SPECIFIC CIRCULAR DICHROISM SPECTRA OF POLY-L-LYSINE COMPLEXES WITH GUANOSINE MONOPHOSPHATES. THE ROLE OF THE 2'OH GROUP

K. G. Wagner and K. Wulff

Dedicated to Professor H. H. Inhoffen on his 65. birthday

Gesellschaft für Molekularbiologische Forschung mbH
BRD - 3301 Stöckheim / Braunschweig

Received September 8, 1970

SUMMARY

The CD spectra of poly-L-lysine in the presence of 5'-GMP, 5'-dGMP, 3'-GMP and 2'-GMP have been measured at pH 7 and at low nucleotide concentrations. The binding of 5'-GMP and 3'-GMP by poly-L-lysine induces a strong negative band in the absorption region of the base and large changes at shorter wavelengths; the binding of 5'-dGMP and 2'-GMP, however, causes only minor spectral changes compared with the spectra of the components. The poly-L-lysine complexes with 5'-GMP and 3'-GMP, which differ with respect to CD spectra, temperature stability and cooperativity of melting, are described.

Of the naturally occurring bases, guanine is somewhat unique in that it has a high tendency for self-association, an ability to form gels at high nucleoside or nucleotide concentrations, and has presumably a higher affinity to hydrophobic bonding with suitable ligands than the other bases (1 - 4). In a previous investigation (5) on the circular dichroism of poly-L-lysine in the presence of different 5'-nucleoside monophosphates, it was found that among the common nucleotides only 5'-GMP causes strong spectral changes relative to the spectra of the respective components. These spectral changes were interpreted to arise from the formation of helically ordered stacks of bound nucleotides, which force the poly-L-lysine to change conformation. Similar data have been re-

ported by Rifkind and Eichhorn (6), who found specific contributions of 5'-GMP to the ORD spectra of basic polyamino acids.

Analysis of the binding of different guanosine monophosphates to poly-L-lysine and poly-L-arginine (7), however, showed that the nature of the 2'-carbon ligand markedly influences the binding behavior and especially the stacking tendency of bound nucleotides. The present work on circular dichroism of poly-L-lysine complexes with different guanosine monophosphates was carried out to study the contribution of the 2'-carbon ligand to the specificity of interaction and to the nature of the complexes formed.

Materials and Methods

Nucleotides were purchased from Boehringer Mannheim GmbH, Sigma-Chemical Co. and N. B. C. Research Biochemicals. Poly-L-lysine HBr with a reported average molecular weight of 80 000 was purchased from Miles-Yeda. The bromide ions were exchanged for fluoride ions by dialysis and the nucleotide and poly-L-lysine solutions were adjusted to pH 7. The CD spectra were recorded in a Dichrographe Model CD 185 (Roussel-Juan), in a 1 mm cell and at a sensitivity of $1 \cdot 10^{-5}$ Δ D/mm. The component solutions were precooled in ice, mixed, incubated in ice overnight and measured at 1^{o} (5).

Results and Discussion

Figure 1 shows the CD spectra of poly-L-lysine in the presence of different guanosine monophosphates, compared with the sum of the spectra of the respective components (dotted lines). It is obvious that binding of nucleotides possessing a free 2'OH group, as 5'-GMP and 3'-GMP, induces strong spectral changes whereas the binding of those nucleotides which lack the 2'OH group (5'-dGMP) or have it blocked by a phosphate moiety (2'-GMP) reveals only minor changes[1]. The strong

[1] With 2'-GMP and 5'-dGMP still higher nucleotide to polymer ratios do not give different results.

