Nucleic Acids Research

Polynucleotides. XLVI. [1] Synthesis and properties of poly (2'-amino-2'-deoxyadenylic acid)

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ABSTRACT

Poly (2'-amino-2'-deoxyadenylic acid) [poly (Aa)] was prepared from chemically synthesized 2'-amino-2'-deoxy-ADP by the catalysis of polynucleotide phosphorylase. Poly (Aa) showed a similar UV absorption spectra to poly(A), but quite different CD spectra at pH 7.0 and 5.7. At the former pH it showed a single negative Cotton band and at the latter a curve with a large splitting of bands. Acid titration of poly(Aa) suggested protonated form below pH 7.0. Temperature absorption profiles and their dependency on sodium ion concentration suggested an ordered structure for poly (Aa) which is stabilized by stacking of bases and intrastrand interaction between 2'-amino and internucleotidic phosphate groups. Poly (Aa) forms a 1:2 complex with poly (U) at neutrality and its Tm was 45° in the presence of 0.15M sodium ion.

INTRODUCTION

Recently a versatile method for synthesizing 2'-azido-and 2'-amino-2'-deoxyadenosine (Chart, X=N$_3$ and NH$_2$) was developed.[2] Using this approach poly (2'-azido-2'-deoxyadenylic acid) [poly(Az)] has been synthesized[3] and its properties have been elucidated. It was found that poly(Az) showed
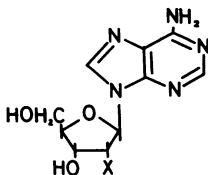
quite similar characteristics to poly(A) in UV and CD spectroscopy, melting temperature (Tm) and complexing with poly(U), in contrast to the previous observation[4,5] that introduction of an azido group in place of 2'-OH of poly (U) enhanced its thermal stability significantly.

We now wish to report on the synthesis and properties
of poly (2'-amino-2'-deoxyadenylic acid) [poly(Aa)].
Interesting features such as the fact that poly(Aa) formed a
protonated ordered structure at pHs lower than 7.0 and a
triple stranded complex with poly (U) in contrast to its
pyrimidine counterpart poly (Ua),[6,7] are described.

Materials and Methods
2'-amino-2'-deoxyadenosine 5'-diphosphate

2'-Azido-2'-deoxyadenosine 5'-diphosphate[3] (747 OD_{260}
units, 49.5 umoles) was dissolved in a mixture of water (5 ml)
and acetic acid (1 ml). To the solution palladium charcoal
(10%, 20 mg) was added and the mixture was stirred under an
hydrogen atmosphere for 1 hr at room temperature.
The catalyst was removed by filtration and the filtrate was
evaporated in vacuo. Traces of acetic acid were removed
by azeotropic distillation with water several times.
The product showed one spot at Rf 1.07 on a paper electro-
phoretogram performed at pH 7.0. AaDP thus obtained was
applied to a column (1.7 x 20 cm) of DEAE-Sephadex A-25
(bicarbonate form) and eluted with triethylammonium bicarbo-
nate (0.15-0.25M, 1l. each) in a linear gradient.
Fractions of 15 ml were collected every 11 min. AaDP was
eluted at the buffer concentration 0.2 M. The yield was 601
OD_{260} units (80 %). Paper electrophoresis: R_{pA} 1.07 (at pH
7.5), R_{pA} 1.00 (at pH 3.5). Hydrolysis of this sample with
alkaline phopshatase gave exclusively 2'-amino-2'-deoxyadeno-
sine and inorganic phosphate.

