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Hetero-association of anticancer antibiotics in aqueous solution: NMR and molecular mechanics analysis

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Abstract

In order to investigate the effect on combinations of aromatic antibiotics used in chemotherapy, the hetero-association of the antitumour antibiotics actinomycin D (AMD) with daunomycin (DAU) or novatrone (NOV) has been studied by the methods of 1D- and 2D 500 MHz ^{1}H-NMR spectroscopy and molecular mechanics calculations. The experimental concentration and temperature dependences of the proton chemical shifts of mixtures of the aromatic drugs have been analyzed in terms of a modified statistical–thermodynamical model of hetero-association to give the equilibrium reaction constants, the thermodynamical parameters (ΔH, ΔS) of hetero-association of AMD with DAU or NOV and the limiting values of proton chemical shifts of the molecules in the hetero-complexes. The most favorable averaged structures of the 1:1 DAU–AMD and NOV-AMD hetero-association complexes have been determined using both the limiting values of proton chemical shifts of the molecules and molecular mechanics methods (X-PLOR software). The results show that intermolecular complexes between DAU–AMD and NOV–AMD are mainly stabilized by stacking interactions of the aromatic chromophores, although the DAU–AMD hetero-complex has additional stabilization, which may be explained by an intermolecular hydrogen bond between a carbonyl group of ring C of DAU and the NH group of D–Val of the pentapeptide side chain ring of AMD. The relative content of each type of molecular complex in the mixed solution has been calculated at different values of the ratio (r) of the initial concentrations of DAU and AMD. It is found that the contributions of hetero-complexes to the general equilibrium in solution are predominant at quite different values of r, *viz*. at $r>12$ for AMD with NOV and at $r>2$ for AMD with DAU, compared to $r>0.3$ for the DAU–NOV system observed previously. It is concluded that anticancer drugs have quite different affinities for formation of hetero-complexes with other aromatic antibiotics in aqueous solution, which may need to be taken into consideration for their use in combination chemotherapy.

Keywords: Hetero-association; Anticancer drugs; Daunomycin; Actinomycin D; Novatrone; NMR spectroscopy

1. Introduction

Combination chemotherapy, i.e. when several drugs are administered either simultaneously or sequentially, has proved to be very effective in the treatment of many human diseases, in particular malignant tumours [1–3]. Aromatic antitumour antibiotics, such as doxorubicin (DOX), daunomycin (DAU), mitoxantrone (also named novatrone, NOV), actinomycin D (AMD), amsacrine (AMSA), are widely used in clinical practice as basic components of combinations of drugs designed for treatment of various types of human cancers. For example, the combination of DOX+AMD is highly effective against various types of sarcomas [4,5], DOX+NOV is mainly used against breast cancer [6], and DOX+AMSA and NOV+AMSA are effective against leukaemia [7,8]. It should be noted that the concentrations of anticancer drugs used in chemotherapy are, as a rule,

