Formation of polymeric chelate bridges between double-stranded DNA molecules fixed in spatial structure of liquid-crystalline dispersions

Yu.M. Yevdokimov[a,*], V.I. Salyanov[a], E. Gedig[b], F. Spener[b]

[a] *Engelhardt Institute of Molecular Biology, The Russian Academy of Sciences, Vavilova Str. 32, 11984 Moscow, Russia*
[b] *Institut fuer Chemo- und Biosensorik, Mendelstrasse 7, D-48149 Muenster, Germany*

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Abstract The formation of cholesteric liquid-crystalline dispersions from DNA–daunomycin complexes in water–salt polyethyleneglycol–containing solutions was investigated. In the case of nonclassical complex formation between DNA and daunomycin (DAU), reactive groups of DAU were used for the formation of polymeric chelate complex with divalent copper ions (-DAU–Cu-...-Cu–DAU-), located between neighboring double-stranded DNA molecules, fixed in spatial structure of liquid-crystalline dispersions. The formation of polymeric chelate complex does not depend upon the sense of helicoidal twist of DNA cholesterics. A many-fold increase in the CD band in the DAU absorption region is specific to this process. A reduction of the divalent copper ions as a result of a redox-process is accompanied by destroying of structure of polymeric chelate complex between DNA molecules and by disappearance of the abnormal CD band in daunomycin absorption region.

Key words: DNA; Daunomycin; Liquid-crystalline dispersion; Chelate complex formation

1. Introduction

A hypothesis on a possibility to use double-stranded DNA molecules fixed in the spatial structure of liquid-crystalline dispersions, as 'building elements' was put forward in [1]. In accordance with this, one can create spatial 'bridges' between DNAs and form three-dimensional bridged architectures containing a large number of molecules of different chemical compounds. From this point of view, the complexes between natural or semi-synthetic biologically active compounds of the anthracycline group and DNA are of special interest. On the one hand, these compounds contain the chemical groups with high reactivity [2], on the other hand, they may interact with DNA molecules by different modes [3,4].

To gain further insight into peculiarities of DNA bridging in the liquid-crystalline state, the polymeric chelate complexes between DNA molecules and daunomycin–copper, fixed in the structure of cholesteric liquid-crystalline dispersions with left- or right-handed helicoidal twist, were synthetized. The spatial structure of the polymeric chelate complex formed could be destroyed by use of reducing agents. It was possible to regulate the amplification of the DAU band in the CD spectra, specific to liquid-crystalline dispersions from these complexes.

2. Materials and methods

Chicken erythrocyte DNA (Reanal, Hungary) was used. After ultrasonic depolymerisation of the original sample, its molecular mass was $(5–8)\times10^5$ Da according to gel electrophoresis measurements in 1% agarose gels.

Antitumor anthracycline antibiotic, daunomycin (DAU) and poly(ethyleneglycol) (PEG) were from Serva and Ferak, respectively. Daunomycin (DAU), DNA, PEG, NaCl and $CuCl_2$ were dissolved in 2 mM sodium phosphate buffer, pH 6.67.

Liquid-crystalline (LC) dispersions of DNA were formed by mixing equal volumes of DNA and PEG solutions as described earlier [5]. LC dispersions of (DNA–DAU) complexes were formed according to the following two methods: (1) the DNA solution was first mixed with PEG (340 mg/ml) solution to form the LC dispersion and then reacted with DAU; (2) the DNA molecules were first complexed with DAU and then mixed with PEG (340 mg/ml) solution to obtain the LC dispersion. The final concentrations of DNA, PEG and NaCl in solutions used were 5 μg/ml, 170 mg/ml and 0.3 M, respectively.

The polymeric chelate complexes of (DAU–Cu) between DNA molecules in LC dispersions were created using the reaction of DAU with copper ions [6]. Aliquots of a 0.01 M $CuCl_2$ (1–10 μl) solution were added into the dichrograph cell containing LC dispersions of (DNA–DAU) complexes. Concentrations of DAU or copper in solution were expressed as total concentration of compound added (C_{tot}). Destruction of polymeric chelate complexes was achieved by a change of valency of copper ions [7,8] as a result of reduction after addition (1–10 μl) of ascorbic acid (Sigma).

The circular dichroism (CD) spectra were taken on a Jobin-Yvon Mark III dichrograph (France); the absorption spectra were recorded using a Specord M40 spectrophotometer (Germany). The CD spectra were expressed in terms of $\Delta A = A_L - A_R$ (optical units) versus λ values.