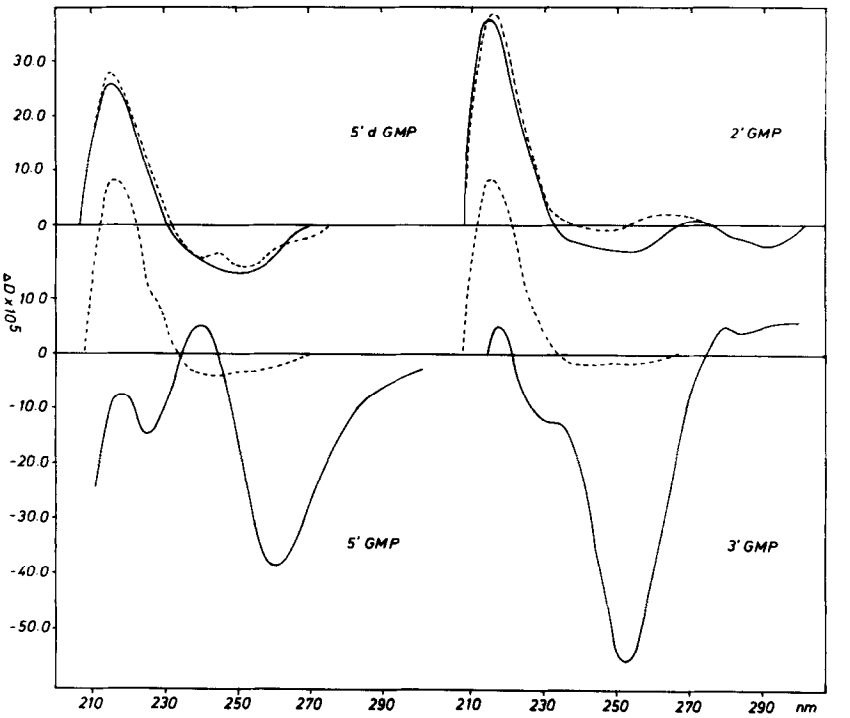

Figure 1: CD spectra of poly-L-lysine in the presence of different guanosine monophosphates recorded at 1°, in 0.02 M KF, pH 7. The concentration of poly-L-lysine was 1.4 mM and of the nucleotides 0.5 mM with the exception of 3'-GMP which was 0.37 mM. Dotted lines are the sum of the CD spectra of the components recorded at the same concentrations and conditions. The number of nucleotides bound per lysine residue (r) determined from the binding isotherms are in the case of 5'-dGMP 0.20; 2'-GMP 0.25; 5'-GMP 0.20; 3'-GMP 0.16.

negative bands found with 5'-GMP and 3'-GMP which are assumed to arise from base stacking are in accord with the previously published results (7) on the binding of guanosine monophosphates by poly-L-lysine and poly-L-arginine, which showed that the nearest neighbor interaction energies of bound nucleotides possessing a free 2'OH group are much larger than those of 5'-dGMP and 2'-GMP. At higher nucleotide to lysine residue ratios than used in the present work, guanosine monophosphates form precipitates with poly-L-lysine. At 0° with 2'-GMP and 5'-dGMP gel-like and transparent

liquid pellets were obtained after centrifugation, whereas 5'-GMP and 3'-GMP gave white precipitates with probably fiber-crystalline order (7).

Figure 2 shows CD spectra of poly-L-lysine complexes with 5'-GMP and 3'-GMP, respectively, and in the inset the maximal heights of the strong negative bands plotted versus the number of bound nucleotides (r), as determined by equilibrium dialysis (7). The CD spectra of the complexes which have been recorded at about equal r values are different: the position of the strong negative band of the 3'-GMP complex is shifted by 5 nm to shorter wavelengths relative to the spectrum of the 5'-GMP complex; the negative shoulder found at the long wavelength terminus of the 5'-GMP spectrum may correspond to the small positive contributions found with the 3'-GMP at the same region. The negative shoulder obtained with 5'-GMP at about 222 nm has been assigned to a poly-L-lysine transition on the basis of the fact that with poly-D-lysine a positive band was obtained at the same position (5). Also, with the 3'-GMP complex the negative shoulder at about 228 nm is inverted to a positive band using poly-D-lysine instead of poly-L-lysine, which again indicates that this is a polypeptide transition, which may arise from a conformational change of poly-L-lysine, due to binding of 3'-GMP (as in the case of 5'-GMP).

Gels are formed at pH 5 and at rather high concentrations of 5'-GMP and 3'-GMP (1, 2, 8-12). The CD spectrum of the 5'-GMP gel (2, 10, 11) was found to resemble the CD spectrum of the 5'-GMP / poly-L-lysine complex considering only the nucleotide contributions (5); however, the 3'-GMP gel spectrum (2) is quite different from the spectrum of the respective poly-L-lysine complex.

