^1H n.m.r. of the DNA-dependent RNA polymerase from *Escherichia coli*

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The ^1H n.m.r. study of the DNA-dependent RNA polymerase from *Escherichia coli* has revealed that the holoenzyme ($\beta\beta'\alpha_2\sigma$) displays two mobile regions: one, observable also in the core enzyme ($\beta\beta'\alpha_2$), is characterized by basic amino acids and its appearance and form depend on ionic strength; the other, specific to the holoenzyme, is characterized by threonine residues and its appearance does not depend on ionic strength.

Transcription of DNA in *Escherichia coli* is catalysed by the enzyme DNA-dependent RNA polymerase, of M_r approx. 450 000 (Chamberlin, 1976), which in the form of holoenzyme (composition $\beta\beta'\alpha_2\sigma$) binds specifically to a promoter site on DNA. After forming a chain of about ten nucleotides, it releases the initiation factor σ , and, in the form of core enzyme (composition $\beta\beta'\alpha_2$), it elongates the RNA chain up to termination.

The ^1H n.m.r. study of this very large protein reveals structural details and conformational variations dependent on protein composition and ionic strength.

Experimental

RNA polymerase was isolated from *Escherichia coli* (Zillig *et al.*, 1970; Meisenberger *et al.*, 1980). Core enzyme and σ factor were purified by phosphocellulose chromatography (Burgess *et al.*, 1969). Holoenzyme was prepared by mixing stoichiometric (1:1) amounts of core enzyme and σ factor. Core-enzyme activity was stimulated 12-fold by addition of σ factor, with phage-T7 DNA as template. The purity of the proteins was >95% as checked by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis; their concentration was determined by the staining procedure (Heil & Zillig, 1970). The samples for the n.m.r. experiments were extensively dialysed against a 1 mM-phosphate buffer in $^2\text{H}_2\text{O}$, pH 7.8, containing 0.05 M-KCl for low-ionic-strength conditions and 0.50 M-KCl for high-ionic-strength conditions. The holoenzyme activity measured at 0.15 M-KCl before and after running the spectra resulted the same. The ^1H n.m.r. spectra were obtained at 200 MHz on the WP-200 Bruker Spectrometer of the 'Servizio NMR—Area della Ricerca di Roma'. The spectra were run on protein solutions at 20 μM concentration at 294 K. The exact

absorption frequency of residual water was measured to suppress it, and its value was also used as internal standard and referred to external 2,2-dimethyl-2-silapentane-5-sulphonate; after each experiment it was verified that the water chemical shift was stable within 2 Hz.

Results and discussion

Holoenzyme and core enzyme dissociated in 6 M-urea gave the same spectra; they were assigned according to Bradbury *et al.* (1972) and used as a reference.

The spectrum of the holoenzyme (Fig. 1) at low ionic strength contains many signals, from which two intense peaks emerge, one attributed to the methyl groups of the aliphatic amino acids and the other attributed most probably to threonine methyl groups. Weak peaks attributed to alanine methyl and very weak bands corresponding to lysine, arginine and glutamate/glutamine are also present. At high ionic strength the holoenzyme displays a more structured spectrum and with much more developed peaks, whereas the threonine-methyl-group peak appears relatively less intense.

Comparison of the spectra of the holoenzyme at high ionic strength with those of core enzyme and σ subunit (Fig. 2) shows that they differ mainly in the peak of the threonine methyl groups, which appears tall and sharp in the holoenzyme spectrum, is lacking in that of core enzyme, and is quite small in the spectrum of the σ subunit.

Comparison of the spectra of the holoenzyme and core enzyme at high ionic strength with those in 6 M-urea and with a spectrum of polylysine (M_r approx. 25 000–30 000) allowed estimation, by scaled measurement of the peak areas, that the sharp signals correspond to about 10% of the total protein signals.

Fig. 1. ^1H n.m.r. 200 MHz spectra of the holoenzyme in 1 mM-phosphate buffer, pH 7.8, at low (a) and high (b) ionic strength
12K scans; gain: (a) $\times 2$, (b) $\times 1$. (*) Impurity. Presumed peak assignments are shown, based on random-coil chemical shift values.

By summarizing these data one can conclude that the enzyme as studied by n.m.r. liberates in solution two portions independent from one another. The first portion, common to both holoenzyme and core enzyme, is rich in aliphatic amino acids and contains also basic amino acids. The form and appearance of this portion in the spectra are largely influenced by ionic strength, i.e. n.m.r. clearly reveals a conformational variation taking place on changing from low to high ionic strength with an increase in intensity and resolution of the signals, and it is known that an increase in ionic strength modifies the behaviour of the holoenzyme, for instance with respect to aggregation phenomena (Berg & Chamberlin, 1970) and with respect to its ability to bind DNA (Kadesch *et al.*, 1980; Strauss *et al.*, 1981).

The second portion is particular to the holoenzyme and is characterized by an intense peak, most probably attributed to threonine methyl groups. The appearance and form of this second portion are only due to the addition of the σ subunit to the core enzyme to form the holoenzyme; it should be mentioned that histones, which display

n.m.r. spectra very similar to those of the bacterial polymerase, have been reported to be unique in undergoing large conformational changes in response to small changes of ionic strength (Hartmann *et al.*, 1977); the polymerase, in fact, displays a similar behaviour.

It is also possible that the variation observed by n.m.r. on addition of σ subunit to core enzyme may well coincide with the conformational transition revealed by fluorimetry (Wu *et al.*, 1976).

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Fig. 2. ^1H n.m.r. 200 MHz spectra of the σ subunit (a) and of the core enzyme (b) in 1 mM-phosphate buffer, pH 7.8, at high ionic strength
12 k scans; gain: (a) $\times 8$, (b) $\times 1$. (*) Impurity.

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