

Influence of the electric field on supramolecular structure and properties of amyloid-specific reagent Congo red

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Abstract Among specific amyloid ligands, Congo red and its analogues are often considered potential therapeutic compounds. However, the results of the studies so far have not been univocal because the properties of this dye, derived mostly from its supramolecular nature, are still poorly understood. The supramolecular structure of Congo red, formed by π - π stacking of dye molecules, is susceptible to the influence of the electric field, which may significantly facilitate electron delocalization. Consequently, the electric field may generate altered physico-chemical properties of the dye. Enhanced electron delocalization, induced by the electric field, alters the total charge of Congo red, making the dye more acidic (negatively charged). This is a consequence of withdrawing electrons from polar substituents of aromatic rings—sulfonic and amino groups—thus increasing their tendency to dissociate protons. The electric field-induced charge alteration

observed in electrophoresis depends on dye concentration. This concentration-dependent charge alteration effect disappears when the supramolecular structure disintegrates in DMSO. Dipoles formed from supramolecular fibrillar species in the electric field become ordered in the solution, introducing the modified arrangement to liquid crystalline phase. Experimental results and theoretical studies provide evidence confirming predictions that the supramolecular character of Congo red is the main reason for its specific properties and reactivity.

Keywords Congo red · Supramolecular dye · Amyloid

Introduction

Congo red has been used for years as the specific amyloid stain (Churukian 2000; Glenner 1981; Heegaard et al. 2000; Hirakura et al. 2000; Howie et al. 2008; Inouye and Kirschner 2000; Jin et al. 2003; Kaminsky et al. 2006; Linke 2000; Navarro et al. 1999). However, the question of how this particular dye can be used as a common specific reagent for amyloid aggregates derived from different proteins, hence composed of different amino acids, still remains unsolved (Chiti et al. 2001; Fändrich et al. 2001; Krebs et al. 2004; Pavlov et al. 2002; Walsh et al. 1999; Westermark 2005).

A new approach to this problem is based on the finding that Congo red—which is known to self-assemble and create supramolecular structures in water solutions—may form complexes with proteins (basically partly unfolded) in a nonstandard way, i.e., not as a single dye molecule but as a supramolecular species (Król et al. 2003, 2005; Piekarska et al. 1999; Stopa et al. 1998, 2003). This means that rod-like or ribbon-like assemblies of this dye may bind proteins

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as single ligands, although they are composed of a number of dye molecules. These supramolecular ligands include several molecules of Congo red interacting with proteins directly, thus tightly bound, and also many attached indirectly (weakly bound) as the dye-dye associated micellar portion (Piekarska et al. 2001; Stopa et al. 2006, 2010).

High plasticity of noncovalently stabilized rod-like or ribbon-like Congo red micellar species facilitates binding, allowing adjusting and fitting of their fibrillar structures to fibrillar structures of amyloids, thus becoming the reason for the common consideration that this dye is the specific amyloid reagent. It may explain the specific properties of this dye. It seems to be of particular importance that the dye in its supramolecular form may recognize amyloid-specific, backbone-derived structures rather than the amino acid sequence, which may differ (Fändrich and Dobson 2002). This is possible since the dye ligands represent noncovalently stabilized supramolecular structures of moderately variable architecture susceptible to many external factors (Jonkheijm et al. 2006; Palmer et al. 2007; Stupp et al. 1997).

The ribbon-like micellar species composed of π - π stacked Congo red molecules are sensitive to the electric field, which generates significant electron delocalization affecting the supramolecular structure of species and the arrangement in solution, and finally influencing their interaction with proteins. Facilitated formation of amyloid-like protein aggregates in the presence of Congo red seems to confirm such a mechanism (Stopa et al. 2010). Experimental and theoretical results supporting the suggested influence of the electric field on Congo red are presented in this article.

Materials and methods

Reagents

All reagents used were of analytical grade.

Congo red

Congo red (97% purity, Sigma-Aldrich, USA) was dissolved in buffer solution of pH 8.6 (0.06 M) or acetate buffer of pH 5.4 (0.1 M). It was then heated in boiling water for 10 min, cooled slowly to room temperature, and used directly in experiments.

The dissociated—non-supramolecular—form of Congo red was obtained in experimental conditions using a buffer with a suitable DMSO concentration for making agarose gel and the appropriate Congo red solution. For the complete Congo red dissociation a 1:4 DMSO to water molar ratio was used (Skowronek et al. 1998), while a limitary

decreased self-assembling tendency was obtained at 1:8 DMSO:water ratio.

Congo red (5 mg/ml) complexation with rhodamine B (1 mg/ml) was performed by heating the mixture in a boiling water bath for 10 min and then cooling slowly to room temperature.

