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REDUCTION OF ARYL AZIDES BY THIOLS: IMPLICATIONS FOR THE USE OF PHOTOAFFINITY REAGENTS

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Aryl azides are rapidly reduced by dithiothreitol at room temperature to the corresponding aryl amines. Glutathione and 2-mercaptoethanol react much more slowly. The relevance of this reaction to experiments involving aryl azide photoaffinity reagents is discussed.

Since their introduction in 1969 as the photolabile group in photoaffinity reagents,[1] aryl azides have been incorporated into a wide variety of photoactivated reagents.[2] One important reason for the popularity of aryl azides has been their chemical stability under physiological conditions in the dark.[2] We have found, however, that aryl azides are rapidly reduced to the corresponding amines by dithiothreitol (DTT) at physiological pH and temperature. The commonly used monothiols, glutathione (GSH) and 2-mercaptoethanol, will also reduce aryl azides, but much more slowly.

Cartwright, et al.[3] have observed that 8-azidoadenosine derivatives are rapidly reduced by dithiols to the 8-amino compounds. However, these authors did not detect the reduction of phenyl azide under the same conditions. Our results indicate that reduction of aryl azides by dithiols is a general reaction.

MATERIALS AND METHODS

Materials. Azides I-IV (Figure 1) were prepared by the action of sodium azide on the corresponding diazonium salt (I,[4] II,[5] III,[6,7,8] IV,[9]). Azide V was prepared by the reaction of 1-fluoro-2-nitro-4-

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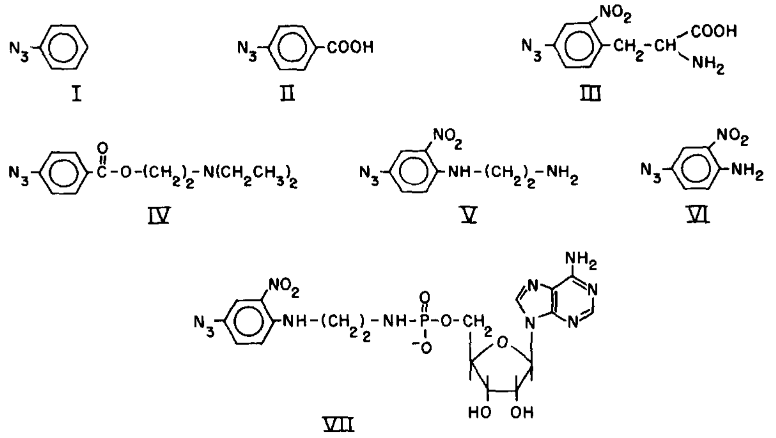

Figure 1. Structures of Aryl Azides Used.

azidobenzene[1] with excess ethylene diamine in dimethylformamide.[10] VI was prepared from 1-fluoro-2-nitro-4-azidobenzene and ammonia.[2] VII[10] was made by coupling V to AMP by the method of Goody and Eckstein.[11] 2-Mercaptoethanol, reduced glutathione, and DL-dithiothreitol were purchased from Sigma.

The following buffers (100 mM) were made in distilled-deionized-distilled water: pH 6.0, 4-morpholinoethanesulfonic acid-KOH; pH 7.0, potassium phosphate; pH 8.0, Tris-HCℓ; pH 9.0, sodium pyrophosphate; pH 10.0, glycine-KOH; pH 11.0 and pH 12.0, potassium phosphate. Stock thiol solutions in buffer were adjusted to the required pH, stored in tightly stoppered vials, and used within 48 h.

Methods. Ultraviolet and visible absorption measurements were made on a Perkin-Elmer PE 575 spectrophotometer. NMR spectra were recorded with a Varian HFT-80 instrument, and infrared spectra on a Perkin-Elmer 727 spectrometer. Unless otherwise stated, all experiments were done at 25°C in the dark.

Thin layer chromatography was performed on fluorescent silica gel plates using appropriate eluants. Approximate half-lives were determined by estimating the extent of reaction visually from the fluorescence quenching of the starting material and product.

Ion-exchange chromatography was performed on washed Dowex 50W resin. The product amines bound to the acid form of the resin and were eluted with aqueous ammonia.

Initial rates for the reductions were measured by the increase in absorbance at 550 nm (for compound V) or the decrease at 250 nm (compound I) in the appropriate buffer.