3. Results and discussion

Fig. 1 illustrates the CD spectra in the visible region, specific to LC dispersions formed in PEG-containing water–salt solution complexed with DAU *before* LC formation and treated with $CuCl_2$, and that of DNA molecules complexed with DAU *after* formation of LC dispersion and then treated with $CuCl_2$.

Two results are evident here. First, formation of the LC dispersion from the double-stranded DNA molecules added first with DAU is accompanied by an appearance of the positive band in the CD spectrum (curve 1) in the region of DAU absorption [9–12], while in the case of the LC dispersion from the original double-stranded DNA molecules with subsequent addition of DAU, the sign of the band in the CD spectrum is negative (curve 3) [9–12]. The equilibrium amplitudes of both CD bands are comparable; they exceed the amplitudes of the CD band specific to the molecular complexes (DNA–DAU) many-fold [9–12] and their values depend only on DAU concentration bounded to DNA.

The reasons for the change in the sign of the CD band specific to LC dispersions have been considered in detail earlier [13–15]. Being applied to LC dispersions formed from (DNA–DAU) complexes, these reasons allow the following statement to be made: DAU molecules may interact with

*Corresponding author. Fax: 007-095-135-14-05.
E-mail: yevd@imb.msk.su

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DNA by two modes. The first mode means a classical intercalation of DAU molecules between the DNA nitrogen bases [4]. The second mode means 'nonintercalative' binding of DAU to DNA [3]. The last process happens only at the certain extent of DNA modification as a result of intercalative DAU interaction, i.e., takes place only at a definite DAU concentration. These modes differ in concrete details of the spatial orientation of the DAU molecules about the DNA base pairs; however, DAU chromophores are perpendicular in respect to the DNA helix for both cases. The interaction between the original right-handed double-stranded DNA molecules at a 'moment' of their spatial approaching, necessary for formation of LC dispersion, is accompanied by formation of DNA cholesterics with left-handed helicoidal twist [5,10]. According to [16,17], the left-handed helicoidal twist is reflected by the negative sign of the band in the CD spectrum, specific to LC dispersions. In contrast, a change of the mode of DAU interaction with DNA molecules results in a change of the interaction energy of these molecules at a 'moment' of LC dispersion formation and, hence, in a change of the sense of helicoidal twist of the spatial structure of dispersion. A positive sign of the CD band means the formation of LC dispersions with the right-handed helicoidal twist of their spatial structure [5,10]. Therefore, a change of mechanism of DAU interaction with DNA *before* formation of LC dispersions is accompanied by change in the sign of the intense band in the CD spectrum [11,18].

Another situation is specific to LC dispersions added with DAU *after* LC formation.The helicoidal spatial structure of LC dispersion, which depends on the interaction energy of original neighboring DNA molecules, is fixed after LC formation because parameters of the secondary DNA structure are not significantly changed and — in the main — correspond to the B-form [1]. The subsequent binding of DAU to DNA, despite the different ways of DAU location near the DNA nitrogen bases, is not able to change the sense of helicoidal twist of the DNA cholesterics. This means, that the sign of the CD band stays negative only, despite the different modes of location of DAU near base pairs. The amplitude of the negative band (curve 3) posseses an equilibrium value as well; it depends only on DAU concentration.

Comparison of curves 1 and 3 in Fig. 1 shows that, in full agreement with the theoretical [13–15] and the experimental data [5,9–11], (DNA–DAU) complexes do form two types of cholesteric LC dispersions which differ in the signs of the amplitudes of the intense bands in the CD spectrum. This difference depends upon the way of the LC dispersion preparation.

An important point follows from a consideration of the concrete details of the different modes of DAU–DNA interaction. According to the first mode, i.e., classical intercalation, the reactive groups of DAU are not accessible for the chemical reactions, while for the second mode, these groups are able to react with different compounds [2].