The registration of the decay of the phenolphthaleine spectral band: Alteration of Congo red acidity upon the influence of the external electric field was studied using phenolphthalein as the pH indicator. The pH alteration was registered spectrophotometrically as the decay of the specific spectral band of the indicator. A special 10-cm-long, 1.5-cm-wide, and 1.8-cm-high vessel was used to follow the process. It was filled with agarose gel made in a solution of 0.15% NaCl and Congo red 0.2 mg/100 ml, adjusted to pH 8.25. A 1.5-cm gap in the gel was left in the middle of the vessel right in the optical path. The vessel was then filled with the above solution with added phenolphthalein. Carbon electrodes were installed at both sides of the vessel, thus being separated from the gap by a 3.7-cm layer of the gel. Direction of the current was changed every 30 s to prevent the migration of dyes. Spectral changes were registered continuously.

Agarose electrophoresis

Standard agarose electrophoresis was performed in 1% gel in 0.06 M veronal buffer, pH 8.6.

Evaluation of self-assembling by measurement of the hydrodynamic radius

Hydrodynamic radii of supramolecular dye assemblies were measured using the Dynamic Light Scattering (DLS) method with a DynaPro MS 800 instrument (Protein Solutions, Inc., USA). Measurements were performed at 25°C in 0.06 M veronal buffer, pH 8.6, 0.15 M NaCl. A Hitachi spectrophotometer was used for optical analyses.

Theoretical calculations

Generation of the micellar structure

Starting structures of Congo red micelles, consisting of four to ten molecules, were generated using in-house software. Congo red molecules were in the amphoteric state with amino and sulfonic groups ionized. All micelles were antiparallel—every other molecule was rotated by 180 degrees around the long internal molecule axis to produce an antiparallel mutual orientation of dipole moments. The protocol of micelle generation is described elsewhere (Roterman et al. 1993; Spólnik et al. 2007) and is based on a fan-like organization with three parameters

describing mutual orientation of molecules. Briefly, a source Congo red molecule is placed with the central benzidine bond in the origin of the coordinate system; the bond defines the x-axis, and a benzene ring plane defines the xy-plane. The positions of other molecules are determined using three transformations: Tx—translation along the x-axis (which expresses the radius of curvature of the fan-like organization—the Tx = 0 produces a stack of molecules), Rz—rotation around the z-axis, and Tz—translation along the z-axis. In all micelles analyzed in this work, Tz was fixed at 3.8 Å—the optimal van der Waals distance between consecutive Congo red molecules, Tx = 0 and Rz = 15 degrees. Micelles created with this set of parameters have optimal stacking of biphenyl fragments—each fragment is rotated by 15 degrees with respect to the lower and upper one. Such an arrangement guarantees low energy conformation of micelles and enables charge-transfer interactions.

Electron density distribution of Congo red

Distribution of electron density for all micelles was calculated for a fixed geometry with the Gaussian 09 package (Frisch et al. 2009) and PM6 semiempirical method (Stewart 2007). Since the studied micelles are large (up to 700 atoms), we could not afford geometry optimization. Distribution of electron density for each micelle was calculated with and without the external electric field. The field was applied along the Z axis (long axis of the micelle, perpendicular to the long axis of a molecule) with the intensity of 0.002 au (approx. 0.1 V/Å). Electron density maps and their differences were calculated with cubgen and cubman utility programs from the Gaussian 09 package. All figures were prepared with the VMD program (Humphrey et al. 1996).

Detection of noise in molecular systems

Since its introduction, molecular information theory (Nalewajski and Parr 2000) has been an interesting tool for describing various molecular properties, e.g., bond order (Nalewajski 2006). The convergence entropy is expressed as:

$$D_{\text{KL}}(p/p^0) = \sum_{i=1}^N p_i \log_2(p_i/p_i^0) \quad (1)$$

where: D_{KL} —convergence entropy, p —probability of a particular observed event, p^0 —probability in reference distribution. The index “ i ” denotes a particular molecule (orbital). N denotes the number of amino acids in the polypeptide chain. With Eq. 1 transformed into the probability distribution expressed by electron density functions, the equation takes the following form:

$$\begin{aligned} H(\psi_k|\chi) &= \sum_i^{\text{AO}} P(\chi_i)P(\psi_k|\chi_i)\log(P(\psi_k|\chi_i)) \\ &= \sum_i^{\text{AO}} n_i/N|c_{k,i}|^2\log(|c_{k,i}|^2) \end{aligned} \quad (2)$$

where: ψ_k —considered k th molecular orbital (MO), χ_i , $P(\chi_i)$ — i th atomic orbital (AO) and its probability, $H(\psi_k|\chi)$, $P(\psi_k|\chi_i)$ —conditional entropy and conditional probability, which determines a chance to find an electron from the i th atomic orbital χ_i on certain k th molecular orbital ψ , n_i —electron population on χ_i , N —number of all electrons in the system, and $c_{k,i}$ —wave function coefficient.

