Ions Modify the Strength of Interaction of Diquat and Paraquat with Some Proteins and Cellulose

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The interaction of the bipyridylium herbicides diquat and paraquat with the proteins gliadin and human serum albumin (HSA) as well as with cellulose and the effect of various ions on the strength of interaction were studied by charge-transfer chromatography. Both compound showed very similar behaviour. The ions decreased in each case the strength of interaction, the effect depended nonlinearly on the ion concentration and was of saturation character. Gliadin showed the weakest interaction. HSA binds the bipyridylium compounds in an ion-dependent manner, the strength of interaction is higher than that of cellulose at higher ion concentration. This finding indicates an ion-mediated interaction of unknown character between the bipyridylium herbicides and HSA.

Introduction

Paraquat and diquat are nonselective contact herbicides extensively used in the up-to-date agrochemical practice. In green plants, bipyridylium herbicides compete for electrons from the primary electron acceptor of photosystem I. The free radicals formed are reoxidized by molecular oxygen giving rise to superoxide anion radicals [1], which can be either a potent oxidant by forming hydroxyl radicals or a reductant by forming singlet oxygen [2]. Bipyridyles cause wilting of leaves due to the light-dependent degradation of biological membranes [3] and induce lipid peroxidation and oxidation of cellular components [4].

Bipyridylium herbicides are also toxic to non-photosynthesizing organisms. The various organisms differently react to paraquat exposure: *E. coli* accumulates the paraquat but *Dunaliella salina* does not [5]. Retention of paraquat to *E. coli* was greatest after exposure at pH 7 than at pH 5. EDTA, 8-hydroxyquinoline and o-phenanthroline prevented the lethal effect of paraquat [6]. The availability of amino acids in the medium also influences the toxicity of paraquat to *E. coli* [7] and the bacteriostatic effect was overcome by amino acids [8]. Salts inhibit the uptake of paraquat in *E. coli* [9].

Paraquat damaged not only membrane structures but also reduced total lung alkaline phosphatase [10] and acetylcholine esterase activity in mammals [11]. The compound is sequestered in the tissues of rats using subcutaneous toxic low dose [12], however, no evidence was found for significant covalent interaction between diquat and tissue proteins or lipids in rats [13]. It was established that N-acetylcysteine may afford some therapeutic effect against paraquat toxicity [14]. An elevation of glutathione level or an increase in glutathione S-transferase activity may also play a certain role in protective mechanisms [15]. By an other research group direct correlation was found between the increase of glutathione peroxidase activity and paraquat resistance [16].

These findings suggest that paraquat and diquat are able to interact noncovalently with proteins. It was supposed that they function as reversible inhibitors for the anionic sites in proteins [11], but it was further proved in other systems that paraquat also binds to cationic sites [17].

The objectives of our work were to study the interaction of paraquat and diquat with cellulose and with the proteins human serum albumine (HSA) and gliadin, to assess the effect of ions on the strength of interaction and to correlate the effect of ions with their physicochemical parameters.

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Materials and Methods

HSA was the product of Human Vaccine Works (Budapest, Hungary), gliadin was prepared from wheat grain as described in Ref. [18–19]. To study the interaction of both bipyridylium herbicides with proteins cellulose powder for thin-layer-chromatography (Merck, Darmstadt, F.R.G.) was mixed with the proteins in 9:1, 19:1 and 99:1 w/w ratios. Layers of 20 × 5 cm (0.25 mm thickness) were prepared from the mixed slurries and after drying their UV spectra were determined with a Model CS-930 Dual Wavelength TLC Scanner (Shimadzu, Kyoto, Japan). Separate experiment were carried out to find the absorption maximum of the adsorbed paraquat and diquat. 5 µl of solutions were spotted on unmixed cellulose layer, the UV spectra were determined as described above.