Poly (2'-amino-2'-deoxyadenylic acid) [poly (Aa)]

A solution(5 ml) containing 2'-amino-2'-deoxyadenosine
5'-DP 4 mM, $MnCl_2$ 2 mM, polynucleotide phosphorylase obtained
from Micrococcus luteus[8] by a method described by Klee and
Singer[9] 45 units/ml and TrisHCl (pH 7.5) 80 mM was incubated
at 37° for 20 hrs. The time course of the polymerization is
shown in Fig.1.
The viscosity of the solution increased significantly during
the incubation. The incubation mixture was extracted with
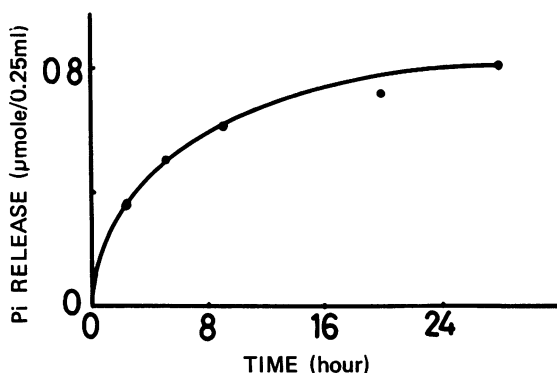a mixture of isoamylalcohol-$CHCl_3$(1:3, vol/vol) for

Fig. 1. Time course of
Aa DP polymerization.

deproteinization and dialyzed against water containing
0.01M Tris-HCl buffer (pH 7.0). The dialyzed solution was
lyophilized, the residue dissolved in a small amount of water,
and applied to a Sephadex G-50 column. As shown in Fig.2,
poly(Aa) was eluted in the void volume. Yield was 32 OD_{260}
units (15 % regardless of hypochromicity).

Physical measurements

UV spectra were taken with a Hitachi 124 spectrophoto-
meter and CD spectra were taken with a JASCO ORD/UV-5 spectro-
meter equipped with a CD attachment. Calibration was per-
formed with d-10-camphorsulfonic acid. Tms were measured with
a Hitachi EPS-3T spectrophotometer equipped with a Komatsu
thermostated cell. Temperature inside the cell was measured
by a Cu-Constantan thermocouple.

RESULTS AND DISCUSSION

UV spectrum of poly(Aa)

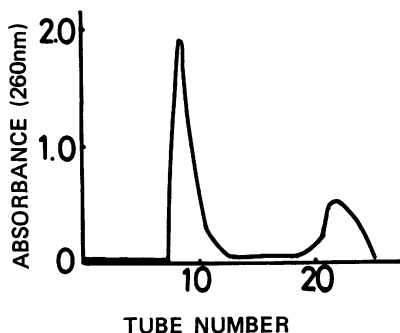The UV spectrum of poly(Aa) was recorded in the presence

Fig. 2. Sephadex G-50
column chromatography of
poly (Aa).

of 0.1M NaCl and 0.05M sodium cacodylate (pH 7.0 and 6.3)
at 25°.   As shown in Fig 3, poly(Aa) showed a spectrum
at neutrality having a single λ max at 258 nm and ε equal
to 11,200. Although the maximum wavelength was almost
identical to that of poly(A)[10], ε was somewhat smaller.
Since the ε value of 2'-amino-2'-deoxyadenosine was reported
to be 15,000 at 258.5 nm[2], hypochromicity of poly(Aa) was
calculated to be 25%. This value is significantly smaller
than that of poly(A) which was reported to be 39% at 260 nm.[10]
This unusually small hypochromicity and negligible hypso-
chromicity may be due to a structure of poly (Aa) with a
lower degree of stacking of adenine bases in the polynucleo-
tide array. At pH 6.3 poly(Aa) showed much lower ε value
presumably due to an acid form described below.

Acid titration of poly (Aa)

     When poly (Aa) was titrated with 0.1N hydrochloric acid
in the presence of 0.15 M NaCl at 15°, the UV absorption
increased sharply at pH 6.7 and reached a plateau at pH 7.6
(see Fig. 4). The midpoint of the transition was at pH 7.0.
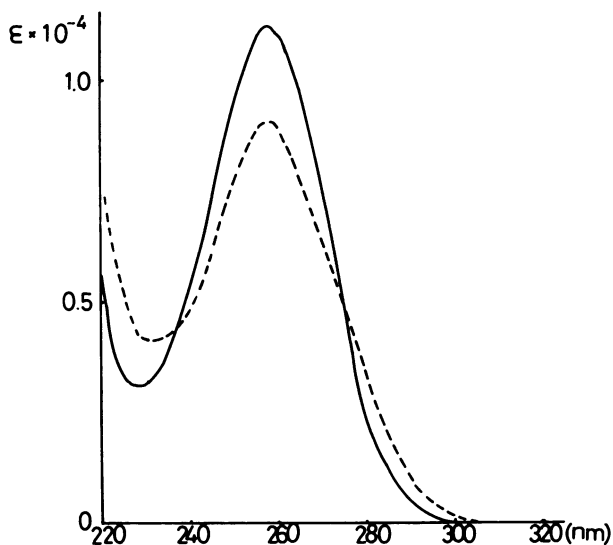The λ max 258 nm of the UV spectra did not change throughout