In this approach the micelle is treated as a communicative channel contaminated with external noise. The average noise can be calculated according to Eq. 2. Different numbers of molecules (4, 5, 6, and 10 molecules) were taken for calculation to estimate the dependence of the noise on the size of a micelle.

Results and discussion

Supramolecularly-derived Congo red properties: the effect of concentration-dependent electrophoretic dye migration

The electrophoretic anodal migration velocity of Congo red fractions increases with increased dye concentration (Spólnik et al. 2007; Stopa et al. 2010). This unusual effect reaches the plateau at high dye concentration. It suggests that Congo red may undergo essential intramolecular alterations as an effect of the increased concentration and/or the influence of the electric field. The association of charge alteration with the increased dye concentration definitely connects this phenomenon to the intensified self-assembling of dye molecules.

This is the obvious result of the concentration-dependent shifting of self-assembling equilibrium towards the formation of more organized supramolecular structures.

The dependence of the charge alteration effect of the dye on its supramolecular properties was shown experimentally by agarose gel electrophoresis of Congo red samples at different dye concentrations and in the presence of DMSO added to disassemble dye micellar structures (Skowronek et al. 1998). The acceleration effect disappears in these conditions, confirming that it results from the supramolecular character of Congo red (Fig. 1).

Conjugation of Congo red molecules is stabilized by π - π interactions. The resulting supramolecular dye fibrils may form species resembling nanowire structures enabling significant electron delocalization upon the influence of the electric field (De Groot and Fuller 1997; Li et al. 2006; Sardone et al. 2006).

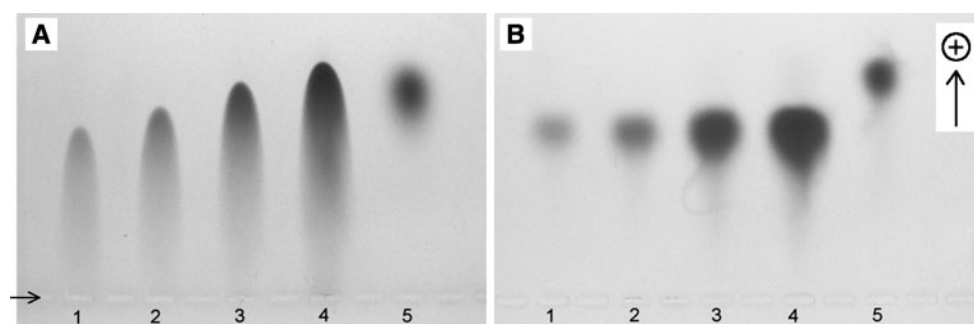


Fig. 1 Agarose gel electrophoresis of Congo red presenting: **a** The phenomenon of concentration-dependent migration velocity of the dye (lanes 1–4, concentrations: 0.5, 1.0, 4.0, and 10 mg/ml, respectively; 7 μ l samples) versus migration of bromophenol blue

(lane 5). **b** The same samples in the presence of DMSO added in order to disintegrate the supramolecular structure of Congo red (see sect. “Methods”)

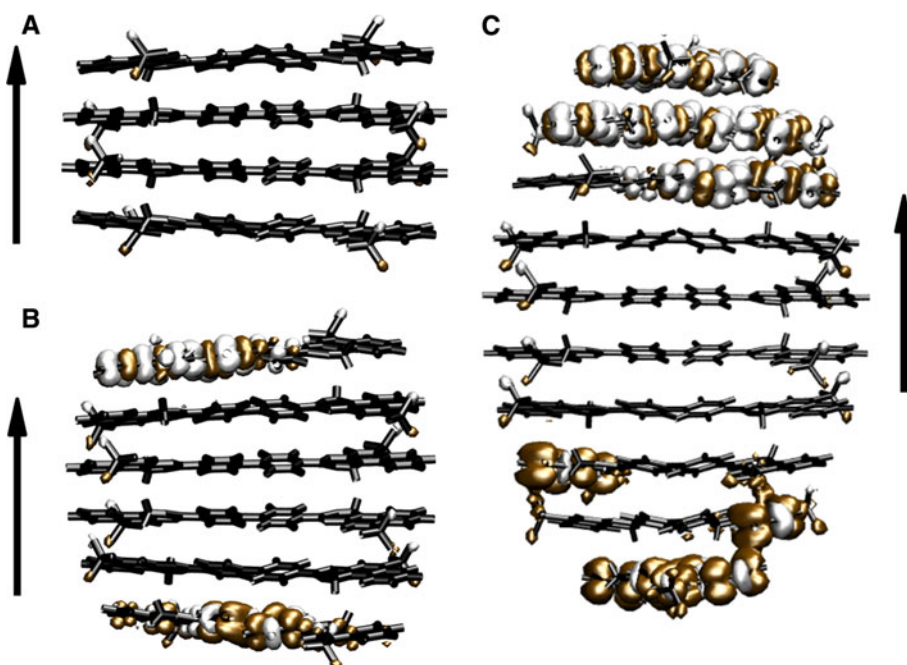
As a consequence, the electric field may induce specific organization of the supramolecular structure since the formation of dipoles and their arrangement facilitate better fitting of Congo red to conditions imposed by the external field (Gomes and Mallion 2001; Kimura 2008; Manjula-devi and Vij 2007; Schenning et al. 2004). In the current work, the effect of the external electric field on the supramolecular organization of Congo red was verified by theoretical calculation.

