PHYSICOCHEMICAL PROPERTIES OF NUCLEOSIDES 3. GEL FORMATION BY 8-BROMOGUANOSINE*

Jean-François CHANTOT** and Wilhelm GUSCHLBAUER
Service de Biochimie, Département de Biologie, CEN Saclay, 91 Gif-sur-Yvette, France

Received 5 July 1969

1. Introduction

Recently, the possiblity of conformational changes in guanosine and related nucleosides has aroused much interest [1—4]. Previous work from this laboratory [3] indicated that protonation of guanine nucleosides might induce a change from the *anti* to the *syn* conformation. It was therefore of interest to investigate the properties of 8-bromoguanosine (BrG) which for steric reasons would preferentially assume a conformation other than *anti* [5]. Since only guanosine and its nucleosides form gels, while the other natural bases which are always in the *anti* conformation do not show this behaviour, it was investigated, if and under which conditions BrG would form a gel. The data on the optical properties of BrG gels show that the *syn* conformation does not hinder the formation of a gel.

2. Material and methods

8-Bromoguanosine (BrG) was synthetized by stirring 1 g guanosine in 30 ml water at room temperature, and adding a saturated aqueous solution of bromine in aliquots of 1 ml. The next aliquot was added only after the colouring had disappeared. After about 15 min the colour persisted. Stirring was continued for $\frac{1}{2}$hr and the resulting precipitate was filtered off. Slow recrystallization from 50 ml water gave long transparent needles in about 80% yield.

Anal. $C_{10}H_{12}BrN_2O_5 + H_2O$:
Calc.: C:31.6; H:3.7; N:18.4; Br:21.0.
Found: C:31.4; H:3.8; N:17.6; Br:20.9.
Decomposes ~ 200°C, ϵ_{261} = 15,700 (pH = 1.7).

Spectroscopic methods were those used previously [3b]. All measurements were made in 0.1 mm Hellma cuvettes.

3. Results and discussion

Fig. 1. shows the optical properties of BrG in solution and as a gel. The absorption spectrum (*C*) shows large hypochromicity and a blue shift upon gel formation, indicative of the formation of an ordered secondary structure. Although Miles [7] had reported a negative Cotton effect for BrG in solution, the ORD (*B*) and CD (*A*) spectra of BrG show a positive first Cotton effect in contrast to other purine nucleosides [8,9] which exhibit a negative Cotton effect; this reflects the preferential *syn* conformation of the compound [6] similarly to 8-iodoguanosine [3a].

Formation of the gel is accompanied by a large increase in optical activity which demonstrates the formation of a highly ordered asymmetric structure, which is possibly helical [6]. The optical activity of the main absorption band can be decomposed into a split exciton band [10], characteristic of base-base interactions (stacking) and could be related to the negative dichroic band of the solution spectrum. A further band with a maximum around 295 nm in the gel spectrum could be either due to a $n \rightarrow \pi^*$ transition

* Part 2, ref. [3c].
** Boursier de Thèse du C.E.A. Les résultats du présent travail feront partie d'une Thèse de Doctorat d'Etat ès-sciences physiques de J.F.C.

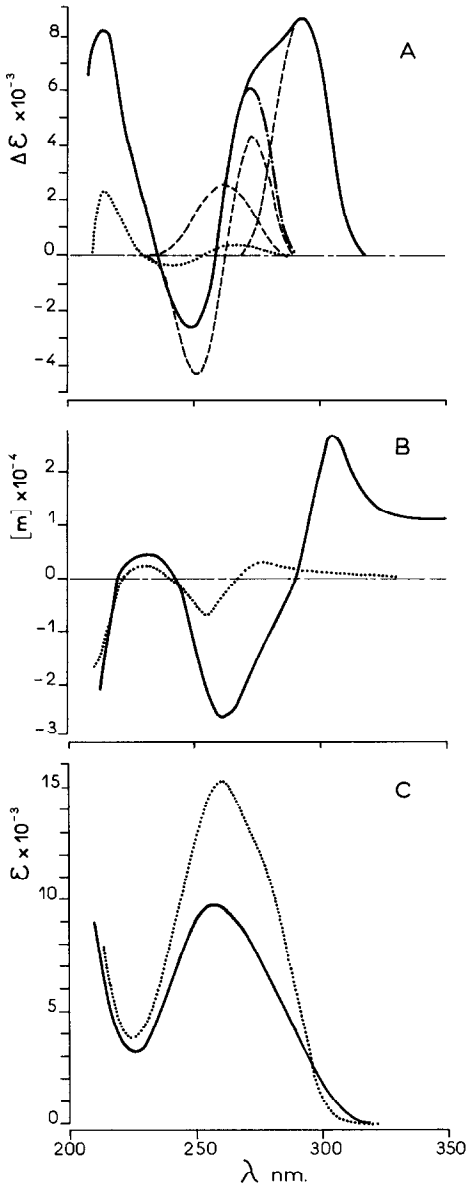

Fig. 1. Optical properties of BrG. — 0°C (gel); . . . 60°C (solution); top: CD; center: ORD; bottom: UV absorbance. - - - exciton band; –.– possible $n \rightarrow \pi^*$ band. [BrG] = 2.2 $\times 10^{-2}$ M, [NaCl] = 0.1 M, pH = 6.5.

or be the increased and shifted positive dichroic band of the solution spectrum.

