SPECIFIC CHANGES OF CIRCULAR DICHROISM SPECTRA UPON INTERACTION OF 5′- GMP WITH POLY-L-LYSINE

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Received April 20, 1970

SUMMARY

The CD spectra of poly-L-lysine in the presence of several 5′-nucleoside monophosphates have been measured at pH 7 and at low nucleotide concentrations. The spectra of the mixtures differ substantially from the sum of the spectra of the respective components only in the case of 5′-GMP. The binding of 5′-GMP induces a strong negative band at about 260 nm and large changes at shorter wavelengths. The spectral changes, which are due to a rather slow kinetic process, indicate that guanosine nucleotides are able to form regular structures upon binding by poly-L-lysine. The formation of these structures is presumably accompanied by a change of the conformation of the polyamino acid.

The interaction of nucleotides with basic polyamino acids, serving as a model system, may contribute to the understanding of interaction specificity in nucleoproteins (1-7). Previous work (8) on the binding of nucleoside monophosphates by poly-L-lysine, studied by equilibrium dialysis, showed that the association constants of 5′-adenosine, 5′-cytidine and 5′-uridine monophosphates are equal, whereas 5′-GMP binds more strongly. Mixtures of poly-L-lysine and 5′-nucleotides exhibit ORD spectra which are different from the sum of the respective component spectra only in the case of 5′-GMP (9, 10). In order to elucidate the contribution of the guanin base to the interaction specificity, CD spectra of poly-L-lysine-nucleotide mixtures have been determined and the spectral changes induced by 5′-GMP have been studied.

Materials and Methods

Nucleotides were purchased from Boehringer, Mannheim; poly-L-lysine HBr was purchased from Miles-Yeda LTD. Stock solutions of poly-L-lysine were prepared by dialysis, first against 0.5 M CH_3COONa and then exhaustively against 0.02 M KF. The nucleotide and poly-L-lysine solutions were adjusted to pH 7. The CD spectra were recorded in a Dichrographe Model CD 185 (Roussel-Jouan), in a cell of 1 mm pathlength, at a sensitivity of $1 \cdot 10^{-5}$ and at 1^{o}.

Results and Discussion

CD spectra of poly-L-lysine in the presence of different 5′-nucleoside monophosphates are shown in Figure 1, compared with the sum of the spectra of the respective components (dotted lines). It is obvious that only the binding of 5′-GMP causes strong changes of the CD spectrum relative to the spectra of the components, inducing a very strong negative band at about 260 nm and significant changes at shorter wavelengths. The changes observed with 5′-AMP, 5′-CMP and 5′-UMP are rather small or within experimental error. With the latter nucleotides the same results were obtained, when the nucleotide to lysine residue ratio was increased from 0.36 (Fig.1) to 0.48. From recent comparative work on CD spectra of polyamino acids (11, 12) it is assumed that at neutral pH poly-L-lysine has some degree of ordered conformation and is composed of locally regular regions of extended helices. The identity of the CD spectra in the case of 5′-AMP, 5′-UMP and 5′-CMP with the sum of the spectra of the components indicates that upon binding the conformation of the poly-L-lysine is not changed and that these nucleotides bind in an unordered manner.