Distribution of electron density

Quantum chemical calculations were performed to study the differences in electron density distribution for Congo red micelles of different length with and without external

electric field applied. Differential maps showing changes in electron density as a function of the field were calculated by subtracting an electron density map obtained without the field from the density map obtained with the field. Graphical representation of the maps for four, six, and ten molecular micelles is shown in Fig. 2. For large micelles, results are as expected—application of an external field imposes separation of charge across the micelle, and there is an electron density buildup along electric field vectors. This separation of charge involves charge transfer between consecutive molecules. As a result, large micelles obtain a non-zero dipole moment in the electric field. However, rather unexpectedly, in case of a small micelle, the external field does not cause the separation of charge. This is further confirmed by the analysis of dipole moments. Figure 3

Fig. 2 Difference of electron density for micelles composed of four (a), six (b), and ten (c) Congo red molecules with and without the external electric field applied. *White areas*—positive values of density difference (more electron density when the field applied), *golden areas*—negative values of density difference (less electron density when the field applied). *Black arrows* indicate the direction of the external electric field



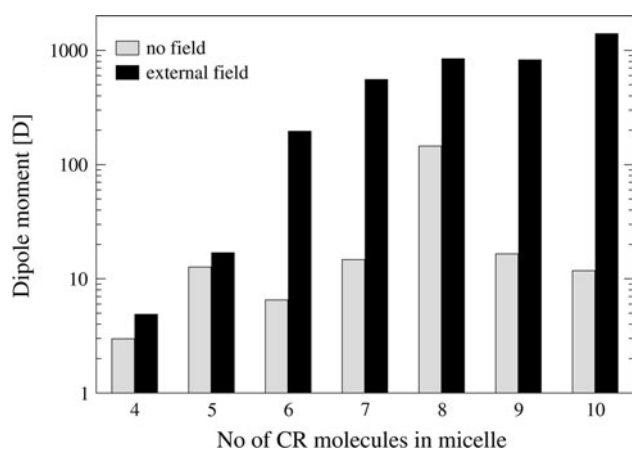


Fig. 3 Total dipole moment of a Congo red micelle [D] with and without the external electric field applied

shows the total dipole moments of all studied micelles with and without the external field applied. Analysis of the figure clearly shows that all micelles have virtually no dipole moment without the field, and separation of charge is only imposed by the external field in large micelles. Micelles composed of four and five molecules are not affected by the field in the same way as larger micelles; there is no charge transfer between consecutive molecules and no dipole moment buildup.

These results indicate that there is a minimal size of a Congo red micelle (in this study consisting of at least six molecules) that is required for charge transfer effects. This is probably due to the fact that a threshold value of electron density must be present before it can be transferred to one end of the micelle by an external field.

Detection of noise in molecular systems

The values of $H(\psi|\chi)$ for two short (4 and 5 molecules) and two long (6 and 10 molecules) micelles are presented in Table 1. We considered only three occupied molecular orbitals with the highest energy from which electrons can be easily moved by the electric field. Computations were performed using the Gaussian 09 package (Frisch et al. 2009).

