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Note

Electron donor–acceptor properties of urea and its role in charge-transfer chromatography

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The ability of urea to form hydrogen bonds is well known. This has been attributed to the positive character of the hydrogen atom covalently bonded to the strongly electron-negative nitrogen atom of the amino group of the urea molecule, which might form the so-called "hydrogen bond" with another electron-dense atom (and thus of negative character), such as the oxygen atom of the carbonyl group of a neighbouring urea molecule or the hydroxyl group of water. Such an event provokes, in aqueous solutions, the disruption of the water structure and is probably the reason for its denaturing effect on proteins and nucleic acids[1]. Also, the hydrophobic interactions that contribute to maintaining the secondary and tertiary structure of these macromolecules can be suppressed by urea. As there is no evidence in favour of a direct interaction between urea and the hydrocarbon chains of hydrophobic compounds[2,3], a model consisting of a "cage-like" structure of urea molecules around the hydrocarbon backbone, preventing its unfavourable contact with the aqueous phase, has been proposed[2,3]. The strength of this structure arises from the hydrogen bonding of the urea molecules within themselves contributing to increase the solubility of hydrophobic substances in water. Thus, it appears that both hydrophobic and/or hydrogen-bonding molecules are affected by the simple ability of urea to form hydrogen bonds.

X-ray studies have shown that the carbon, nitrogen and oxygen atoms of urea lie in a plane[4]. Its lone pair of electrons are thereby expected to be located above and below its molecular plane, as occurs with aromatic structures. Such a configuration may explain the ability of urea to form chelate complexes with metals. Chelate-like complexes of urea with organic compounds can also originate from charge-transfer interactions, for example between an electron-deficient molecule (acceptor) and the electron-rich molecule of urea (donor)[5,6]. It has been noticed, for instance, that in the interaction of urea with dyes[7], aromatic amino acids[8] and the bases of nucleotides[9–14], a kind of charge-transfer complex is formed. Moreover, the addition of urea to histidine, phenylalanine, tryptophan, tyrosine and related compounds in solution usually causes some shifts in their spectra where the major adsorption band corre-

sponds to π–π transitions[5]. Hence it is not surprising that urea could bind directly to the active site of enzymes where these amino acids are often involved[15–17]. The formation of coloured and fluorescent compounds on mixing urea with some diketones[18], on the other hand, indicates also that some electron transitions may occur between them. Thus, urea is able to form charge-transfer complexes with a number of structures and affect their association by such a mechanism[19–25].

As an illustration, the effect of urea on the retention of adenosine on acriflavin- and/or pentachlorophenyl-Sephadex is shown in Fig. 1. The action of urea in this instance, which is discussed in detail elsewhere[26,27], has been interpreted as being due to its electron donor–acceptor properties acting competitively against the tendency of adenosine to complex with such supports[26,27].

Another example is given in Table I, which concerns the effect of different compounds on the adsorption of homopolynucleotides on acriflavin-Sepharose[28]. Stock solutions containing 100 μg/ml of the different homopolynucleotides (Sigma, St. Louis, Mo., U.S.A.) were mixed with 5 μl of the corresponding ^{3}H-labelled nucleotides (New England Nuclear, Boston, Mass., U.S.A.). All the homopolynucleotides were of molecular weight above 100,000. A 100-μl volume of the corresponding mixture was applied to Pasteur-pipette columns containing 200 μl of acriflavin-Sepharose 4B gel (prepared according to ref. 27). The buffer for the preparation of the samples and equilibration of the columns (10 m*M* Tris·HCl, pH 7.6) was mixed with the corresponding compounds indicated in Table I. After application of the sample, the columns were washed five times the bed volume with the buffer and the total radioactivity on the eluent was determined by liquid scintillation using Instagel (Packard, Downers Grove, Ill., U.S.A.) in an ABAC-SL40 Intertechnique scintillation counter. The values shown correspond to the percentage of material adsorbed with respect to the total amount applied. All the experiments were run in triplicate at 20° and the standard deviation was about 5%.

As shown in Table I these negatively charged polymers are adsorbed rapidly on the positively charged acriflavin-Sepharose at low ionic strength and neutral pH.

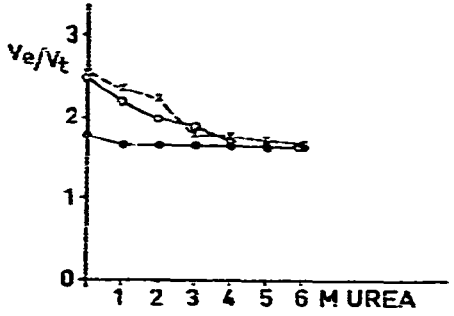

Fig. 1. Effect of urea concentration on the retention of adenosine on acriflavin-Sephadex (○), pentachlorophenyl-Sephadex (×) and Sephadex (●). A sample of 50 μl of adenosine (3 mg/ml in 10 m*M* Tris–HCl buffer, pH 7.6) was applied to the chromatographic columns (bed volume V_t about 12 ml; column dimensions 15 × 1 cm I.D.) at 20° and a constant flow-rate of 10 ml/h. V_e/V_t values were calculated by measuring the volume V_e at the appearance of the maximum absorption band at 254 nm.

