ULTRAVIOLET ABSORPTION SPECTRUM OF RNA POLYMERASE

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1. Introduction

One of the criteria of purity of a preparation of RNA polymerase (EC 2.7.7.6) is its 280/260 mμ absorbance ratio. Enzyme preparations with a ratio below 1.65 are assumed to contain some contaminating nucleic acids or their derivatives [1]. However, no nucleic acid was detected in a purified enzyme preparation of absorbance ratio 1.25 [2], either by the Dische method (limit of detection 1%) or the orcinol procedure (limit of detection 0.6%). From the data of Warburg and Christian [3], a ratio of 1.25 indicates the presence of more than 1.5% nucleic acids (by weight). Perchloric acid hydrolysis and paper chromatography of 5 mg of an enzyme preparation of absorbance ratio 1.4 buffer A (mM) (10 tris-HCl, pH 7.9; 10 $MgSO_4$; 1 2-mercaptoethanol, 0.1 EDTA), could not reveal the presence of purine or pyrimidine bases in the hydrolysate [4]. This paper reports the influence of buffer ionic strength on the ultraviolet absorption spectrum of highly purified enzyme.

2. Materials and methods

RNA polymerase was isolated from *E. coli* MRE 600, by a procedure to be described in detail later, but essentially according to the method of Chamberlin and Berg [5]. Besides certain minor modifications, our procedure contains an additional protamine sulphate fractionation to separate enzyme molecules having a full complement of the sigma factor [6] from the minimal enzyme. The purified enzyme incorporated 0.19 μmoles of ATP per minute per mg protein under the following conditions: the reaction mixture (0.5 ml) contained (mM): 50 tris-HCl, pH 7.66, at 37°; 5 mercaptoethanol; 0.16 each of GTP, CTP and UTP; 0.16 ^{14}C-ATP; 10 $MgCl_2$; 0.16 KCl; 10 μg of enzyme and an optimal concentration of T_4DNA (7 μg). After 10 min incubation at 37°, the sample was prepared for radioactivity measurement as described earlier [7]. Absorption spectra were obtained using a Shimadzu 50L multipurpose recording spectrophotometer. Dialysis of enzyme solutions was carried out in boiled dialysis tubing [8], which was rinsed thoroughly with the dialysis buffer before us.

3. Results and discussion

Fig. 1 shows the ultraviolet absorption spectrum of

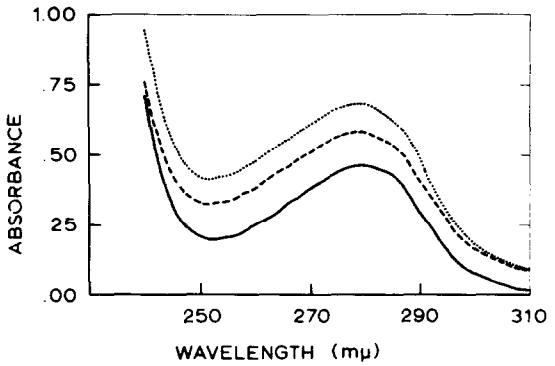

Fig. 1. Ultraviolet absorption spectrum of purified RNA-polymerase in buffer A of different ionic strength. ——: enzyme in buffer A containing 0.22 M KCl; - - -: enzyme in buffer A containing 0.22 M KCl dialysed for 1 hr against this buffer containing 0.1 M KCl; . . . .: enzyme in buffer A containing 0.22 M KCl dialysed for 1 hr against this buffer in the absence of KCl.

a fresh enzyme preparation in buffer A containing 0.22 M KCl. The 280/260 mμ absorbance ratio calculated from this spectrum is 1.8. This enzyme preparation after 1 hr dialysis against buffer A containing 0.1 M KCl gave a different spectrum (fig. 1). Increased UV absorption of the enzyme in this buffer is more marked in the region of absorption minimum (251 mμ), than in the region of absorption maximum (280 mμ). The 280/260 mμ absorbance ratio calculated from this spectrum is 1.5. This change in the absorption of the enzyme due to a variation of the ionic strength of the buffer is a reversible process, because the enzyme in the low ionic strength buffer, when precipitated with ammonium sulphate and redissolved in buffer A containing 0.22M KCl showed an absorption pattern identical with the original spectrum. Reversion to the original spectrum can also be achieved by dialysis against buffer A containing 0.22 M KCl for 4 hr.

Dialysis of the enzyme against buffer A without KCl resulted in a greater increase in the absorbance of the enzyme (fig. 1). The degree of this increase is dependent on the extent of dialysis. The increase in absorption under these conditions is also more pronounced in the region of the absorption minimum. Prolonged dialysis against this buffer, however, resulted in a gradual loss of enzyme activity.

The influence of ionic strength on the spectrum of the enzyme was also observed in buffer C [9], (50 mM tris-HCl, pH 7.9; 0.1 mM EDTA; 0.1 mM dithiothreitol; 5% glycerol). Dialysis of the enzyme against buffer C containing 0.1 M KCl (fig. 2) resulted in the same pattern of spectral change observed in buffer A; the change is very slow in this buffer, probably due to some stabilizing effect of glycerol. The 280/260 mμ absorbance ratio after 2 hr dialysis is 1.55 in this low ionic strength buffer.

Light scattering caused by a protein solution affects its absorption spectrum to a certain degree, but the change observed here cannot be explained in terms of light scattering alone for the following reasons: First, in these experiments the sample cuvette was placed close to the photomultiplier, thereby greatly minimizing the light scattering effect. Second, the light scattering influence on the observed spectrum would cause a steady increase in the absorption as the wavelength decreases.

Attention has been drawn to the influence of pH

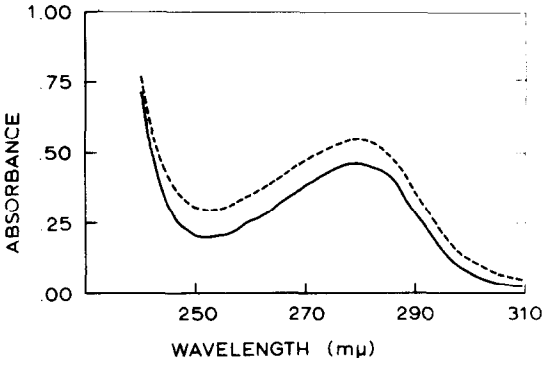

Fig. 2. Effect of KCl on the ultraviolet absorption spectrum of RNA polymerase in buffer C. ——: enzyme in buffer C containing 0.22 M KCl; - - - -: enzyme in buffer C containing 0.22 M KCl dialysed for 2 hr against this buffer containing 0.1 M KCl.

on the absorption of a protein solution [10]; all spectra shown here were measured at pH 7.9. If the observed spectral change was caused solely by the exposure of certain hidden aromatic amino acid residues of the protein, the effect should have been more marked around 280 mμ. However, a conformational change of the protein, resulting in the exposure of certain aromatic amino acids and secondary changes in the peptide backbone, (helix-coil transition) could result in the kind of spectral change shown here. It would be interesting to investigate the correlation between this spectral change and the aggregation of the enzyme in buffers of low ionic strength [8].

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