Enhancing effect of intercalated rhodamine B on the concentration-dependent electrophoretic migration of Congo red

An essential support of the hypothesis that the charge alteration effect is caused by electron delocalization within supramolecular Congo red species comes from studies concerning the properties of Congo red-rhodamine B complex. Rhodamine B, as well as many other planar aromatic ring organic molecules, may be intercalated by

Table 1 Conditional entropy [nat] for three occupied molecular orbitals with the highest energies (including HOMO, ψ'/ψ'' first and second below in energy scale)

	4 Congo red molecules	5 Congo red molecules	6 Congo red molecules	10 Congo red molecules
ψ^{HOMO}	4.66e-3	2.90e-3	2.46e-3	1.38e-3
ψ'	4.65e-3	2.76e-3	2.58e-3	1.24e-3
ψ''	3.52e-3	2.76e-3	2.40e-3	1.58e-3

The level of noise for ten Congo red molecules in micelles is highest in comparison with smaller micelles (lower number of Congo red molecules). The gradual lowering of the number of nats suggests the dependence on micelle size. The dislocation of electrons can be seen in Fig. 2

Congo red micellar structures. The positive charge of the molecule significantly facilitates such complexation.

Interestingly, the binding of rhodamine B by Congo red results in the increased anodal electrophoretic migration velocity of the complex compared to pure Congo red (Fig. 4). This result is surprising, as the migration velocity of free rhodamine B is close to zero in the experimental conditions used and, consequently, the decrease of migration velocity should rather be expected upon combining of both dyes. Even the low content of rhodamine B involved in complexation exerts an evident effect on the electrophoretic migration, indicating that the complex constitutes a new integrated system and its properties are not just an average of dye properties.

The capacity of Congo red micellar species to intercalate rhodamine B is limited. On average, no more than one

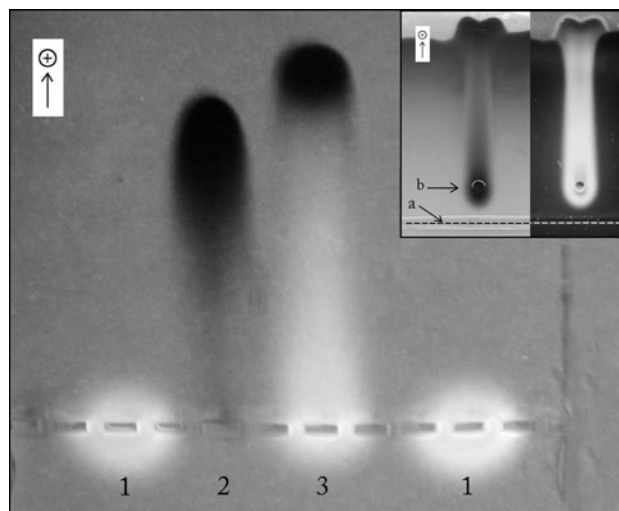


Fig. 4 The acceleration of Congo red migration upon the influence of intercalated rhodamine B molecules seen in UV. 1 Rhodamine B; 2 Congo red; 3 Congo red-rhodamine B complex. Inset The accelerated migration of Congo red caused by complexation of rhodamine B met on the way of migration and manifested as a bulge on the front of Congo red—seen in visible (left) and UV light (right). Arrows: a starting line for Congo red, and b starting point for rhodamine B

rhodamine B molecule is incorporated per 13–14 molecules of Congo red in a complex—as concluded from analysis of the fraction migrating towards the anode (see Fig. 4). The excess of rhodamine B used for complexation remains at the starting point. The increased negative charge of Congo red migrating in electrophoresis after rhodamine B complexation may be the direct result of combining the dyes, or it appears, but not until being induced by the applied electric field. In the latter case, the increased polarizability of Congo red species arising from the π - π stacking and conjugation of dye molecules and additionally facilitated by rhodamine B complexation would be the reason for charge alteration by making the supramolecular system highly susceptible to the electric field. To solve this problem the complexation of Congo red and rhodamine B was performed under the pH control, but without the influence of the electric field. This was done using the dyes dissolved in salt solution (0.9%) adjusted to the same pH value of alkaline character (pH 8.25) in order to avoid the buffering effects of containing carboxylic group rhodamine B (Mchedlov-Petrosyan and Kholin 2004). The experiment (Fig. 5) showed no pH effect, which could explain the accelerated Congo red migration, thus indicating that only electron delocalization induced by the electric field in electrophoresis is the reason for the charge alteration. Hence, the increased Congo red acidity arising upon the rhodamine B complexation observed in electrophoresis cannot appear as the direct effect of dye combination. It rather reflects an essential alteration of dye properties allowed by the facilitated electron delocalization within the supramolecular system and generated by the electric field (Hunter et al. 2001; Pergamenschik et al. 2006).