TABLE I

EFFECT OF BUFFER COMPOSITION ON THE ADSORPTION OF HOMOPOLYNUCLEOTIDES ON ACRIFLAVIN-SEPHAROSE 4B

Component	*Poly-(G)*	*Poly-(A)*	*Poly-(C)*	*Poly-(U)*
NaCl (1 *M*)	96	98	0	0
Urea (6 *M* + 0 *M* NaCl)	98	90	98	65
Urea (6 *M* + 0.4 *M* NaCl)	94	20	0	0
Formamide (90%, v/v + 0 *M* NaCl)	97	90	98	25
Formamide (90%, v/v + 0.4 *M* NaCl)	79	32	31	0
NaN_3 (1 *M*)	92	20	13	15
Na_3ClO_3 (1 *M*)	87	86	0	27
NaSCN (1 *M*)	86	20	12	0
SDS (1%, w/v, + 0.4 *M* NaCl)	0	0	0	0

By increasing the salt concentration it is possible to prevent adsorption of the pyrimidine polynucleotides, whereas the purine polymers still bind strongly to the adsorbent. If urea is added to the saline buffer (10 m*M* Tris·HCl, pH 7.6, 0.4 *M* sodium chloride), poly(G) adsorption still occurs, but poly(A) adsorption is drastically reduced. The adsorption of poly(G) on acriflavin–Sepharose can be prevented only by SDS. In other words, poly(G) adsorption on acriflavin-Sepharose cannot be abolished by urea at a concentration as high as 6 *M*. It is therefore suggested that the adsorption strength of the different homopolynucleotides on acriflavin-Sepharose decreases in the order poly(G) > poly(A) > poly(C) > poly(U).

There is some evidence in favour of charge-transfer interactions occurring between nucleotides and acriflavin[27]. With polynucleotides, for example, it has been found that the adsorption of poly(A) on acriflavin-Sepharose is prevented by poly(U), provided that the conditions for poly(A)–poly(U) complexing to occur are adequate[28]. That is, double-stranded nucleic acids apparently show no significant affinity for acriflavin-Sepharose. This observation is similar to that made by other workers when using unsubstituted celluloses in the purification of poly(A)-containing nucleic acids[38]. In the latter instance, the adsorption mechanism was ascribed to charge-transfer interactions between the aromatic groups of the lignin in cellulose and the poly(A) segment of the corresponding ribonucleic acids[38]. Urea is able to disrupt the charge-transfer association between poly(A) and either cellulose or acriflavin-Sepharose by forming a complex which, in the case of acriflavin, causes some phosphorescence on the dye molecule[7]. Further evidence for the importance of the electron density of compounds forming charge-transfer complexes is shown by the stability of dimers of the series $HOH\cdots OCR_2$ (where R can be H, CH_3, NH_2 or F). As has been observed, the π-donating and/or σ-withdrawing effects of such substituents determine the order of dimer stability: $(NH_2)_2 > HNH_2 > (CH_3)_2 > CH_3 > H_2 > HF > F_2$ (refs. 6, 29). Therefore, urea should form stronger complexes than formamide with acceptor molecules[6].

The ability of ions such as SCN^- and ClO_3^- to prevent the adsorption of homopolynucleotides on acriflavin-Sepharose, on the other hand, also appears to be in agreement with their chaotropicity[30–36] (increasing chaotropic effect: SCN > ClO_3 > urea > Cl). Hence, the description proposed by Gutmann[6] in the sense that

the efficiency of chaotropic ions to disrupt biomolecular interactions can be related to their strong electron donor–acceptor properties is pertinent in this connection.

Both urea and SCN^- are known to favour the solubilization of hydrophobic substances in aqueous systems[1,31], and therefore their denaturing action towards proteins and nucleic acids has been discussed mainly in terms of disruption of hydrophobic bonds[1,30–36]. However, the charge-transfer interactions that may occur within the structures of biomolecules[37] cannot be ignored. Recently, it has been possible to show that the charge-transfer complexing abilities of a variety of molecules can be exploited for purification purposes[26,27]. In this way, amino acids and oligonucleotides, and also higher polymers such as proteins and polynucleotides[28,39], can be fractionated by charge-transfer chromatography according to the strength of their complexes with selected aromatic ligands[26–28]. Desorption or elution of adsorbed materials in these systems can often be attained either biospecifically or by using solutes with structures that partly resemble those of the specific ligand[39–40]. Other compounds that differ in chemical composition and structure with respect to the ligand in question, but show similar interacting abilities, can also be used very efficiently as eluents. This seems to be the case with urea, formamide and some chaotropic solutions[27,28]. As shown in Fig. 2, the electronic configurations of these molecules are strikingly similar, being planar compounds with electron-rich clouds and strong chelating properties. The charge-transfer complexes that can be formed with these molecules are greatly stabilized by their resonance energies[5,6] and thus possess a large capacity to interfere in the charge-transfer complexation occurring between other compounds. They may also be useful as eluents in chromatographic systems involving charge-transfer complex formation.

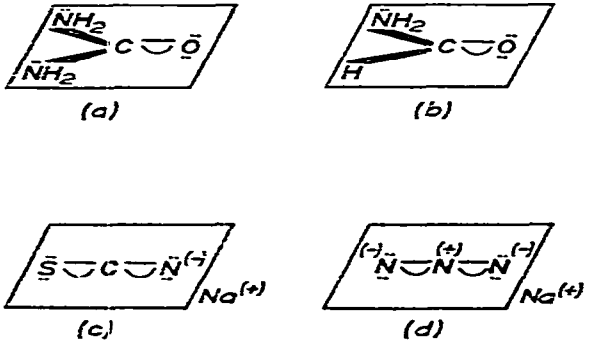

Fig. 2. Structures of (a) urea, (b) formamide, (c) sodium thiocyanate and (d) sodium azide.

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