Fig. 3. UV absorption spectra of poly(Aa),— at pH 7.0, --- at
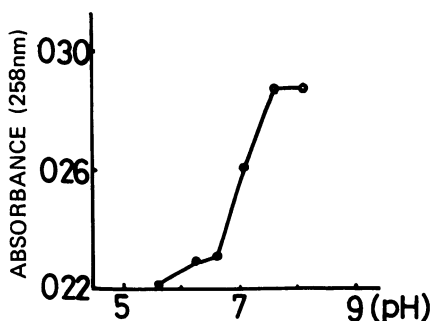   pH 6.3.

Fig. 4. Acid titration of
poly(Aa).

the titration and below pH 5.5 a precipitation occurred,
which disturbed the UV measurements. These phenomena imply
that poly (Aa) must be completely protonated at the $2'-NH_2$
group at around pH 6.7 and stacking of bases may be increased,
despite the fact that no protonation at the N^1-atom of the
adenine ring as reported in the case of poly(A), occurred.[11]

CD spectrum of poly(Aa)

As shown in Fig. 5, the CD spectrum of poly (Aa) taken
at pH 7.0 and 24° showed a simple curve resembling that of
monomer AaDP. If it was taken at pH 5.7 two troughs at
280 and 235 nm and a peak at 252 nm appeared. This fact may
suggest that poly (Aa) exists as a random coil structure
at pH 7.0 and at pH 5.7 it transformed to an ordered
structure upon protonation at the $2'-NH_2$ group. This type
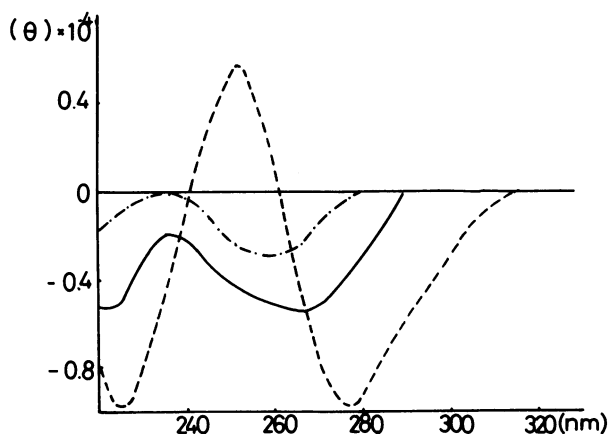

Fig. 5. CD spectra of AaDP and poly(Aa), ——poly(Aa) at pH 7.0.
---poly(Aa) at pH 5.7, -·-·- AaDP at pH 7.0.

of structure has not been reported previously with pyrimidine
$2'-NH_2$ polynucleotides.[6,7]

   To examine the thermal stability of this ordered
structure of poly(Aa), CD spectra were recorded at 20°, 46°
and 60° in the presence of 0.15M sodium ion at pH 6.3.
As shown in Fig 6, the curve at 20° closely resembled that
shown in Fig. 5, which had two troughs at 274 and 225 nm and
a peak at 252 nm. Raising the temperature to 46° this spect-
rum changed to one resembling the monomer spectrum and
this is also the case for the 60° spectrum. Therefore, the
$2'-NH_2$ protonated acid form of poly (Aa) at 20° decomposed
by thermal perturbation and a random structure might be
formed above 46°.