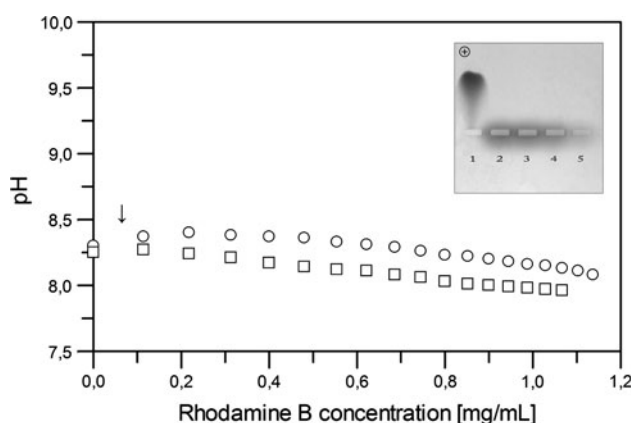


Fig. 5 The effect of rhodamine B added to Congo red (open circle) or to the salt solution (open square) on the pH. The arrow points to the Congo red/rhodamine B molar ratio found for the migrating complex in Fig. 4. Inset Agarose electrophoresis of 1 Congo red (5 mg/ml) and rhodamine B at different concentrations shown as 2–5 (1.2, 1.03, 0.8, 0.48 mg/ml, and 1.0 Congo red 5 mg/ml) showing no migration dependence on concentration in the condition used

The electric field-induced Congo red acidity registered by color decay of the pH indicator phenolphthalein

The conjugation of face-to-face stacked Congo red molecules leads to the formation of supramolecular fibrillar structures that, upon the influence of the electric field, become univocally oriented dipoles because of the delocalization of π electrons within the micellar column built from stacked aromatic rings of the dye (Barbara et al. 1996; Nakano and Yade 2003). Electron delocalization in turn affects polar groups of Congo red, altering their proton dissociation constants and, consequently, the charge. This is manifested as the accelerated electrophoretic migration of the dye toward the anode in the agarose gel at increasing dye concentration, indicating the concentration-dependent acidity of Congo red.

To investigate the effect of the electric field we registered the increasing acidity of Congo red solution when the dye was affected by the external electric field by measuring the color decay of the added pH indicator—phenolphthalein. Phenolphthalein was used as an indicator because of its nonplanar structure, inappropriate for complexation with Congo red, and because this dye when added to Congo red solution was not observed to affect its electrophoretic migration, confirming the unaltered initial properties of both dyes. A vessel suitable for the experiment was constructed allowing spectrophotometrical registration of color decay under the influence of the electric field (see sect. “Methods”).

The increasing acidity registered as the color decay of phenolphthalein proceeded for about 10 min, indicating that charge alteration is not an instant process; hence, increasing acidity of Congo red is not solely the result of electron delocalization. It is also likely associated with alteration of the supramolecular structure, which is a slow process (Fig. 6). This experimental result implied that the influence of the electric field involves two apparently independent processes: electron delocalization and some structural modification of dye species. The increased stacking interaction of Congo red molecules due to charge delocalization over the π -stacked supramolecular system, however, most likely favors the attachment of new dye molecules, modifying the structure of dye species.

Effect of the increasing intensity of the electric field on Congo red self-assembling

The concentration-dependent electrophoretic migration velocity of Congo red was interpreted as the manifestation of its supramolecular character (see Fig. 1). This effect was formerly observed to increase with the increasing voltage, suggesting voltage-dependent self-assembling (Stopa et al. 2010). To verify this effect DMSO was added to Congo red

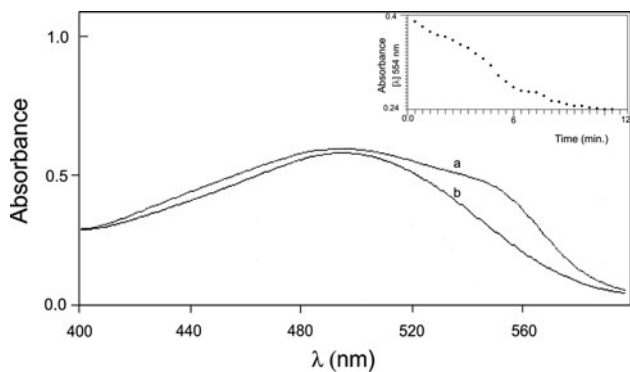


Fig. 6 The color decay of phenolphthalein in the presence of Congo red influenced by the electric field. *a* Absorption spectrum of Congo red + phenolphthalein; *b* spectrum of Congo red. *Inset* The decay of phenolphthalein color (measured at 554 nm) upon the influence of the electric field

solution until the investigated low voltage (50 V) effect of migration dependence on dye concentration disappeared (water:DMSO = 8:1). Then the voltage was increased twice. In these conditions only a slight acceleration of the dye migration velocity was observed (in particular at higher dye concentrations). The recovery of the effect appeared, however, at 300 V. This confirms the assumption that the electric field may induce self-organization of Congo red (Fig. 7). The freedom of the electron movements increasing significantly in the conjugated system of dye molecules and electron delocalization induced by the electric field enhance the stability of supramolecular Congo red structures and stimulate their growth by the attachment of new molecules. This may further explain the phenomenon of concentration-dependent migration of Congo red in electrophoresis.