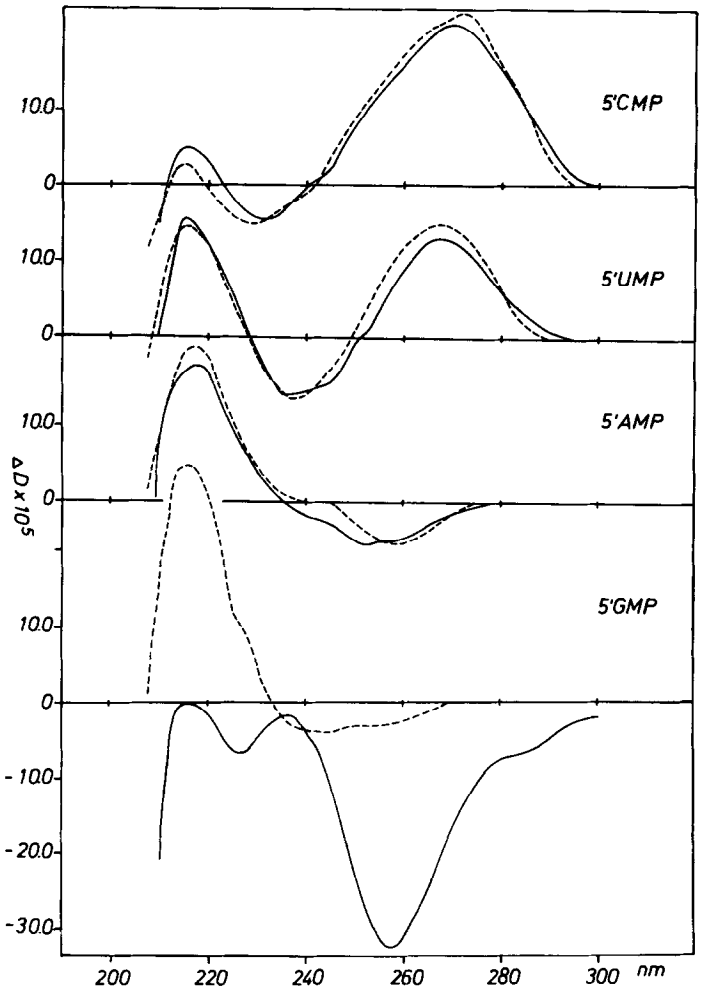

Figure 1: CD spectra of poly-L-lysine in the presence of different 5′-nucleoside monophosphates recorded at 1°, in 0.02 M KF, pH 7, in a 1 mm cell. The concentration of poly-L-lysine was 1.4 mM and of the nucleotides 0.5 mM Dotted lines are the sum of the CD spectra of the components recorded at the same concentrations and conditions.

It was observed that the spectral changes caused by binding of 5′-GMP are due to a rather slow kinetic process. An illustration of this behavior is shown in Fig. 2, where the component solutions have been precooled in ice, mixed, filled in a precooled cuvette and measured at 1° at different times after mixing. Figure 2 shows that the process underlying the

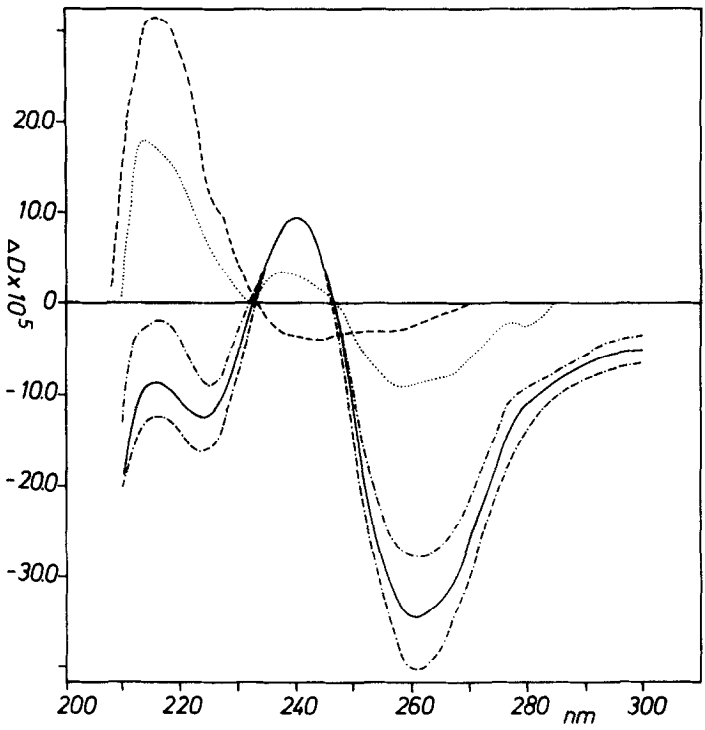

Figure 2: CD spectra of a mixture of 5´-GMP and poly-L-lysine at the same conditions and concentrations as in Figure 1, recorded at different times afte mixing; ····· 6 min, —·—·— 60 min, ——— 120 min, —·—·—·— 240 min after mixing; – – – – – sum of the component spectra.

spectral changes is a slow one, lasting several hours[1]. The kinetics of this process are under further investigation.

However, binding of the charged nucleotides by the charged poly-L-lysine should occur very fast. One may therefore assume that the spectral changes indicate a slow transition between two conformational states of the polylysine-nucleotide complex. The first state obtained immediate-

[1] In the subsequent experiments the solutions were mixed as described above and incubated in ice overnight before recording the CD spectrum. Solutions which have been mixed at room temperature or temporarily incubated at 30° and then overnight at 0° exhibit a somewhat different CD spectrum. This difference is illustrated by comparing the spectrum of the GMP-polysine complexe of Figure 1, where the solutions have been mixed at room temperature, with those of Figures 2 and 3.

ly after mixing should contain the poly-L-lysine in its original structure and the guanosine nucleotides bound in an unordered fashion. The strong negative band at about 260 nm, formed by the slow process, has to be assigned to a guanine transition and indicates that the second state contains the nucleotides in an ordered, presumably helical, conformation.