Temperature-absorption profiles of poly (Aa)

   The temperature absorption profiles of poly(Aa) at
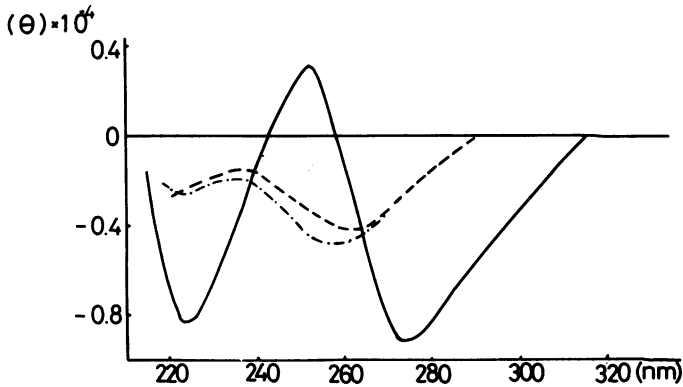various pH's were recorded in Fig. 7. At pH 7.0 in the

Fig. 6. CD spectra of poly(Aa) taken at 20°(—), 46°(---)
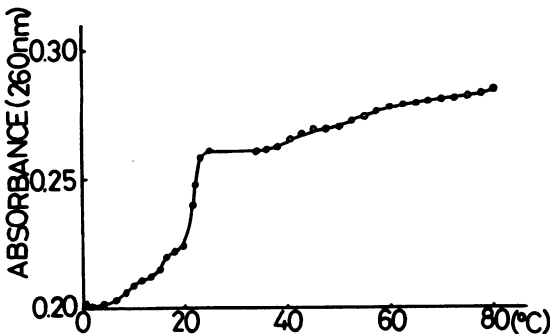   and 66°(-·-·-).

Fig. 7. Temperature-
absorption profile
of poly(Aa) taken at
7.0.

presence of 0.1M NaCl and 0.05M sodium cacodylate, the
absorption steeply increased at 20° and reached a pleteau at
24°, then it gradually increased up to 80°. This provides a
Tm of 22° at least for the first melting step. When the pH
goes down to 6.3 (Fig. 8), poly(Aa) showed a similar type
of curve, though the transition point moved to 38°.
At pH 5.7 the curve (Fig. 9) showed a clear transition point
at 53-55° and about an 80% increase in absorption.
This may account for the complete melting of poly (Aa) acid
form. At pH 5.0 the polymer precipitated presumably due to
double protonation both at the 2'-amino and N^1 of the
adenine ring.

These facts imply that by the protonation of poly (Aa)
initially at the 2'-amino group with decreasing pH a
partially protonated structure was formed and this structure
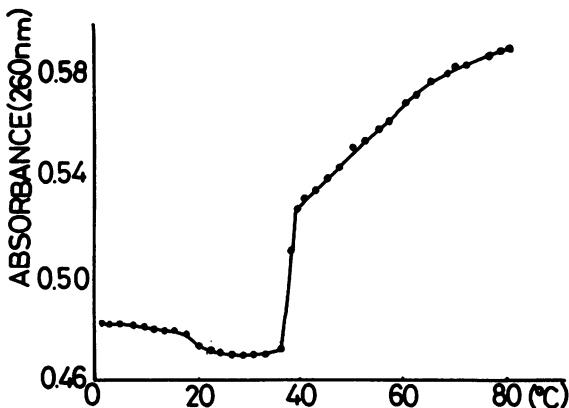changed to a "fully protonated" one at pH 5.7. Since pK of

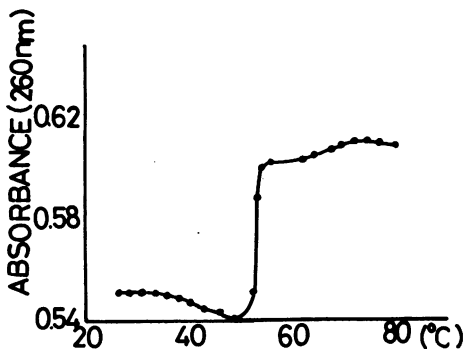Fig.8.Temperature-
absorption profile
of poly(Aa) taken
at pH 6.3.