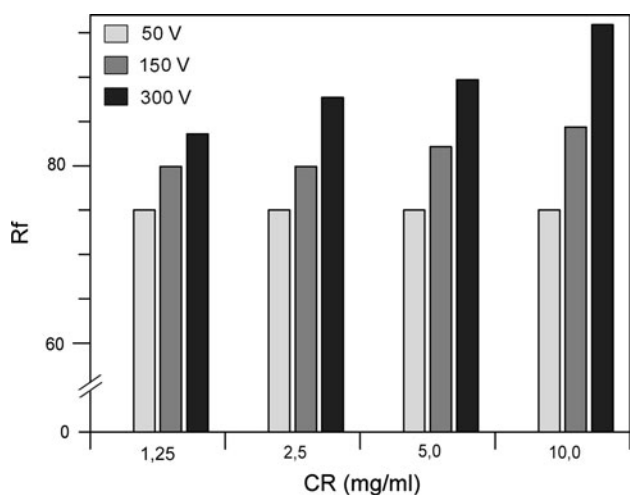


Fig. 7 The recovery of the suppressed by DMSO concentration-dependent Congo red migration effect after using the electric voltage to influence supramolecular dye systems. (DMSO:water ratio equal 1:8)

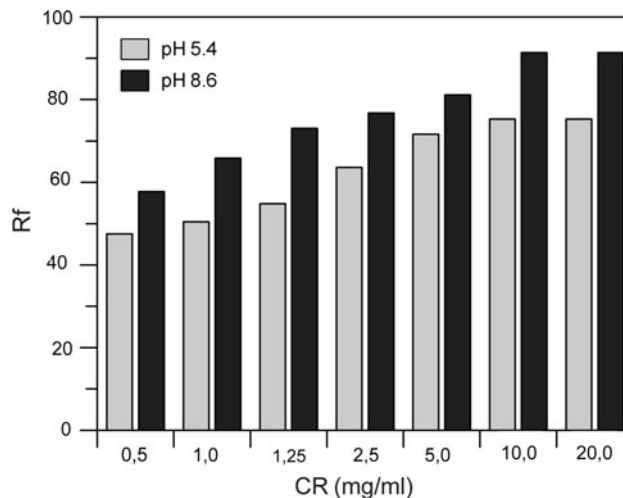


Fig. 8 The effect of different pHs on the relative electrophoretic mobility of Congo red (vs. bromophenol blue) studied at different dye concentrations. The migration difference corresponds to environmental pressure (pH) on the proton-binding tendency to sulfonic and amino groups

The role of Congo red polar groups in increasing the acidic character of the dye upon electric field influence

The mechanism of increasing Congo red acidity upon the influence of the electric field may be explained by the altered dissociation equilibria of the dye sulphonic and amino groups affected by delocalization of electrons in the supramolecular form of the dye.

The direct connection of the charge alteration effect with polar groups of the dye was revealed by the comparison of the relative electrophoretic migration of Congo red (measured vs. bromophenol blue) at different pH values—5.4 and 8.6, thus at higher and lower tendency to accept protons by Congo red proton binding groups (Hunter et al. 2001; Mignon et al. 2005a, b; Sinnokrot and Sherrill 2003, 2004; Venkataraman et al. 2006).

The resulting different relative electrophoretic mobility of Congo red at different buffer pH values, higher at 8.6 and lower at 5.4, confirms the direct involvement of amino and sulphonic groups in charge effects revealed by electrophoretic analysis of Congo red (Fig. 8).

Structural background of the concentration-dependent migration effect of Congo red in electrophoresis

The concentration-dependent migration of Congo red in electrophoresis indicates structural reasons for this phenomenon. The effect is clearly the evidence of qualitative changes that may derive from the alteration of the supramolecular dye structure. The most probable change is the elongation of ribbon-like micellar structures.

Supramolecular Congo red species have a fibrillar character, and hence, in contrast to globular micelles, their size cannot be univocally determined. Depending on the concentration, they may grow or become shorter.

