STRUCTURAL CHANGES OF POLYRIBOINOSINIC ACID INDUCED BY A STEROIDAL DIAMINE, IREHDIAMINE A

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It has been shown (Mahler, H.R. et al., 1966) that a steroidal diamine, irehdiamine A, (IDA), diamino 3 β-20 α pregnene 5, interacts with deoxyribonucleic acid (DNA). Some parameters of this interaction have been determined (Saucier, J.M., Lefresne, P., and Paoletti, C., 1967). We have extended these studies to the effects of IDA on polyriboinosinic acid (poly I) which is known to exist at low ionic strength and at an acid or neutral pH under a coiled form with little secondary structure (Rich, A., 1958; Sarkar, P.K., and Yang, J.T., 1965). When added to poly I at pH 7.5 (Tris-HCl 5 mM), the steroid is able to organize the structure of the polynucleotide as shown by UV absorbance and optical rotatory dispersion (ORD).

MATERIALS AND METHODS

Poly I was purchased from Miles Chemical Co.; its concentration was determined spectrophotometrically, using E (248 mμ) = 10.4 x 10^3 as the molar extinction coefficient. IDA ($C_{21}H_{38}N_2Cl_2$, $2H_2O$ = 425.5) was kindly provided by Professor M.M. Janot and Dr. R. Goutarel (Centre de Recherches sur les Substances Naturelles, CNRS, Gif sur Yvette, France). All the solutions were in Tris-HCl 5 mM, pH 7.5 and poly I concentration was 6.4 x 10^{-5} M, unless otherwise stated.

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Absorption measurements were performed with a Zeiss PMQII spectrophotometer. ORD measurements were done in a Cary 60 recording spectropolarimeter with a strain-free quartz cell.

RESULTS

(a) UV Absorbance

An hypochromicity at 248 mμ and a very slight shift of the poly I maximum absorbance are induced by IDA (Fig. 1); the importance of the optical effect depends on the wave length. The variation of the OD_{248} with IDA concentration is slightly sigmoidal; the hypochromicity is maximum for a steroid to polynucleotide molar ratio of 0.78 above which absorbance increases again (Fig. 2A); the OD_{320} also increases for the highest IDA concentration for which some slow changes leading to the poly I precipitation become apparent. The hypochromicity is reversed by increasing ionic strength (Fig. 2B). Spermine,

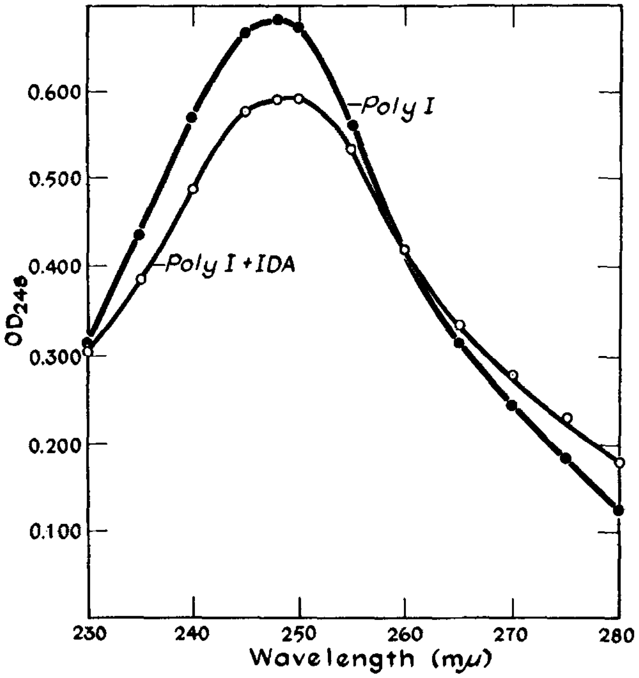

Figure 1. UV absorption spectrum - IDA: 7 x 10^{-5}.

used as a control, induces a moderate red shift of the poly I spectrum but no 248 mμ hypochromic effect is observed at a concentration of 24.8×10^{-6} M. At higher concentrations, this polyamine leads to a slow precipitation of the polynucleotide.