RESULTS AND DISCUSSION

The reactions of compounds II - VII with DTT were followed by thin layer chromatography (tlc). Only one product was found in each case. The half-lives of these azides were found to be similar (approximately 5-10 min for III - VII and ∿ 15 min for II), using 2 mM aryl azide and

10 mM DTT at pH 8.0. In the presence of higher concentrations (50 mM) of a monothiol (GSH or mercaptoethanol), only traces of reduction product could be detected after 4 h at pH 8.0 and 25°C. Prolonged incubation (24 h) of V (the most readily reduced azide) leads to ∿ 60-70% reduction by GSH compared with ∿ 10-20% by mercaptoethanol.

The reduction of I was followed by monitoring the ultraviolet absorbance at 250 nm. The half-life of I at pH 8.0 in the presence of 50 mM DTT was found to be 5.5 min at 25°C. All the aryl azides investigated were found to be stable for days in the absence of thiols under these conditions. Azides II, V, and VI were reduced with DTT on the milligram scale. A gas (presumably N_2) was evolved and each azide was converted quantitatively to a single product, as judged by tlc. These products were isolated by ion-exchange chromatography and were identified as the corresponding amines by NMR, ultraviolet and infrared spectroscopy, and by tlc. The remaining amines were characterized by tlc against authentic samples of amine (III and IV), by infrared (III), and by ultraviolet (I and VII) spectroscopy.

To characterize the reduction more thoroughly, kinetic studies were undertaken with compound V. The rate of reduction is first order in both azide and DTT, and the second order rate constant is 0.41 $M^{-1}\ s^{-1}$ at pH 8.0 and 30°C. The initial rate of reaction of GSH and 2-mercaptoethanol are 0.002 and 0.001 times that of DTT, respectively. The same difference in rates between monothiols and DTT has been qualitatively verified for I - IV, VI, and VII. GSH appears to reduce all the aryl azides at approximately twice the rate of mercaptoethanol.

The rate of reduction is pH-dependent (Figure 2), in agreement with the results of Cartwright, *et al.*[3] The apparent lack of reactivity of the fully protonated dithiol argues against a concerted reduction followed by prototropy and loss of N_2, but there are a number of reasonable pathways involving the addition of the monothiolate

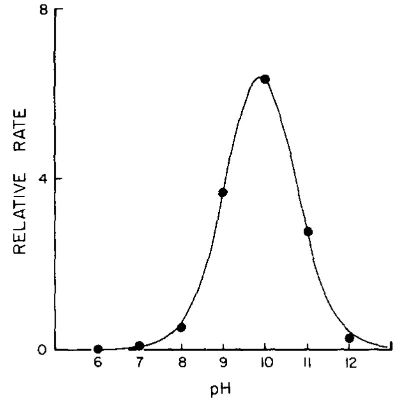

Figure 2. pH-Dependence of the Reduction of Compound V by Dithiothreitol. For details, see the text. The line is theoretical, for apparent pK_a values at 9.1 and 10.7.

to the azide that are consistent with this pH-dependent behavior (for one of these, see ref. 3). The second order rate constant for the reduction of V at pH 9.0 is approximately 2.9 M^{-1} s^{-1} at 30°C, which can be compared to the value of 7.41 M^{-1} s^{-1} at 25°C observed by Cartwright, et al.[3] for the reduction of 8-azidoAMP at pH 8.9. In contrast to these authors, however, we have found that phenyl azide is also reduced by dithiols at a rate that is only ten times slower than that found for compound V under equivalent conditions. This implies that phenyl azide is reduced about 25 times more slowly that 8-azidoAMP, which possibly made the phenyl azide reduction more difficult to observe.[3] The relatively faster rates of reduction of electron-deficient aryl azides lends some support to a pathway involving rate-limiting nucleophilic addition to the azide group.

The results presented here show that for experiments involving aryl azide photoaffinity reagents in biological systems that require thiols for activity, DTT should not be used. Monothiols should be used with appropriate caution. [For experiments where thiols are not required for activity, we have found that low concentrations of monothiols are useful as efficient scavengers[9] in photoaffinity labeling experiments using aryl azides.] In addition, our results suggest that in photolabeling studies with reagents that are very tightly bound by their receptors, DTT might be used to reduce any unbound reagent immediately prior to photolysis, thereby decreasing the level of non-specific labeling.

We are currently investigating the generality of this reaction. Preliminary results indicate that alkyl azides are also rapidly reduced by DTT under very mild conditions.

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