Face-to-face stacking of Congo red molecules creates a supramolecular structure that is convenient for the electron delocalization along the ribbon-shaped micelle. The defined electron delocalization induced by the electric field turns ribbon-shaped dye micellar structures into dipoles. Since neither the dipole moment nor the length of noncovalently stabilized supramolecular structures of Congo red can increase permanently, the curve presenting the concentration-dependent migration velocity of this dye in electrophoresis is non-linear, aiming finally, at higher dye concentrations, to the plateau (Fig. 9). As a consequence, only the approximate size of supramolecular species can be considered in measurements. The micellar species of Congo red comprise 10–15 molecules (for dye concentrations 0.5–4 mg/ml) as determined by dynamic light scattering (DLS) measurements. As suggested by experimental results, these values seem to increase when influenced by the electric field.

The question of why the negative charge of Congo red micellar species increases with the increasing concentration of the dye, as registered in electrophoresis, may be explained based on the behavior of its polar substituents—the NH_2 group in particular. The pK of the Congo red NH_2 group (about 5) (Spólnik et al. 2007) determines its being uncharged at neutral and alkaline pH. It may, however, accept the proton in the assembled form of dye molecules being induced by the negative charge of sulfonic groups belonging to neighboring dye molecules.

The decreased electron density, which is the effect of electron delocalization generated by the electric field, favors the dissociation of protons from both amino and

sulfonic groups. However, more molecules seem to be affected because of polarization of their bonds in dipoles formed from longer micellar structures (hence at higher dye concentration) than in shorter ones. As a result, their acidity increases more.

Conclusion

The structural basis of Congo red interaction with amyloids is still an unsolved problem, although it has been studied since the recognition of amyloidosis as a clinical unit. The high tendency of Congo red molecules to self-assemble and form supramolecular structures makes properties of this dye promiscuous and difficult to define. Progress in understanding Congo red-amyloid interactions has come with the finding that the dye may bind proteins as a supramolecular ligand and not in the standard way as individual dye molecules (Roterman et al. 1998; Stopa et al. 1998; Piekarska et al. 2001). Still, however, the dye and its interaction properties need further study. This paper is one step along the way. The properties of Congo red modified by the electric field are described here. Face-to-face stacking of Congo red molecules leads to the formation of columnar micellar structures (Skowronek et al. 1998; Król et al. 2005). The conjugation of aromatic rings of the dye molecules in a face-to-face manner allows the involvement of their π electrons in complexation. The complexation in turn increases the possibility of charge delocalization over the stacked system. Such a structure becomes susceptible to the influence of the electric field, which imposes a significant delocalization of electrons and thus causes the formation of defined dipoles (Mignon et al. 2004). The dipolar character of Congo red species increases with increasing size. This process can possibly occur at increasing dye concentrations through the induction of self-assembling. Increasing dye electrophoretic migration velocity with increasing concentration is evidence of this phenomenon. However, the effect of simultaneously increasing the size of dye assemblies and their negative charge still needs to be clarified. This effect, which is revealed by the electric field, indicates that electron delocalization alters the proton dissociation capability of the dye sulfonic and amino groups, favoring dissociation when the electron density of dye molecules is decreasing. The effects derived from electron delocalization over conjugated systems are noted to increase with increasing the range of possible electron movements (He et al. 2005; Kondo et al. 2004; Nakano and Yade 2003; Schenning et al. 2004). Also the number of molecules in Congo red species affected by delocalization of electrons under the external field seems to be relatively greater in larger dipoles than in smaller ones. This effect may explain the

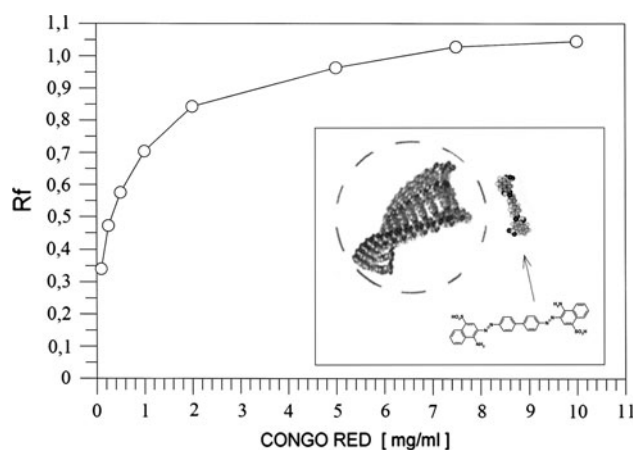


Fig. 9 The curve representing migration of Congo red at different increasing concentrations (measured as Rf). *Inset* Congo red micellar structure and single dye molecule—space-filling model

increased migration velocity and acidity of Congo red in the electric field. The freedom of electron movements and their delocalization upon the electric field enhance the stability of micellar structures and favor further self-assembling of dye molecules. Understanding of this phenomenon may help in future studies of Congo red complexation with amyloids.

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