Several points can be deduced from fig. 2 which shows the stability diagram of BrG. On the one hand, gel formation is dependent on the presence of a minim-

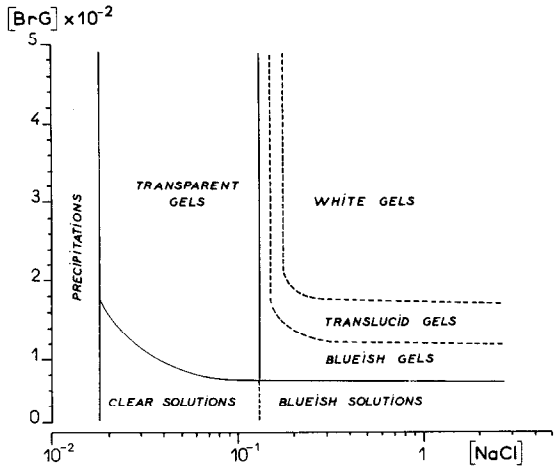

Fig. 2. Stability diagram of BrG.

al concentration of an electrolyte. Instead of NaCl many other salts can be used (see fig. 5). Further the ionic strength delimits the existence of gels: above 0.12 M NaCl the gels become increasingly turbid, while below 0.02 M NaCl precipitation occurs. On the other hand, at a given salt concentration, a minimal threshold concentration of BrG is necessary for gel formation, in accordance with the biocolloid theory of Peticolas [11]. This concentration dependence (fig. 3) manifests itself by abrupt changes of ϵ_{max} and by the appearance of an ordered structure, the melting point of which increases linearly up to a limiting value.

As has been demonstrated in fig. 2, the ionic strength has strong influence on the gel. Fig. 4 shows the increase in T_m as a function of sodium and potassium ion concentration. Interestingly enough there is a large difference between the melting points measured in KCl and those measured in NaCl solution. The increase in T_m in NaCl shows a slope ($\Delta T_m / \Delta \log [Na^+] = 21°$) similar to that observed for DNA and double-stranded polynucleotide complexes [12] (while it is considerably smaller in KCl) despite the absence of phosphate groups. This can apparently not be explained by polyelectrolyte theory [13]. If one increased the chloride concentration in a clear gel in NaCl by adding HCl, turbidity occurred. The work of Robinson and Grant [14] appeared therefore of great interest. These authors have studied the effect of anions on the solubility and conversely on the

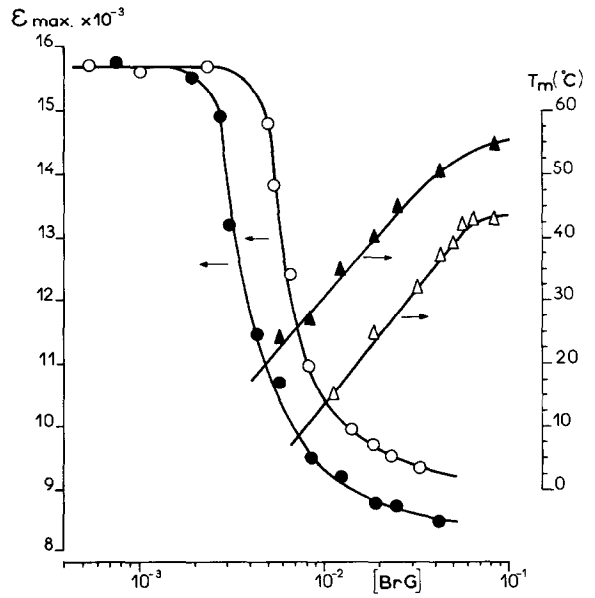

Fig. 3. Change of ϵ_{max} (○) and T_m (△) as a function of BrG concentration. Filled symbols: 0.1 M KCl; open symbols: 0.1 M NaCl.

Table 1
Influence of anions on gel formation by BrG.

Anion	Aspect of gel (at 5°C)	T_m
CH_3COO^-	Transparent	39°
CCl_3COO^-	Opaque	38°
F^-	Transparent	36°
Cl^-	Transparent	40°
Br^-	Opaque	42°
I^-	Opaque	46°
ClO_3^-	Transparent (becomes blueish after heating)	40°
NO_3^-	Blueish	39°
$PO_4H_2^-$	Transparent	39°
SO_4^{--}	Opaque	45°
SCN^-	Opaque	47°
CO_3H^-	Opaque soft gel (pH≈8.6)	26°
$B_4O_7^{--}$	No gel	–

[BrG] = 1.9×10^{-2}, [A^-] = 0.1, cation: K^+, all at pH = 6 except CO_3H^-.