As the bipyridylium herbicides were easily detectable even at the highest protein:cellulose ratio (1:9 w/w), this mixed sorbent was used in the experiments to study the interaction between the bipyridylium herbicides and the proteins. The use of unimpregnated cellulose layer as reversed-phase sorbent was motivated by the theoretical considerations stating that any layer may behave as a reversed-phase layer when the stationery phase is less polar then the mobil phase [20]. The validity of the hypothesis outlined above was proved to be true for unimpregnated cellulose [21]. Paraquat and diquat were separately dissolved in methanol at the concentration of 2 mg/ml. 5 µl of this solution was spotted onto the plates. Distilled water and LiCl, NaCl, KCl, CsCl, $MgCl_2$ and $CaCl_2$ solutions in the concentration range of 0.125–6 mM were used as eluents. Unmixed cellulose layers served as control. After development the plates were dried at 105 °C and the maximum of the herbicide spots was determined with the same TLC scanner at 270 and 310 nm for paraquat and diquat, respectively. The R_M values characterizing the lipophilicity were calculated in each case according to the equation given by [22]:

$$R_M = \log (1/R_f - 1).$$

As it was assumed that the protein and salt concentration and the interaction between proteins and salts may influence simultaneously the R_M value of paraquat and diquat we used stepwise regression analysis to elucidate this problem [23]. The calculations were carried out twice, the R_M values of diquat and paraquat being separately the dependent variables. In both cases the salt concentration (mM), the square of salt concentration, the cation charge, the cation radius, the concentration of HSA and gliadin (w %) in the support, as well as the characteristics of ions multiplied by the protein concentrations (altogether 12 variables) served as independent variables. The number of accepted variables was not limited, the significance level for each independent variables was set to 95%. When the coefficient of variation of the parallel determinations was higher than 8%, the data were omitted from the calculations.

Results and Discussion

Paraquat and diquat show a characteristic absorption maximum at 270 or 310 nm (Fig. 1) that is their spots can be easily determined even at the higher protein/cellulose ratio, the absorbance of the layer being low at these wavelengths. The spots of both herbicides were symmetric and did not show any tailing in each chromatographic system (Fig. 2).

The salts decreased the R_M value of paraquat and diquat on each layer (Fig. 3). The retention behaviour of bipyridylium herbicides was similar on cellulose and on cellulose + HSA surfaces at lower salt concentration range. At higher salt con-

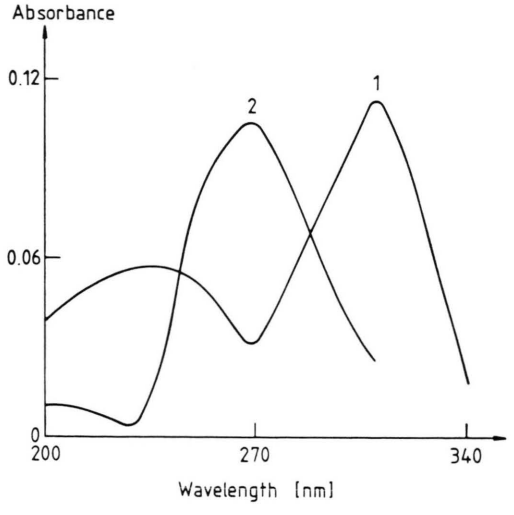

Fig. 1. Ultraviolet absorption spectra of 10 µg diquat (1) and paraquat (2) adsorbed on cellulose:gliadin (9:1 w/w ratio) surface.

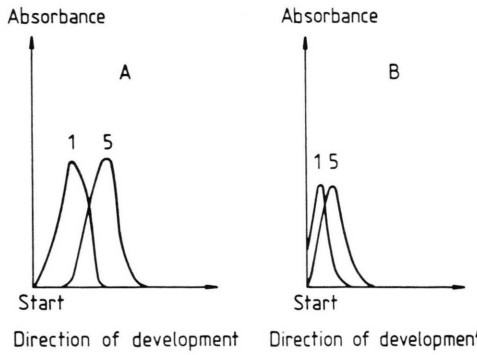

Fig. 2. Effect of NaCl concentration in the eluent on the mobility of 10 μg diquat (A) and paraquat (B) on unmixed cellulose layer. 1. 1 mM NaCl, 5. 5 mM NaCl.