If the hypothesis about two modes of DAU interaction with the DNA is correct, one can expect to detect the 'presence' of DAU molecules capable of chemical reactions, in particular, of complex formation, at the certain DAU concentration for both ways of formation of LC dispersions. Very specific to the reactive groups (quinone carbonyls and hydroxyls) of anthracyclines is the formation of chelate complexes with di- or trivalent ions [2,6,19–22]. The structure of these complexes

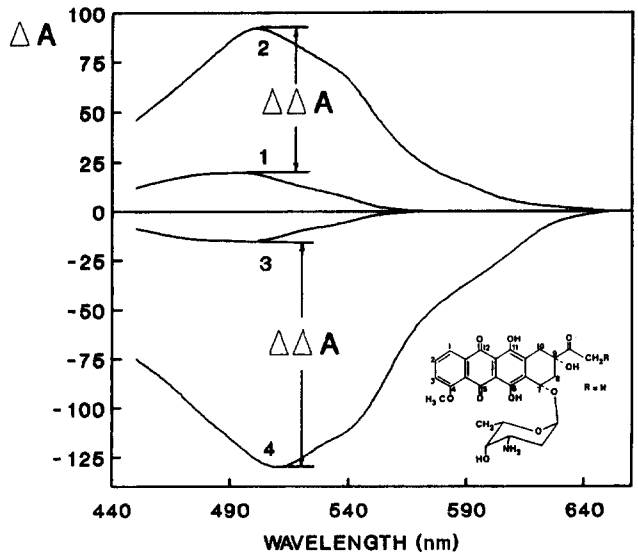

Fig. 1. The CD spectra for LC dispersions formed from DNA molecules complexed with DAU (formula of DAU is shown) *before* LC formation (curve 1) and treated with $CuCl_2$ (curve 2) and that of DNA complexed with DAU *after* formation of LC dispersion (curve 3) and then treated with $CuCl_2$ (curve 4). DAU concentration = 14.3×10^{-6} M; $CuCl_2$ concentration = 5×10^{-6} M; concentrations of DNA, PEG and the other conditions — see Section 2; ΔA in mm, 1 mm = 1×10^{-5} opt. unit.

can be explained by a concept of the metal chelate, giving rise to an intramolecular charge transfer to the anthraquinone nucleus [2,20]. Physico-chemical properties of such complexes is a function of the electron donating abilities of the metal ions [2,20].

Fig. 1 demonstrates the second result obtained. The addition of copper ions to LC dispersions of the (DNA–DAU) complexes (under conditions used) leads to 5–7-fold increase in the equiliblium amplitudes of the CD bands ($\Delta\Delta A$) in the region of DAU absorption for the both cases, despite the signs of bands.

Fig. 2A shows the dependence of the 'extra-increase' ($\Delta\Delta A$) of positive as well as negative bands upon the DAU concentration for LC dispersions at fixed concentration of copper ions. The 'extra-increase' of both bands begins only at the certain (critical) concentration of DAU ($C_{tot} \approx 5\text{–}6\times10^{-6}$ M). One can stress, that the interaction between DAU and DNA takes place under conditions below the critical DAU concentration as well, and is reflected by an intense (ΔA) band in the CD spectrum (see Fig. 1). The most interesting point is the dependence for positive band (curve 2). An appearance of positive band in the CD spectrum of DNA LC dispersion added with DAU is a 'visible' evidence for change of the mode of DAU orientation about DNA nitrogen bases (see above). This allows DAU molecules to react with copper ions [2,6]. However, the 'extra-increase' of the positive band begins only after the certain 'critical' DAU concentration. If one can accept, that 'extra-increase' of the CD band proves the formation of a (DAU–Cu) complex near the DNA surface, then Fig. 2A demonstrates the necessity to accumulate a certain amount of DAU molecules in structure of the formed complex to get the 'extra-increase' of the CD band.

In principle, the same situation is correct for the LC dispersions with the negative CD band (curve 4). However, here,

an addition of DAU molecules is not accompanied initially by a 'visible' evidence in the mode of DAU orientation. Only 'extra-increase' of the CD band ($\Delta\Delta A$) reflects a formation of (DAU–Cu) complexes. Coincidence of the shape of the dependence in this case with that of LC dispersion with positive band shows again, that for 'extra-increase' of the CD band it is necessary to accumulate a certain amount of DAU molecules in complex formed.

Fig. 2B compares the dependences of the 'extra-intensity' for positive and negative bands for LC dispersions upon concentration of $CuCl_2$ (at fixed concentration of DAU for both solutions). One can see that addition of $CuCl_2$ up to concentration about 2×10^{-6} M does not result in an increase in the amplitudes of positive or negative band. However, if $CuCl_2$ concentration exceeds 2×10^{-6} M, a strong increase in the amplitudes takes place for both cases. This 'extra-intensity', as well as in the Fig. 2A, reflects the formation of (DAU–Cu) complexes [21,22] near the DNA surface.