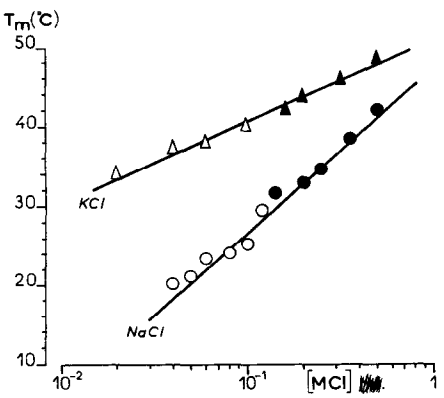

Fig. 4. Change of T_m of BrG gels as a function of ionic strength. [BrG] = 1.8×10^{-2} M; ○ NaCl; △ KCl. Open symbols: clear gels; filled symbols: blueish or translucid gels.

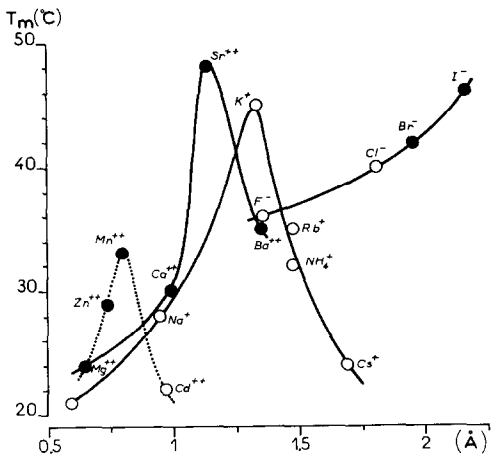

Fig. 5. Variation of T_m of BrG gels with different ions. The results have been plotted as a function of the crystal radius of the ions. The variations of the cations were measured in the corresponding 0.1 M chloride solution, [BrG] = 2.4×10^{-2} M, while those of the anions were measured in the corresponding 0.1 M potassium salt solutions, [BrG] = 1.9×10^{-2} M.

activity coefficients of bases and nucleosides. When applied to BrG only minor effects on the melting points of the gel were observed (table 1), although the general trend is similar to that observed by Robinson and Grant [15], i.e. the large "salting out" anions

(Cl_3CCOO^-, ClO_3^-, I^-) have the tendency to give blueish or opaque gels, while the smaller anions (F^-, Cl^-) (fig. 5) give clear gels. There is however a very large influence of the cations. In fig. 5, the T_m's of BrG gel, as a function of the crystal (ionic) radii of several mono- and divalent cations are plotted. In each series, one cation shows a peak in the increase in T_m, K^+, Sr^{++} and Mn^{++}, respectively, as a function of the crystal radius of the cation. The salt effect and this anomaly in each series are not understood and particularly surprising in the light of the results of Zimmer and Venner [15] on DNA where the T_m decreased linearly with the crystal radius of the cation. On the other hand, Hamaguchi and Geiduschek [16], in their study of DNA denaturation by various alkali salts, could not demonstrate any significant correlation between melting of DNA and denaturing effect. It might well be that the cation anomaly is due to a differential penetration and therefore binding capacity of the cation for the gel.

Acknowledgements

The authors are indebted to Dr. P.Fromageot for his continuous interest and encouragement. Dr. A.E.V.Haschemeyer gave much appreciated advice and directed our attention to the work of Robinson and Grant. We thank Dr. Y.Courtois and Mr. G.Melcher for many helpful discussions.

References

[1] A.E.V.Haschemeyer and H.M.Sobell, Acta Cryst. 15 (1965) 125.
[2] A.E.V.Haschemeyer and A.Rich, J. Mol. Biol. 27 (1967) 365.
[3] W.Guschlbauer and Y.Courtois, FEBS Letters 1 (1968) 183.
Y.Courtois, P.Fromageot and W.Guschlbauer, European J. Biochem. 6 (1968) 483.
W.Guschlbauer and M.Privat de Garilhe, Bull. Soc. Chim. Biol. 51 (1969) 00
[4] G.Koyama, K.Maeda, H.Umezawa and Y.Itaka, Tetrahedron Letters (1966) p. 597.
[5] H.M.Sobell and E.Reich, in preparation.
[6] M.Gellert, M.N.Lipsett and D.R.Davies, Proc. Natl. Acad. Sci. U.S. 48 (1962) 2013.
[7] D.W.Miles, Ph. D. Thesis, University of Utah (1967).
[8] J.T.Yang, T.Samejima and P.K.Sarkar, Biopolymers 4 (1966) 623.
[9] T.R.Emerson, R.J.Swan and T.L.V.Ulbricht, Biochem. Biophys. Res. Commun. 22 (1966) 505.
[10] I.Tinoco, J. Chim. Phys. 65 (1968) 91.
[11] W.L.Peticolas, J. Chem. Phys. 40 (1960) 1463.
[12] A.M.Michelson, J.Massoulié and W.Guschlbauer, Progr. Nucl. Acid Res. 6 (1967) 83.
[13] C.Schildkraut and S.H.Lifson, Biopolymers 3 (1965) 195.
[14] D.R.Robinson and M.E.Grant, J. Biol. Chem. 241 (1966) 4030.
[15] C.Zimmer and H.Venner, Naturwissenschaften 45 (1962) 86.
[16] K.Hamaguchi and E.P.Geiduschek, J. Am. Chem. Soc. 84 (1962) 1325.