Reports

Competitive Hydration of Quinazoline at the Montmorillonite-Water Interface

Abstract. *Ultraviolet spectroscopic analyses of suspensions of quinazoline and Na^+-saturated montmorillonite have indicated that covalent hydration of the monovalent organic cation is inhibited at or near the clay surface. A similar lack of hydration was observed in solutions of quinazoline and high-ionic-strength calcium chloride. The inhibition is attributed to a local competition between quinazoline and the inorganic cations for water of hydration.*

The characterization of the properties of water and ions in the immediate vicinity of clay surfaces has long been a perplexing problem. Because of their complexities and inherent instabilities, clay-water-ion systems have largely evaded conclusive experimental attack. Recent evidence (*1*) of a drastically enhanced degree of dissociation in clay-adsorbed water relative to that in pure bulk water (which is not represented by average suspension properties) suggests the need for measurement techniques that are selective for the properties of water immediately adjacent to the surfaces. Earlier investigators (*2*) have employed infrared spectroscopy to study adsorbed organic species as molecular monitors of the clay surface environment. These efforts have been limited to systems of very low water content and high adsorbate concentrations. In the work reported here we have attempted to avoid these restrictions by using ultraviolet (UV) spectroscopy.

With the use of UV measurements it is possible to study suspensions as well as dry clay films with such sensitivity that the clay surfaces need not be heavily loaded with adsorbate to obtain well-resolved spectra (*3*). By selecting an organic adsorbate that undergoes a reaction with water in the vicinity of a clay surface, direct information about the properties of water in this region can be obtained. Quinazoline is well suited for this purpose because, when protonated, it forms a covalent hydrate by reversibly adding water across the C=N bond in ring positions 3 and 4. The resulting product is recognized by well-defined spectral changes (*4–6*). Since the neutral quinazoline molecule does not covalently hydrate, the hydration reaction at the surface can be initiated by inducing protonation after mixing the organic species with the clay.

Three types of quinazoline-montmorillonite mixtures were investigated: system 1 was prepared with neutral quinazoline, system 2 by protonating the quinazoline with dilute HCl before mixing with the clay, and system 3 by protonating the quinazoline after mixing with the clay. Hereafter, systems 2 and 3 will be referred to as pre- and postprotonated, respectively. The < 2-μm fraction of a montmorillonite (*7*), which had been separated by sedimentation, washed with distilled water, and percolated through with a Na^+-saturated Rexyn AG-50 (*8*) resin column, was used throughout. The final concentrations of clay and quinazoline were 0.88 and 0.0060 mg/ml, respectively. We also prepared similar quinazoline systems with hectorite substituted for montmorillonite and other systems containing quinazoline and saturated solutions of NaCl or $CaCl_2$ in place of the clay.

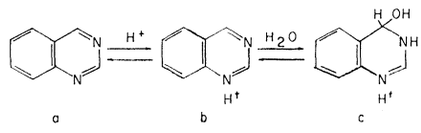

Fig. 1. Equilibria showing the protonation and subsequent hydration of quinazoline.

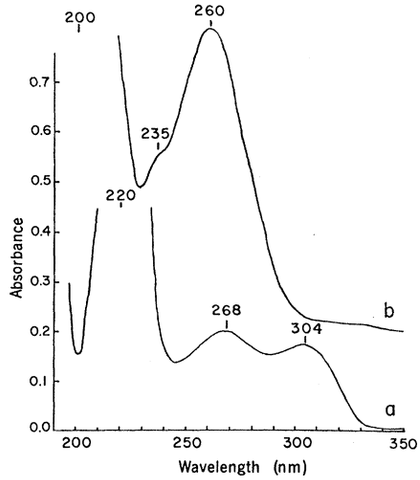

Fig. 2. The UV spectra of neutral (curve a) and protonated (and hydrated) (curve b) quinazoline solutions.

Spectra were recorded at room temperature on a Perkin-Elmer 356 UV-visible spectrophotometer fitted with an R189 photomultiplier tube. All quinazoline-clay spectra were referenced to a blank clay suspension adjusted to the sample *p*H. The $CaCl_2$- and NaCl-quinazoline spectra were referenced to to the pure salt solutions.