Practically constant values of amplitiudes of both bands below a 'critical' $CuCl_2$ concentration, proves that for the 'extra-increase' of the CD bands it is necessary to accumulate a definite number of copper ions in the content of complexes formed.

Taking into account the reasons for the appearance of the intense bands in the CD spectra of LC dispersions added with coloured compounds, in particular DAU [15], as well as the dependence of 'extra-intensity' of the CD bands upon DAU and copper concentrations, a comparison of Fig. 2A,B demonstrates that the 'extra-intensity' of the amplitude of the CD band is connected with an increase in concentration of anisotropically oriented DAU molecules. Hence, for the amplification of the CD bands of LC dispersions formed from (DNA–DAU) complexes, a few requirements are necessary: specific orientation of DAU molecules near DNA surface, definite concentrations of DAU molecules and copper ions in the content of complexes formed.

The hypothetical structure reported in [23] represents the polymeric chelate complex as (DNA–DAU–(Cu–DAU–Cu-...-Cu–DAU–Cu)$_n$–DAU–DNA). This structure allows to include variable amounts of DAU molecules and copper ions. A number of properties has been used to characterize the structure of polymeric chelate complex: (1) principal ability of DAU molecules to form polymeric chelate complexes with divalent copper [6,19,21,22]; (2) possibility of including in the structure of the complex up to 10 anthracycline molecules [19]; (3) flat spatial structure of (anthracycline–Cu) complexes [6,19].

The stabilization of the polymeric chelate complex is reached here by 'bridging' the chelate system DAU–(Cu–DAU–Cu-...-Cu–DAU–Cu)$_n$–DAU between two neighboring DNA molecules, whose mutual spatial orientation is fixed in the structure of cholesteric LC dispersions.

Analogous sigmoidal shapes of curves, shown in Fig. 2B, testifies that a formation of stable polymeric chelate bridges between DNA molecules, i.e., (-Cu–DAU–Cu-...-Cu–DAU–Cu-)$_n$, begins only after reaching of the certain lenght of the planar structure of the polymeric linker formed between DNA molecules which, in turn, depends on the number of DAU molecules (anisotropically oriented near DNA surface) and on the number of copper atoms, as well as on the average distance between DNA molecules in the spatial structure of the LC dispersion.

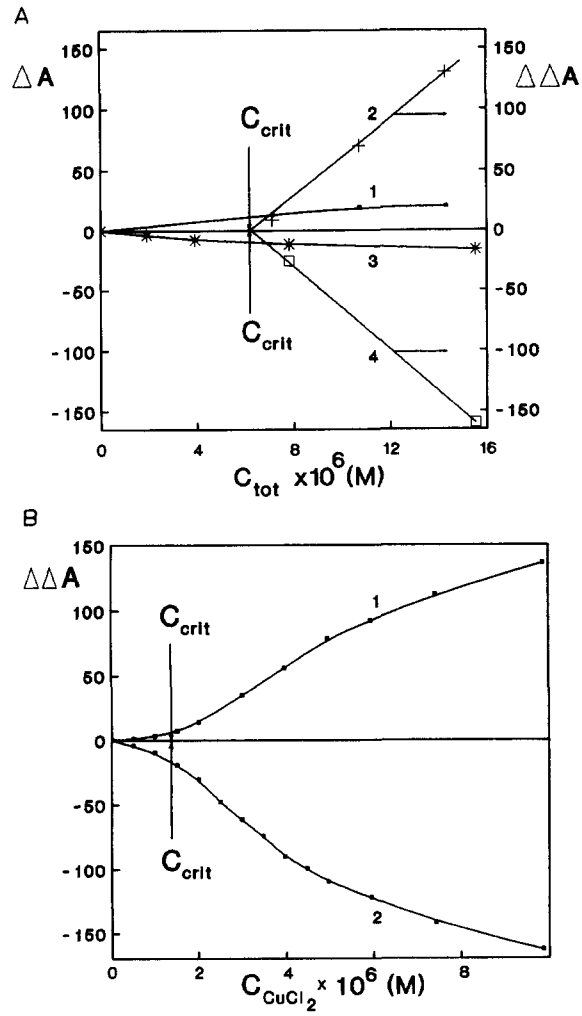

Fig. 2. The dependences of 'extra-increase' ($\Delta\Delta A$) for the positive (curve 2) and the negative (curve 4) bands in the CD spectra (λ 505 nm) of LC dispersions formed from DNA–DAU complexes upon DAU (A) and $CuCl_2$ (B) concentrations. Curves 2 and 4 in (A) were taken in presence of 1×10^{-5} M $CuCl_2$; curves 1 and 3 reflect the dependences of amplitudes for positive and negative bands (ΔA) versus DAU concentration without addition of $CuCl_2$; curve 1 in (B) was taken for the positive, curve 2 for the negative band; DAU concentration in (B) = 14.3×10^{-6} M; ΔA in mm, 1 mm = 1×10^{-5} opt. unit.