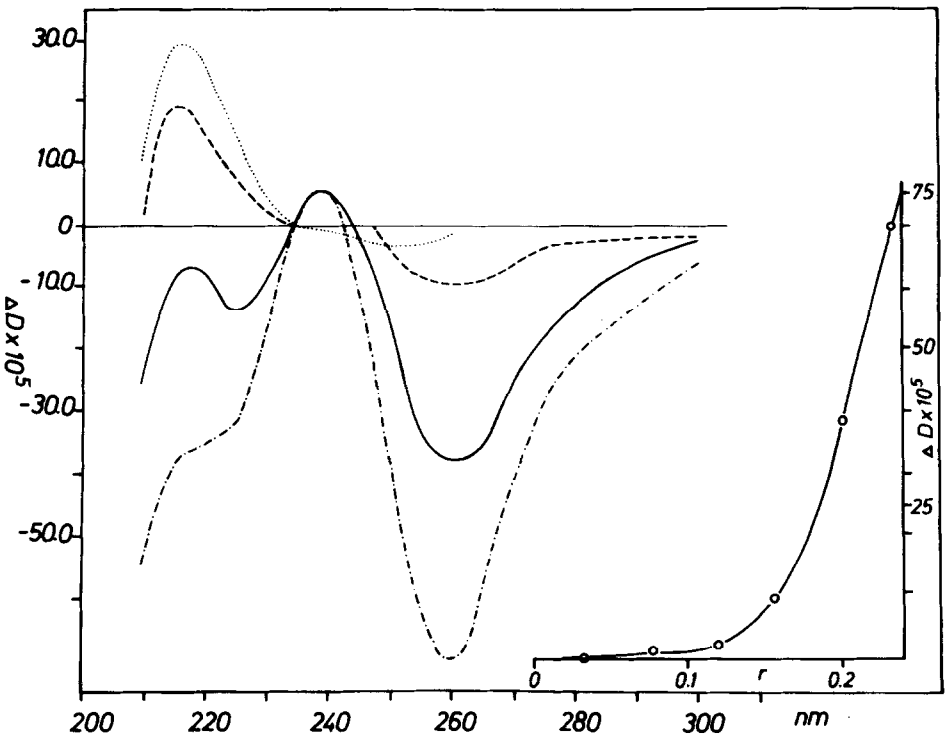

Figure 3: CD spectra of poly-L-lysine in the presence of different concentrations of 5′-GMP. Conditions and concentration of poly-L-lysine as in Figure 1 (see also footnote 1). Concentrations of 5′-GMP: ····· 0.27 mM; – – – – – 0.37 mM; ——— 0.5 mM; —·—·—· 0.60 mM.
In the inset the Δ D at 260 nm are plotted versus the number of nucleotides bound per lysine residue (r).

The formation of the ordered polylysine-GMP complex is a cooperative process. Fig. 3 shows CD spectra of poly-L-lysine in the presence of increasing 5′-GMP concentrations. From the binding isotherm obtained by equilibrium dialysis (8) the number of nucleotides bound per lysine re-

sidue (r) are calculated. In the inset of Fig. 3 the heights of the negative band at 260 nm are plotted versus these values. The expression of the 260 nm band, assumed to indicate the formation of the ordered GMP structures, begins at a threshold r value and raises in a strong cooperative manner. Above r values of about 0.3 the polylysine-GMP complex becomes insoluble and precipitates.

Guanosine nucleotide solutions are known to form gels and helical selfstructures at pH 5 and at much higher nucleotide concentrations than used in our case (13, 14). Brahms and Sadron (15) obtained CD spectra of such gels which had an ionic strength dependence. The CD spectrum measured in 0.2 M NaCl, showing a strong negative band at 272 nm and a small positive band at 241 nm, is qualitatively similar with the spectra of Figures 2 and 3, when the strong negative band at about 260 nm and the weak positive band at 240 nm are considered. The strong negative band is, however, shifted to a shorter wavelength relative to the spectrum of the GMP gel. One possible explanation may be the fact that $\pi \rightarrow \pi^*$ transitions are shifted to shorter wavelengths by replacing a polar solvent by an nonpolar one. The GMP selfstructure obtained in our case may be imbedded in or may interact with the framework of the poly-L-lysine which may represent a more unpolar surrounding. It is also conceivable that this shift is a result of slight differences in the dimensions of the presumably helical nucleotide conformation, depending whether this helix is part of the gel or part of the polypeptide-GMP complex.

Fig. 3 further shows that with increasing r values the characteristic poly-L-lysine band centered at 217 nm decreases and disappears, whereas a shoulder at about 220 nm is built up; there is also evidence for a stronger negative band below 210 nm. The CD spectrum of 5´-GMP gel at high

ionic strength shows an additional negative band at 214 nm (15), which may correspond to a band centered below 210 nm in the case of the GMP-polylysine complex, taking into account a shift similar to the 260 nm band. The negative shoulder at about 220 nm has no counterpart in the CD spectrum of the gel. The spectrum of a complex of 5′-GMP with poly-D-lysine also shows a strong negative band at about 260 nm but a positive band at about 223 nm. It is possible that this positive band obtained with poly-D-lysine originates from the same transition as the negative shoulder at about 220 nm obtained with poly-L-lysine, which may indicate that this transition is to be assigned to the polylysine component of the complex.

In summary these findings lead to the following conclusions: a) Upon binding of 5′-GMP by poly-L-lysine the bound nucleotides are ordered by a slow and cooperative process to a structure which resembles the structure obtained in 5′-GMP gels at high ionic strength and which may possess a helical conformation (13, 14). b) The process which establishes the ordered GMP structure does result in a change in the conformation of the poly-L-lysine. It may be possible that the negative shoulder abtained at about 220 nm coincides with the long wave negative band of the α-helix (16), but further evidence has to be collected before this supposition can be proved.

ACKNOWLEDGEMENT: We are indebted to Mrs. M. Kühl and the Deutsche Forschungsgemeinschaft.

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