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relatively small in blood plasma [1] so that one might not expect substantial interactions between drug molecules in vivo. However, it is significant that stock concentrations, C_0, (or doses of antibiotics to be administered) depend on chemotherapeutic regimen used but are typically in the millimolar range [1]. For example, for the antibiotic AMD C_0 ca. 0.4 mM, for the anticancer drugs NOV and DAU C_0 is 1–4 mM, and for other antibiotics concentrations of stock solutions may be 1–2 orders higher [1]. It has been shown that at millimolar concentrations in aqueous solution, there is a strong tendency for aromatic anticancer drugs to exhibit self-association forming dimers (AMD) and higher order aggregates (DOX, DAU, NOV), which are stabilized by stacking interactions of the aromatic chromophores [9–12]. It has been also shown that different types of biologically-active aromatic molecules form stacked hetero-associated complexes in aqueous solution, e.g. the above-mentioned anticancer antibiotics may complex with caffeine [13,14], riboflavine [15], chlorophylline [16] and aromatic dyes [17,18]. Hence it is likely that self- and hetero-association reactions of aromatic anticancer drugs take place in the stock solutions used for combination chemotherapy, which may affect the pharmacokinetics of these drugs and consequently their medico-biological activity. Moreover, the rate of metabolic activation/deactivation processes of antibiotics in the hetero-complex may be different from the kinetics of transformation of free drug in a biological fluid. It has been proposed that such a phenomenon is likely to be the preferred mechanism to explain the rate of degradation of DOX and other aromatic carcinogens on binding with riboflavin [19,20]. In some cases the hetero-association of aromatic molecules may lead to formation of stable hetero-complexes in solution as they are energetically more favorable than self-association of the constituent drugs. Our previous investigations have shown that stacked complexes between the antibiotic DAU and the aromatic dyes, proflavine and ethidium bromide, are additionally stabilized by intermolecular hydrogen bonds in aqueous salt solution [17,18]. Hence it is likely that physico-chemical investigations of the distinctive features of the interactions between aromatic antibiotics under physiological conditions are very important for the development of chemotherapeutic regimes involving combinations of these drugs.

In this work 500 MHz ^{1}H NMR (1D and 2D) spectroscopy and molecular mechanics methods have been used to investigate the formation of hetero-complexes between the aromatic antitumour antibiotics actinomycin D (AMD) and either novatrone (NOV) or daunomycin (DAU) in aqueous salt solution. The structures of AMD, DAU and NOV are shown in Fig. 1. The thermodynamic parameters of complexation of NOV–AMD and DAU–AMD in aqueous salt solution have been determined from the experimental concentration and temperature dependences of proton NMR chemical shifts of the interacting molecules, and the results compared with those for similar studies on the hetero-

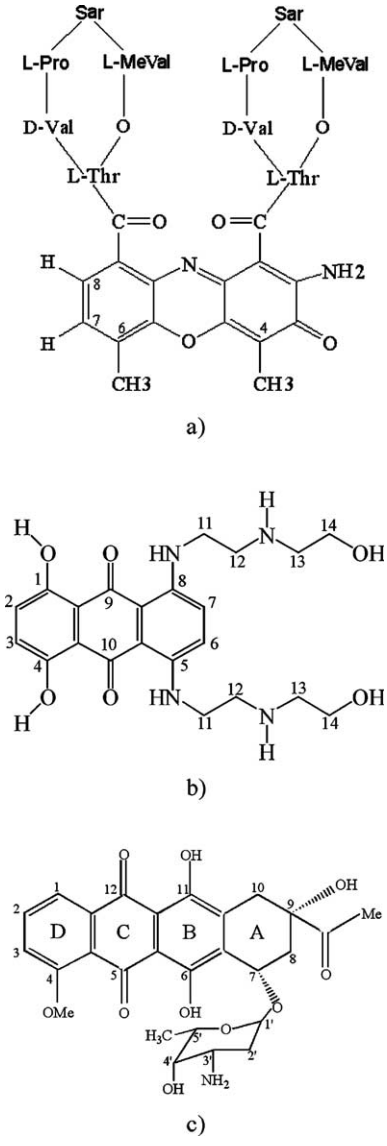

Fig. 1. Chemical structures of the anticancer antibiotics: a) actinomycin D (AMD); b) novatrone (NOV); c) daunomycin (DAU).

association of NOV and DAU under the same solution conditions [21]. Such investigations provide information on the nature of the physical forces involved in the complex-action of aromatic antitumour antibiotics and give some insight into the molecular basis of the pharmacological activity of drugs used in combination chemotherapy.

2. Materials and methods

2.1. NMR spectroscopy

The antitumour antibiotics (Fig. 1), actinomycin D (AMD) and novatrone (1,4-dehydroxy-5,8-bis [[2-(2-hydroxyethyl) amino] ethyl] amino]-9,10-antracenedione, NOV), were purchased from “Sigma” and daunomycin (DAU) from “Fluka” and were all used without further