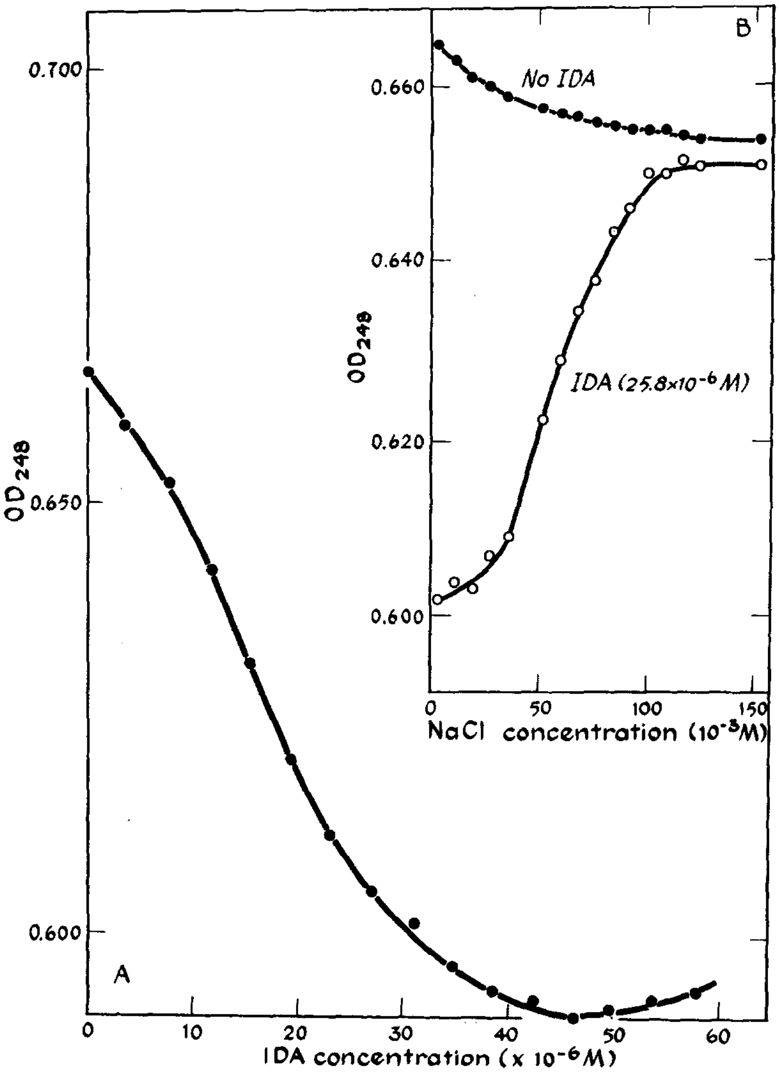

Figure 2. Poly I UV absorbance as a function of IDA (A) and NaCl (B) concentration.

A. Increasing volumes of an IDA concentrated solution (2.35×10^{-3} M) was added with an Agla micrometer syringe in a determined volume of poly I (6.4×10^{-5} M).

B. NaCl added after IDA - poly I interaction in Tris-HCl 5 mM, pH 7.5.

All measurements against poly I alone. IDA does not absorb over this spectrum range.

The absorbance of poly I alone is only slightly temperature dependent in our pH and ionic strength conditions; in the presence of IDA, there is, on the contrary, a sharp and cooperative transition with an apparent T_m of 39.5° C. (Fig. 3).

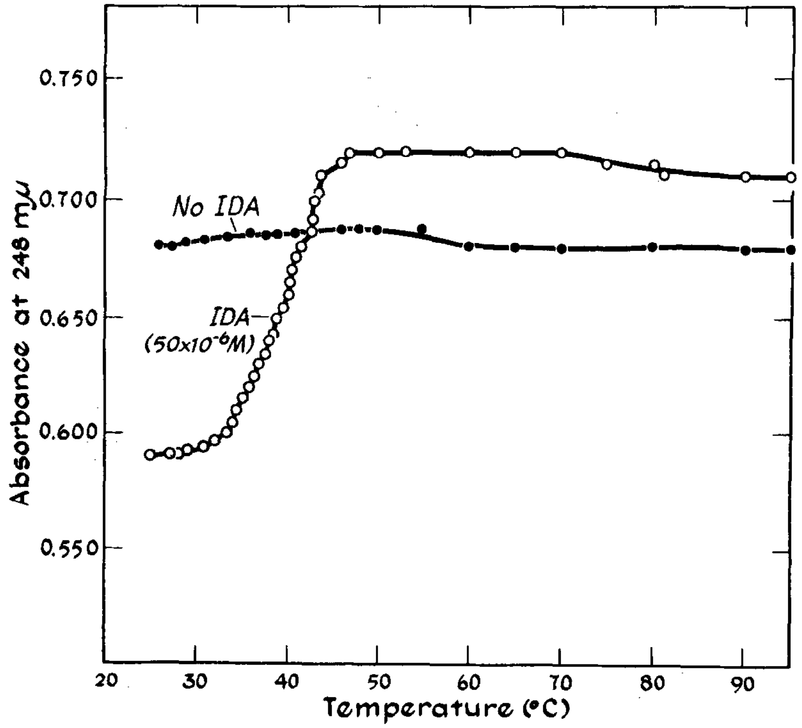

Figure 3. Poly I-IDA UV absorption as a function of temperature. The raise of temperature was 1° C every 120 sec.

(b) ORD

Poly I alone exhibits multiple Cotton effects under 300 mμ with two troughs (276 mμ and 244 mμ) and one peak (258 mμ) as described by Sarkar and Yang (1965) (Fig. 4). Addition of IDA leads to drastic changes: the ORD spectra displays multiple Cotton effects but shows one peak (246 mμ) and two troughs (264 mμ and 236 mμ) and is more similar to the normal polynucleotide spectra (Samejima, T., and Yang, J.T., 1964). Mg^{++}, at a similar molar concentration as the steroid one, and spermine at a 24 x 10^{-6} M concentration modified the magnitude of the optical effect but does not change the position of the peak and of the two troughs of poly I alone.