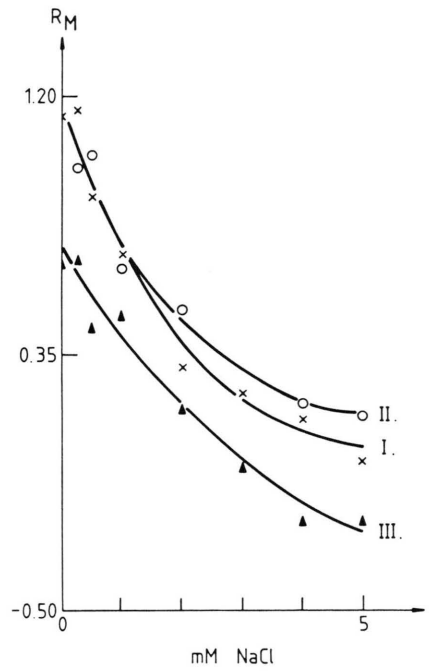

Fig. 3. Effect of NaCl concentration on the R_M value of 10 μg diquat. I. Cellulose, II. Cellulose + human serum albumin (9:1 w/w ratio), III. Cellulose + gliadin (9:1 w/w ratio).

centrations HSA more strongly retained the herbicides than cellulose did. This finding indicates that HSA shows a higher affinity to bind paraquat and diquat than cellulose and the binding depends on the ionic environment. The curve of cellulose + gliadin is fairly parallel to that of cellulose suggesting that the ions have similar effect on the herbicide–cellulose and on the herbicide–gliadin interaction. The retention was always lower on gliadin than on cellulose. That means the binding on cellulose is stronger than on gliadin. The considerable difference between the binding character of both proteins suggests that the binding of paraquat and diquat to proteins may be highly selective and may markedly depend on the structure of protein. This observation is in good correlation with our earlier results. It was proved by charge-transfer-chromatography that paraquat and diquat readily bind to polar (dibasic and dicarboxylic) amino acids in aqueous systems [24] and the binding is of noncovalent character. Differential scanning calorimetry detected direct interaction between the bipyridyles and the proteins papain and lysozyme in water and the strength of interaction depended strongly on the structures of proteins investigated [25].

The type of salt also has a considerable impact on the interaction (Fig. 4). The monovalent cations decrease the lipophilicity to a lesser extent than divalent ones indicating the role of ion charge in the interaction. This finding suggests that the interaction of paraquat and diquat with proteins is mainly of hydrophilic character. The R_M value did

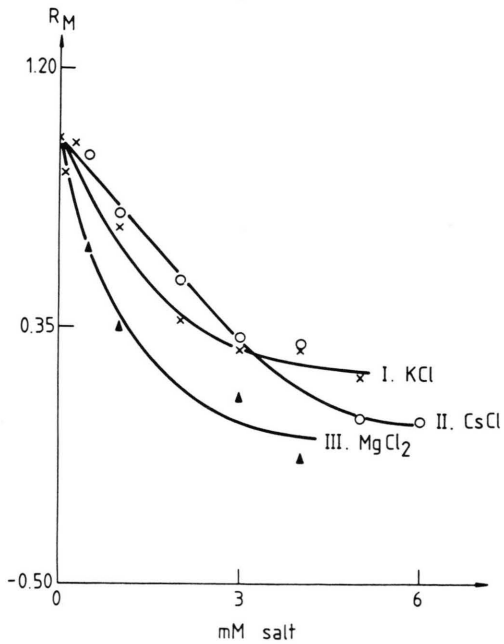

Fig. 4. Effect of salt concentration on the R_M value of 10 μg diquat on cellulose + human serum albumin layers. I. KCl, II. CsCl, III. $MgCl_2$.