Obviously, the properties of the 'crosslink' DAU–(Cu–DAU–Cu-...-Cu–DAU–Cu)$_n$–DAU should be regulated not only by the distance between DNA molecules.

As was mentioned above, the properties of (anthracycline–metal) complexes are a function of the electron donating properties of metal ions. This means, that the valency of copper ion will also affect the properties of the polymeric chelate 'crosslink'. From this point of view, it was interesting to study the optical properties of the LC dispersions of DNA molecules crosslinked by polymeric chelate complexes in the presence of reducing agents, for instance, ascorbic acid [7,8]. (It is necessary to stress that ascorbic acid can act both as a reducing agent and as a chelating agent for divalent copper ions.)

Fig. 3A shows the changes in the amplitudes of both bands after addition of ascorbic acid into solution. One can see that in both cases addition of ascorbic acid results in a sharp decrease in the amplitudes of the bands. Fig. 3B demonstrates

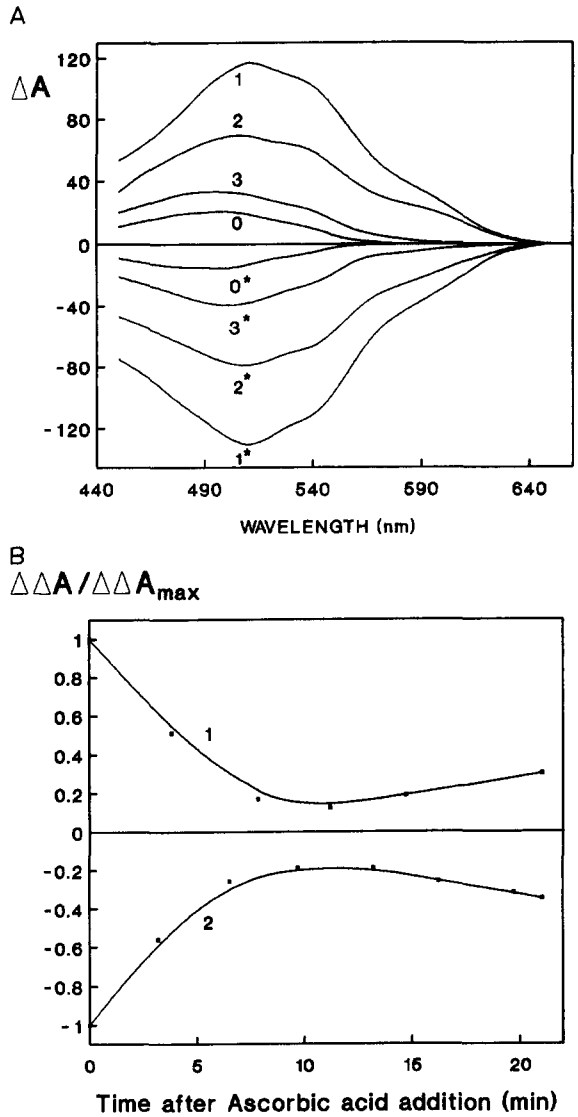

Fig. 3. The CD spectra (A) for the right-hand (upper panel) and left-hand helicoidal twisted (lower panel) LC dispersions added with DAU (curves 0, 0*), $CuCl_2$ (curves 1, 1*) and then with ascorbic acid (curves 2, 2* and 3, 3*) and the change (B) of relative values of 'extra-increase' in positive (curve 1) and negative (curve 2) bands (λ505 nm) in the CD spectra of LC dispersions versus time of ascorbic acid addition. DAU and $CuCl_2$ concentrations — see Fig. 1; ascorbic acid concentration = 2.5×10^{-6} M; upper panel in (A), curves 2 and 3 were taken 4 and 11 min after ascorbic acid addition, respectively; lower panel in (A), curves 2* and 3* were taken 3 and 13 min after ascorbic acid addition, respectively. For contrast, the changes for negative band in (B) (curve 2) are presented as 'negative values' of ($\Delta\Delta A/\Delta\Delta A_{max}$); ΔA in mm, 1 mm = 1×10^{-5} opt. unit.

that these changes are relatively quick and they do not depend upon the signs of the bands. However, after reaching the minimal value of amplitude, which is appoximately equal to amplitude of the band without addition of copper ions (see Fig. 1), an increase in the amplitudes of both bands begins. (This shows that the addition of ascorbic acid causes a reversible process.)