A further difference in the interaction of these two nucleotides with

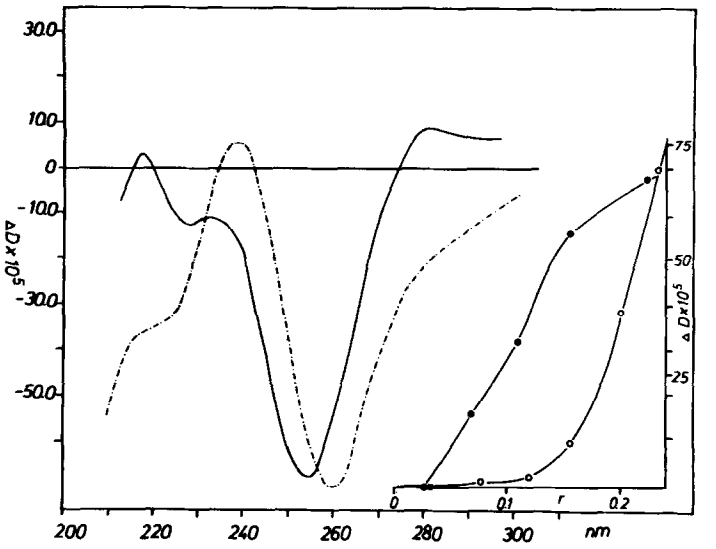

Figure 2: CD spectra of mixtures of poly-L-lysine with 5'-GMP and 3'-GMP, respectively. Conditions as in Figure 1. ———— CD spectrum with 3'-GMP, concentration of poly-L-lysine 1.4 mM, of 3'-GMP 0.45 mM, number of bound nucleotides per lysine residue (r) 0.22. —·—·—·—·— CD spectrum with 5'-GMP, concentration of poly-L-lysine 1.3 mM, of 5'-GMP 0.60 mM, r equal to 0.23. In the inset the Δ D of the strong negative band are plotted versus the number of nucleotides bound per lysine residue (r): ● with 3'-GMP, O with 5'-GMP.

poly-L-lysine is illustrated in the inset of Figure 2. The formation of the strong negative band as a function of the number of nucleotides bound per lysine residue is in the case of 5'-GMP a strong cooperative process, whereas with 3'-GMP this process is more complicated and begins at much lower r values.

Figure 3 shows the temperature stability of the two complexes; the maximum heights of the strong negative bands are plotted versus temperature. With the 5'-GMP complex the negative shoulder at about 222 nm, assigned to a poly-L-lysine transition, was also used. The complex with 3'-GMP is rather stable and "melts" uncooperatively with a transition midpoint of about 42°. The 5'-GMP complex, however, melts

at about 20^{o} and has a temperature profile which indicates a cooperative melting process which is biphasic. The cooperative transition of the 5'-GMP complex is in accord with the cooperative formation of this complex by successively adding bound nucleotides (Figure 2, inset). It is interesting to compare Figure 3 with the melting behavior of the respective nucleotide gels (1, 9), which is in both cases cooperative;

