.

Thus, we conclude that the simple expedient of converting a free chlorine residual to monochloramine reduces artifact production by at least ten-fold. Dechlorination is another means of preventing the production of mutagenic artifacts. However, many of the common dechlorination agents are nucleophiles which create the risk of destroying bona fide mutagenic electrophiles[2,12], so in some cases conversion of chlorine residuals to monochloramine may be the best expedient.

Where a water supply is connected directly to a column for monitoring, it is not possible to convert free chlorine to monochloramine. Some idea of the magnitude of artifact production is necessary so that appropriate corrections may be applied. While measurement of artifact production under actual column operating conditions is the only way to determine this correction, such an assay requires up to one day for column operation and preparation of the concentrate, and two days for the Ames assay. Measurements of kinetic rates of reaction can be done much more quickly and can suggest the magnitude of artifact production. However, there are limitations in extrapolating from kinetic studies to column performance.

The free chlorine residuals passed through XAD-4 at 25°C and pH 7.4 were completely consumed. Thus any condition (*e.g.*, lower pH) that increases the rate of reaction between free chlorine and XAD-4 may not generate more mutagenic artifacts. Monochloramine was partially consumed upon passage through XAD-4 at ten bed volumes per hour (contact time = 0.1 h) while in the kinetic study, a stirred monochloramine solution 40 times the volume of resin showed little reaction in 4 h. Therefore, the kinetic studies may underestimate the amount of mutagenic artifact production by slowly reacting species.

The kinetic studies demonstrate an expected increase in rate with lower pH and higher temperature. However, the pH effect (Fig. 2) appears to be greater than expected using the maximal assumption of hypochlorous acid being responsible for all of the reaction, with no contribution by hypochlorite. Perhaps a species dependent on $[H^+]$ is involved as well. Suggestions for the participation by H_2OCl^+ or Cl_2 in chlorination reactions have been made[15].

The impact of Cl^- has import for saline waters. It also suggests that the equilibria:

$$H^+ + OCl^- \rightleftarrows HOCl$$

$$HOCl + H^+ + Cl^- \rightleftarrows Cl_2 + H_2O$$

may be important because of Cl_2 participation in the reaction.

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