Albert *et al.* (*5*) have characterized the protonation (species b) and subsequent covalent hydration (species c) of quinazoline (species a) as shown in Fig. 1. They employed fast-reaction techniques to evaluate equilibria involving the three species and demonstrated the dependence of the pK_a (the negative logarithm of the acid constant) on the protonated species involved. In weak aqueous acid solution (*p*H 2) species c is dominant. Spectroscopically, this species is characterized by a strong 260-nm band (Fig. 2). Albert *et al.* showed that there is a reduction of covalent hydration in concentrated sulfuric acid solutions (8*N*). The anhydrous cation (that is, not covalently hydrated) is spectroscopically distinguished from the hydrated counterpart by a strong 240-nm absorption band which is diminutive in the covalent hydrate.

The spectra of neutral, pre-, and postprotonated quinazoline-montmorillonite systems are shown in Fig. 3. The

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neutral and preprotonated preparations gave spectra nearly identical to their solution counterparts. However, in the postprotonated system the major portion (approximately 75 percent) of the quinazoline cations are anhydrous, as indicated by the strong 240-nm absorption.

The initial character of the protonated species is determined by the preparation sequence. Upon aging, the postprotonated system retains essentially all of its anhydrous character whereas the preprotonated quinazoline dehydrates (that is, loses covalently bound water) considerably over a 24-hour period in the presence of the clay. When the protonated systems were centrifuged, the quinazoline cations remaining in the decantate were covalently hydrated. Hectorite-quinazoline suspensions behaved like the montmorillonite counterpart initially, but aging in the hectorite-quinazoline systems is compounded by the chemical reactivity of the hectorite lattice (*9*). The use of hydrogen-saturated clays makes it possible to protonate the quinazoline in suspension without adding additional HCl; systems consisting of hydrogen-saturated hectorite and quinazoline or hydrogen-saturated montmorillonite and quinazoline behaved in much the same way as their postprotonated Na^+ analogs. Quinazoline in $CaCl_2$ (Fig. 4) or NaCl solution behaved as in clay suspensions but only under highly viscous, saturated salt conditions.

The preponderance of anhydrous cations in the presence of the clay may be the result of (i) changes in the activity of the quinazoline cations (hydrous or anhydrous) or water in clay suspensions relative to the activity in dilute acid solution or (ii) stereochemical blocking of the cationic site of covalent hydration by surface adsorption.

The clay would be expected to perturb the activity of the hydrous and anhydrous cations equally; therefore, any such changes would not alter the covalent hydration equilibrium. Moreover, the spectrum of a given species is not altered appreciably by surface adsorption. This finding is indicative of minimal electronic perturbation of the molecules themselves. The hypothesis that there is surface stereochemical blockage of quinazoline hydration may be ruled out because the behavior of quinazoline in electrolytes is similar to its behavior in neutral solution, and because of the dehydration effect of the clay surface in preprotonated systems. The most plausible explanation for the enhanced presence of the anhydrous cation in clay suspensions consistent with quinazoline behavior in electrolyte solutions is a substantially reduced activity of water. Water in the vicinity of a clay surface is bound by hydration forces of the exchangeable cations and the surface itself. Quinazoline cations cannot compete favorably with these inorganic species for water. Water is energetically unavailable for the covalent hydration reaction. In the saturated salt solutions, there is little unbound water available for covalent hydration. In dilute clay suspensions, the drastically reduced water reactivity is probably a property only of the surface and near-surface regions. This hypothesis is supported by the centrifugation results. The anhydrous quinazoline cations in the postprotonated system resulted from either (i) protonation of quinazoline within the surface region, or (ii) protonation outside the surface region followed by cation diffusion into the surface region prior to hydration, or (iii) protonation outside the surface region followed by hydration, and diffusion into the surface region with subsequent loss of bound water.