Fig. 4 exemplifies the relative decrease in the amplitude of the negative band after addition of different concentrations of ascorbic acid. One can see, that minimal concentration of ascorbic acid, which induces the change in amplitude of band is about 5×10^{-7} M.

Comparison of Figs. 3 and 4 allows the following conclusion to be made. Because Cu^+ ions are not able to form stable complexes with DAU, a transition Cu^{2+}–Cu^+, as a result of a reduction, is accompanied by rapid destruction of the polymeric chelate bridge DAU–(Cu–DAU–Cu-...-Cu–DAU–Cu)$_n$–DAU, fixed between DNA molecules, by disappearance of the 'extra' number of DAU molecules oriented anisotropically and, hence, by disappearance of the 'extra-intensity' of bands in the CD spectrum. The location of the places in structure of polymeric chelate complex, where reduction of copper ions takes place, is unimportant for this process. This 'destroying' effect of ascorbic acid depends only on its concentration in the solution. Subsequent autooxidation (Cu^+–Cu^{2+}) under action of oxygen in a solution [7,8] restores the initial polymeric chelate bridge between DNA molecule and, hence, restores as well the 'extra-intensity' of band in the CD spectrum in region of DAU absorption.

It is necessary to add that chelate formation, as a result of interaction between EDTA and Cu^{2+} ions, is accompanied by destruction of polymeric chelate complex as well, but in this case a disappearance of the 'extra-intensity' of the band in the CD spectrum is nonreversible.

Hence, data obtained prove the hypothesis on the regulated formation of polymeric chelate bridge DAU–(Cu–DAU–Cu-...-Cu–DAU–Cu)$_n$–DAU between DNA molecules fixed in the spatial structure of cholesteric LC dispersions, despite different senses of their helicoidal twist.

Therefore, using neighboring DNA molecules, fixed in structure of the LC dispersion, it is possible to generate stable spatial structures containing copper ions and DAU molecules. This approach might be of use in molecular pharmacology for the introduction of high concentrations of DAU molecules into cells. In addition, the many-fold amplification of the op-

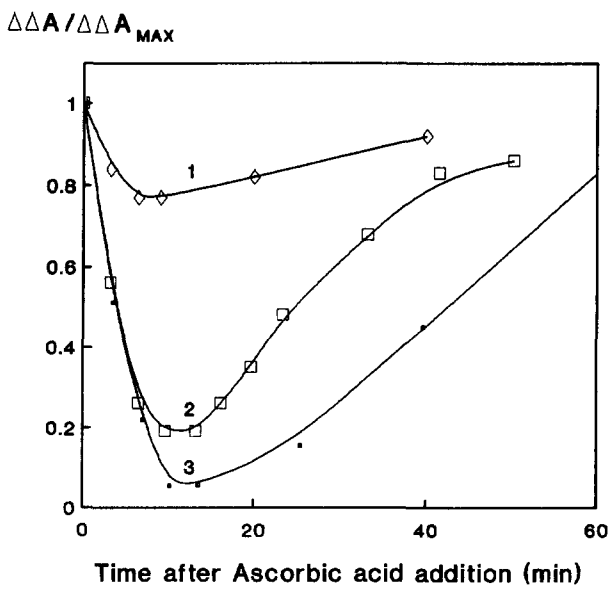

Fig. 4. The change of relative value ($\Delta\Delta A/\Delta\Delta A_{max}$) of 'extra-increase' in negative band (λ 505nm) in the CD spectra of LC dispersions versus time of addition of ascorbic acid. DAU, $CuCl_2$ concentrations and the other conditions — see Fig. 1. Ascorbic acid concentrations: curve 1, 5×10^{-7} M; curve 2, 2.5×10^{-6} M; curve 3, 5×10^{-6} M.

tical signal, observed for bridged structures, will be useful to produce biosensing units for sensor devices based on the LC dispersions formed by DAU–DNA complexes.

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