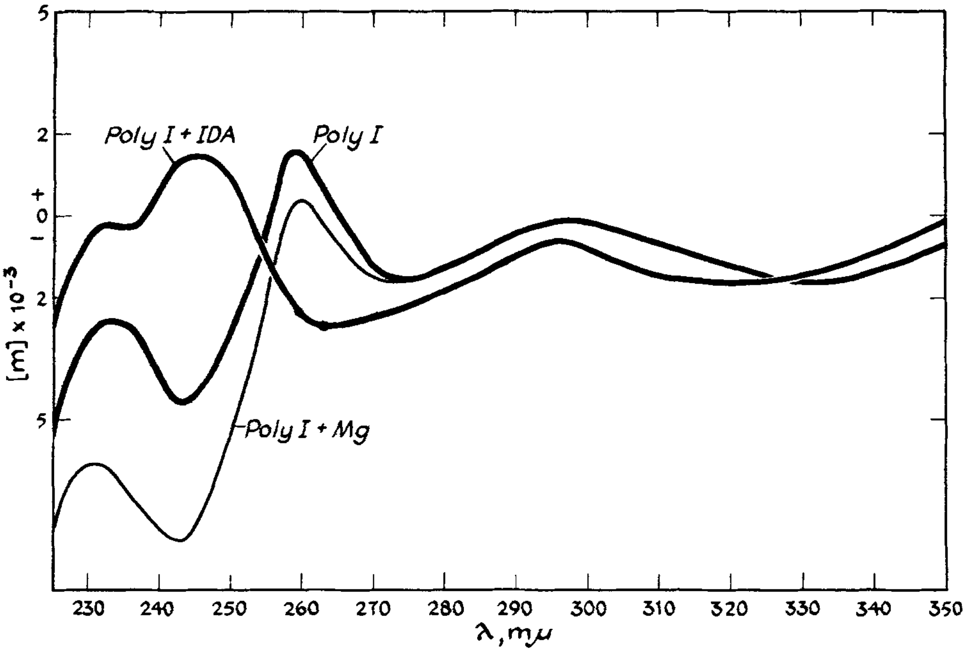

Figure 4. Ultraviolet rotatory dispersion.
Light path: 1 cm - Temp.: 25° C.
Data expressed in terms of mean residue rotation, or molar rotation for mononucleotide, (m) = 10 a/dM, where a is the measured rotation in degrees, d the light path in decimeters and M the molar concentration on the basis of UV absorption. The steroid has no rotatory effect. Poly I = 64 x 10^{-6} M; IDA = 66 x 10^{-6} M; $MgCl_2$ = 60 x 10^{-6} M.

DISCUSSION

The data presented here provide evidence for the existence of at least two steps in the interaction between poly I and IDA at low ionic strength and neutral pH. The first step which has only been studied here is characterized by the formation of an organized structure of poly I. Such a conclusion is warranted by the hypochromic effect, the cooperative melting of the IDA-poly I complex, as contrasted with poly I alone, and the extensive changes in ORD spectrum which can be interpreted as due to bases stacking as predicted on theoretical grounds by Tinoco (1964). Moreover, some preliminary data

(Paoletti, C., unpublished) show that the endonuclease of Neurospora crassa (Linn, S., and Lehman, I. R., 1965), active only on polynucleotides lacking an ordered structure, is progressively inhibited in a steroid concentration range where the previous changes are observed.

Electrostatic forces come into play in the conformational changes leading to the new structure as proved by the disappearance of the optical effect with increasing ion concentration. It is likely that the steroidal diamine neutralizing the negative phosphate charges on the polymer, weakens the forces of mutual repulsion and allow some base stacking along the polynucleotide backbone leading to the structural organization and to the optical and ORD effects. These changes seem to be specifically induced by the steroid diamine as demonstrated by the absence of ORD modifications with another polyamine, spermine. Nevertheless, the true nature of such a specificity remains unknown; it could either be related to the rigidity of the steroid nucleus which stands in contrast with the free chain rotation of the aliphatic amines and would allow the two amino groups to lie at a constant distance (11.6 Å) favorable for the interaction on the polynucleotide or be due - more interestingly - to the configuration of this steroid structure able to exchange some specific forces with the polynucleotide bases.

The actual structure of the new complex presently described is not known; it probably involves the regular and repetitive spacing of the hypoxanthine residues along the phosphodiester chain. The steroid is engaged in the complex and takes part of its stability since poly I is reversed to its poorly organized structure when IDA is removed by competition with sodium ions at such a concentration (around 100 mM) where the competing self organization induced on poly I by the ionic strength increase is not yet significant. The occurrence of a poly I_3-like structure as demonstrated for poly I at high ionic strength (Rich, A., 1958; Doty, P. et al., 1959) is ruled out by the ORD data since the poly I-IDA complex displays a very different spectrum from the one given by poly I_3 (Sarkar, P.K., and Yang, J.T., 1965).

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