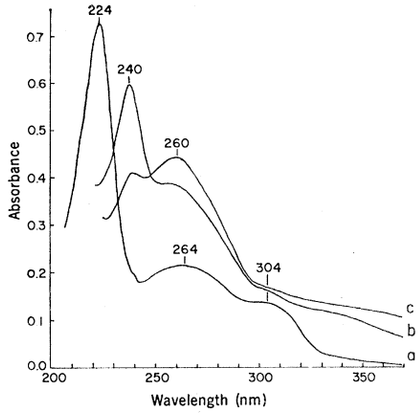

Fig. 3. The UV spectra of neutral (curve a), postprotonated (curve b), and preprotonated (curve c) suspensions containing quinazoline and Na^+-saturated montmorillonite.

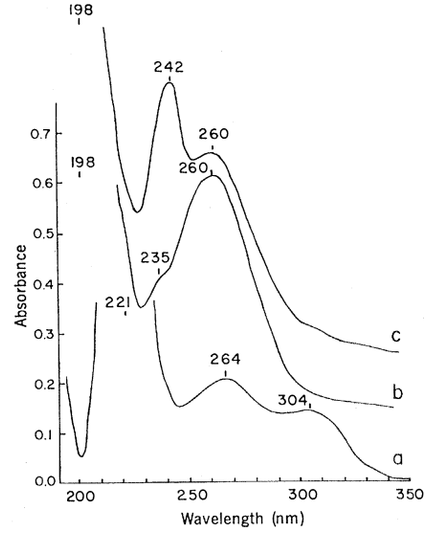

Fig. 4. The UV spectra of neutral (curve a), preprotonated (curve b), and postprotonated (curve c) quinazoline in 30 percent (by weight) $CaCl_2$ solutions.

In the preprotonated system, the covalently hydrated molecules establish an adsorption equilibrium relative to the clay surface. Those molecules lying within the surface region tend to lose covalently bound water—those outside the surface region tend to retain it.

The future possibilities of using quinazoline or other covalently hydrating organic species as molecular monitors in evaluating the properties of water adjacent to clay surfaces are numerous. Past study techniques have generally involved measurements that were sensitive to the average bulk suspension properties. Recent evidence (*10*) suggests that the properties of water at or near clay surfaces vary in reasonably discrete layers outward from the surface. Therefore, the eventual resolution of the properties of surface water will require the development of analytical methods which have greater selectivities for water in particular regions near the surface. The quinazoline study reported here is unique because it involves a direct reaction with water that can be initiated at the surface and can be easily followed with the use of modern UV-spectroscopy equipment. Because measurements are taken in the UV range, dilute suspensions as well as very dry clay films may be studied. The sensitivity of the method eliminates the need for heavy surface loading with the organic species under study.

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Murine Leukemia: A Virus-Induced Autoimmune Disease?

Abstract. *Thymocytes from mice carrying Moloney murine leukemia virus since birth are cytotoxic for normal syngeneic fibroblasts; they are much less cytotoxic for the same cells infected with this virus. The cytotoxic thymocytes appear to increase in number with age of the carrier mice and are present both during preleukemic and leukemic periods. These results suggest that lymphomas in carrier mice result from a sequence of events initiated by intrathymic destruction of normal cells by virus-infected cells, and culminating in the unrestricted proliferation of autoaggressive clones in the thymic cortex.*

The concept that clones of lymphoid cells with antiself activity may be involved in the pathogenesis of both autoimmune and lymphoproliferative disorders is not new (*1*). It has been proposed that viruses might trigger the proliferation of these clones of lymphocytes, particularly when normal immune surveillance mechanisms are disturbed (*1, 2*). These hypotheses still lack necessary experimental support, although there is evidence that autoimmune reactivity, viruses, and oncogenesis may be related (*3*). We now report that, during the course of murine leukemia virus (Moloney Strain, MuLV-M) leukemogenesis, thymus cells of congenitally infected mice, both in preneoplastic and neoplastic periods kill normal syngeneic fibroblasts in an in vitro assay but spare identically derived but MuLV-M infected fibroblasts.