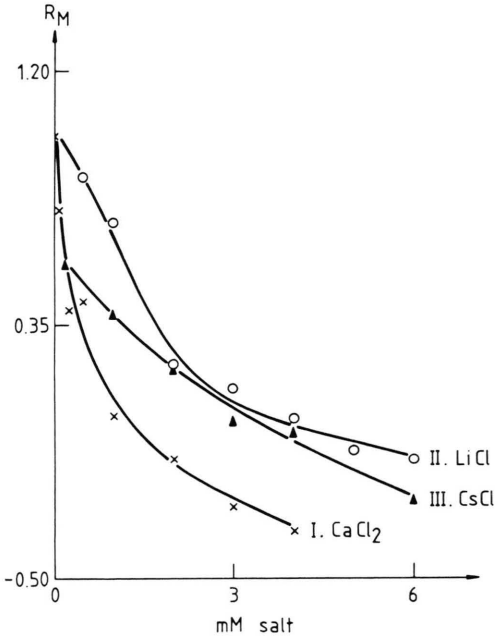

Fig. 5. Effect of salt concentration on the R_M value of 10 µg paraquat on cellulose + gliadin layers. I. $CaCl_2$, II. LiCl, III. CsCl.

not decrease linearly with the growing salt concentration, the relationship was of saturation character (Fig. 5). This result can be explained by the supposition that the dissociated ions (the salts are practically completely dissociated in water at such a low concentration) compete for the adsorption sites with the bipyridyles. When the majority of the binding sites is occupied the further increase of salt concentration has not any effect on the retention of the bipyridyles investigated.

The results of stepwise regression analysis are compiled in Table I and II for paraquat and diquat, respectively. The equations selected by stepwise regression analysis fit well to the experimental data, the significance level was in both cases over 99.9% (see calculated F values). The equations explain about 83–85% of the total variance, that is the independent variables account for the overwhelming majority of the change of the R_M value (see r^2 values). Both equations are highly similar, that means that the binding of paraquat and diquat to cellulose and to proteins are governed by similar physicochemical forces and the differences in their effect and toxicity cannot be explained by their binding to cellulose and to these two proteins. The regression coefficient of the concentration of various salts added to the eluent (x_2) is negative that is the salts significantly decrease the lipophilicity. This result is in perfect accordance with

Table I. Effect of various external parameters on the lipophilicity of paraquat. Results of stepwise regression analysis. $R_M = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_1x_4$ where x_1 = concentration of various salts in mM, x_2 = cation charge, x_3 = gliadin concentration in the support, x_4 = human serum albumin concentration in the support. $n = 118$, $a = 126.9$, $F_{calc.} = 105.8$, $r^2 = 0.8256$

Parameter	Number of independent variables				
	1	2	3	4	5
b	−41.1	−21.9	−1.40	3.95	0.42
s_b	3.38	3.20	0.38	0.62	0.15
path coeff. %*	53.66	8.87	4.97	28.08	4.42

* (Path coefficients are dimensionless numbers indicating the relative impact of the independent variables on the dependent variable.)

Table II. Effect of various external parameters on the lipophilicity of diquat. Results of stepwise regression analysis. $R_M = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1^2 + b_5x_2x_3 + b_6x_4x_5$ where x_1 = salt concentration in mM, x_2 = cation charge, x_3 = gliadin concentration in the support, x_4 = human serum albumin concentration in the support, x_5 = cation radius.
$n = 106$ $a = 118.7$ $F_{calc.} = 91.2$ $r^2 = 0.8468$

Parameter	Number of independent variables					
	1	2	3	4	5	6
b	−44.7	−19.1	−3.84	4.79	1.53	0.86
s_b	3.60	3.88	0.94	0.69	0.67	0.34
path coeff. %*	46.83	6.53	10.99	26.39	6.31	2.95

* (Path coefficients are dimensionless numbers indicating the relative impact of the independent variables on the dependent variable.)

our previous qualitative conclusions and lends support to the hydrophilic character of the interaction. The growing cation charge (x_2) also decreases the retention of diquat and paraquat. The negative regression coefficient of the gliadin concentration (x_3) indicates that gliadin binds the bipyridylium herbicides to a lesser extent than cellulose does. This finding lends support to the assumption that cellulose in the plant cell wall may play a role in the binding of the bipyridylium compounds. However, this fact do not play any role in the paraquat resistance of some weeds, because the molecular structure of the cellulose in the cell wall of different plants is similar.

The interaction of HSA with the bipyridyles is strongly ion-dependent. In the presence of ions HSA binds the compounds more strongly than cellulose does. This phenomenon is not well understood. We suppose an ion-mediated hydrophilic binding or a salting out effect facilitating the approximation of diquat and paraquat to the active binding center of the HSA.

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