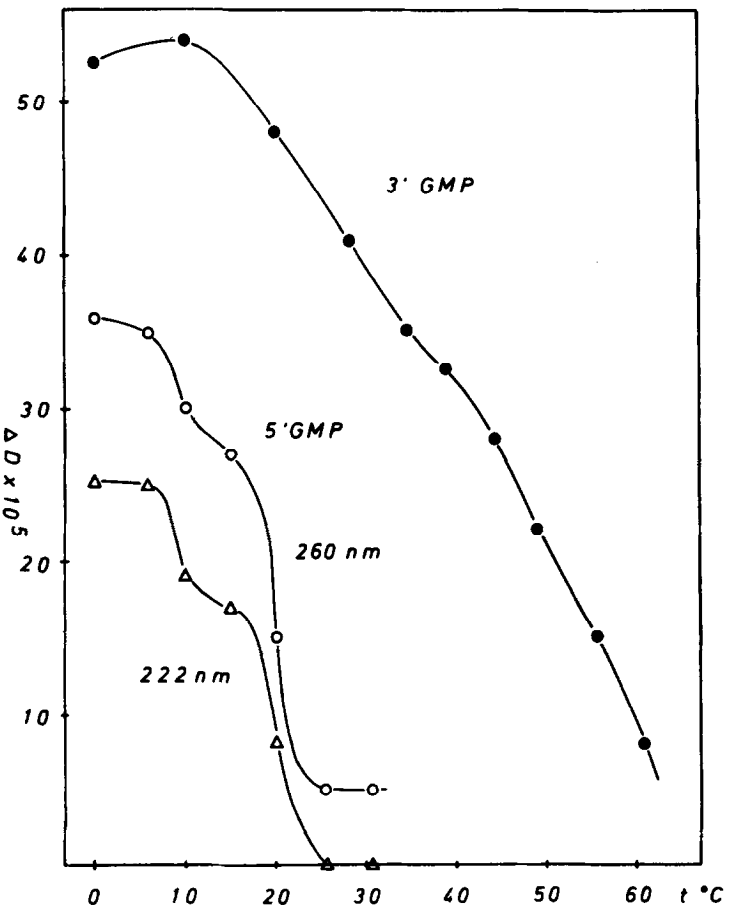

Figure 3: Melting behavior of poly-L-lysine complexes with 3'-GMP and 5'-GMP, respectively. ● complex with 3'-GMP; the Δ D of the strong negative band are plotted versus temperature. 5'-GMP: O the Δ D of the strong negative band is used for plotting; △ the Δ D of the shoulder at 222 nm, assumed to be a polypeptide transition, is plotted versus temperature. The concentration of poly-L-lysine was 1.4 mM, of 3'-GMP 0.4 mM (r 0.19), and of 5'-GMP 0.57 mM (r 0.21).

the transition midpoints are rather low, for 3'-GMP at about 11^{o} and for 5' GMP at about 14^{o}.

The ability of a guanosine nucleotide to form a stacked conformation when bound to poly-L-lysine and to induce a conformational change in the polyamino acid requires the presence of the 2'OH group. The 5'-GMP complex reveals rather cooperative features both with respect to the formation and "melting"of ordered nucleotide stacks. The interaction of 3'-GMP with poly-L-lysine leads to a more stable complex, and the formation of a stacked conformation begins at lower concentration of bound nucleotides compared with the 5'-GMP complex. One may speculate that the steric features of stacked 3'-GMP allow a stronger hydrophobic interaction of the bases with the poly-L-lysine component, or a stronger interaction of the phosphates with the protonated ε-amino groups than is possible in the 5'-GMP complex. A stronger mutual interaction may explain the fact that the CD spectra of the poly-L-lysine complex and the gel are different in the case of 3'-GMP. However the case of 5'-GMP, where the spectra are similar, may indicate weaker mutual penetration and weaker mutual interaction but probably a better fitting between the ordered nucleotide stacks and the assumed ordered polypeptide conformation. The latter supposition is supported by the CD spectrum of the 5'-GMP complex which reveals a rather strong negative shoulder at 222 nm compared to the spectrum of the 3'-GMP complex (Figure 2).

ACKNOWLEDGEMENTS: We are much indebted to Mrs. M. Kühl for skilful technical assistance and the Deutsche Forschungsgemeinschaft for support.

REFERENCES

1. Gellert, M., Lipsett, M.N., and Davies, D.R., Proc.Natl.Acad.Sci. U.S. 48, 2013 (1962)
2. Guschlbauer, W., Chantot, J.F., Sarocchi, M.-Th., and Thiele, D., studia biophysica, Berlin, in press
3. Wagner, K.G., and Arav, R., Biochemistry, 7, 1771 (1968)
4. Crothers, D.M., and Ratner, D.I., Biochemistry, 7, 1823 (1968)
5. Wulff, K., Wolf, H., and Wagner, K.G., Biochem.Biophys.Res. Comm. 39, 870 (1970)
6. Rifkind, J.M., and Eichhorn, G.L., Biochemistry, 9, 1753 (1970)
7. Arav, R., and Wagner, K.G., Eur.J.Biochem. 13, 267 (1970)
8. Peticolas, W.L., J. Chem. Phys. 40, 1463 (1964)
9. Sarkar, P.K., and Yang, J.T., Biochem. Biophys. Res. Comm. 20, 346 (1965)
10. Brahms, J., and Sadron, Ch., Nature, 212, 1309 (1966)
11. Homer, R.B., and Mason, S.F., Chem. Comm. 332 (1966)
12. Iball, J., Morgan, C.H., and Wilson, H.R., Nature, 199, 688 (1963)