We used male mice from a colony of C3/HeJ mice (Jackson Laboratories) carrying MuLV-M since birth (carriers). Normal C3H/HeJ mice served as controls. C3H/HeJ embryo cell lines of common origin infected with MuLV-M or not infected were used as target cells for thymocyte cytotoxicity assays. By an infectious center assay, 90 to 100 percent of the infected target cells were producing infectious MuLV. Uninfected target cells were negative for virus. The establishment of the carrier colony, the target cell lines, and the infectious center assay have been described (*4*).

Thymocyte suspensions were prepared from the pooled thymus glands of three to four carrier or normal mice (*4*). Both weanling (4 weeks old) and adult (12 weeks old) mice were used, depending on the experimental design. After purification of the cells on Ficoll-Hypaque gradients (*5*), adherent cells were removed by incubating the cells recovered from the gradient interface in plastic culture dishes for 30 minutes at 37°C. The procedure was repeated once, and the remaining nonadherent cells were then used. Thymocyte viability was 98 to 100 percent (trypan blue exclusion). These cells had the morphological appearance of small- to medium-sized lymphocytes. Thymocyte extracts were prepared by sonic disruption of cell suspensions (Raytheon Sonifier; 3 amp for 2 minutes).

The thymocyte cytotoxicity assay (*4*) measures both cytotoxicity and inhibition of cell replication. The cells were placed in Falcon Microtest II plates (approximately 150 target cells per well). After 18 hours, thymocytes (5×10^5) were added to each well. The plates were incubated for 48 hours, the thymocytes were removed, and the target cells were fixed, stained, and counted. When uninfected fibroblasts are placed in Falcon Microtest II plates 48 ± 1.4 percent of the cells survive for 18 hours whereas 39 ± 1.4 percent of infected fibroblasts survive for 18 hours after their addition to the wells. These differences are significant ($P < .01$ by paired *t*-tests) and are reflected in Tables 1 and 2. However, the differences in the number of cells that survive do not interfere with interpretation of the table, since interpretation of reductions in number of target cells by carrier thymocytes should always be compared with reduction of that same target cell by normal thymocytes (group comparisons in Tables 1 and 2 should be made horizontally).

Thymocytes from adult carrier mice were combined in vitro with syngeneic normal or MuLV-M infected target cells. These thymocytes were cytotoxic for normal target cells, but less so or not at all for infected target cells ($P < .01$ by paired *t*-test) (Table 1). The

Table 1. Reactivity of thymocytes from MuLV-M carrier and normal C3H/HeJ mice against infected and noninfected target cells. Each mean value (± standard error) was derived from at least ten replicate observations. Both cell lines are routinely passed in parallel and are used at the same passage level for a given experiment; NT, not tested.

Target cells	Mean number of target cells remaining and reduction (%) after reaction with thymocytes							
	Adult normal	Reduction*	Adult carrier	Reduction	Weanling normal	Reduction	Weanling carrier	Reduction
Infected	63 ± 3.3	0	58 ± 3.6	8	75 ± 6.3	0	74 ± 3.3	0
Noninfected	108 ± 8.6	0	26 ± 2.5	76†	112 ± 8.3	0	70 ± 4.5	38†
Infected	18 ± 1.9	0	21 ± 1.4	0	17 ± 1.2	6	18 ± 9.0	0
Noninfected	38 ± 14.6	0	8 ± 0.2	79†	38 ± 1.1	0	28 ± 1.0	26†
Infected	55 ± 4.8	0	62 ± 6.3	0	NT		NT	
Noninfected	62 ± 5.8	0	39 ± 3.2	37†	NT		NT	
Infected	45 ± 2.7	0	40 ± 3.3	11	NT		45 ± 3.6	0
Noninfected	157 ± 13.1	0	35 ± 2.8	78†	NT		50 ± 3.6	68†

* Percent reduction of infected or noninfected target cells is relative to the reduction by normal thymocytes on infected or noninfected target cells. † Significant differences ($P < .01$ by paired *t*-tests) compared with the effect of adult normal or weanling normal thymocytes on noninfected target cells.