Fig. 9. Temperature-
absorption profile of
of poly(Aa) taken at
pH 5.7.

the adenine base is around 3.5[12] and the λ max 258 of poly
(Aa) does not show any change in this pH area, the protona-
tion at the adenine base could be excluded.

Dependency of Tm on cationic concentration

As shown in Fig. 10 the Tm value at pH 7.0 was then
measured at various cationic strengths. At 0.04, 0.15 and
0.35 M sodium ion concentration Tm's were slightly lowered,
28°, 22° and 20°, respectively. This phenomenon is in
contrast to the fact that in other polynucleotides Tm increa-
sed with increasing cationic concentration.[13]

It may be deduced from this experiment that in the poly
(Aa) acid form the ordered structure is stabilized by intra-
molecular interaction between protonated 2'-amino group and
internucleotidic phosphate dissociation. This situation
could be observed in a Corey-Pauling-Koltun model of poly (Aa),
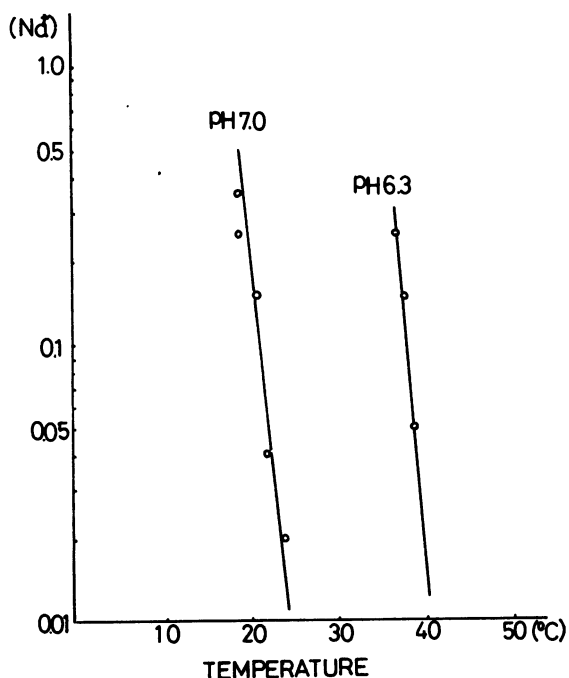though the exact structure must await X-ray diffraction study.

Fig. 10. Depending of Tm of poly(Aa) on sodium ion
concentration.

Complex formation of poly(Aa) with poly (U)

    A mixing experiment of poly (Aa) with poly(U) was performed at 0.15M sodium ion concentration and pH 7.0. Polynucleotides of 0.04M concentration each were mixed in various ratios, heated to 80° for 2 min and annealed at 20° for 15 hrs. As shown in Fig. 11, inflexion points appeared at a ratio of 1:2 for poly (Aa) vs poly (U) at wavelengths of 250, 260 and 270 nm. This indicated that a three-stranded complex poly (Aa)·2poly(U) was formed in these conditions as in the case of poly (A)·2poly(U).[14] Separate experiments at 0.04M sodium ion concentration gave fluctuating curves and no definite results could be obtained.

    Formation of this type of complex was further supported by the CD spectrum of poly (Aa) 2 poly (U) as shown in Fig. 12
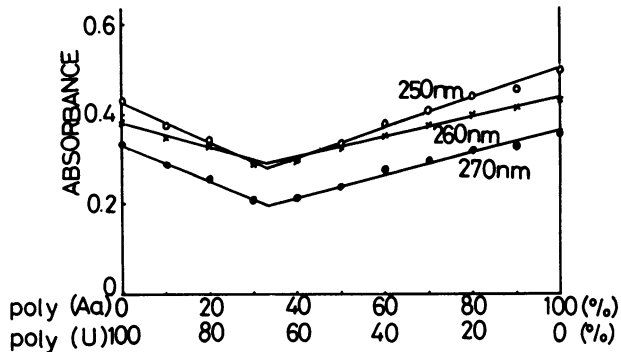

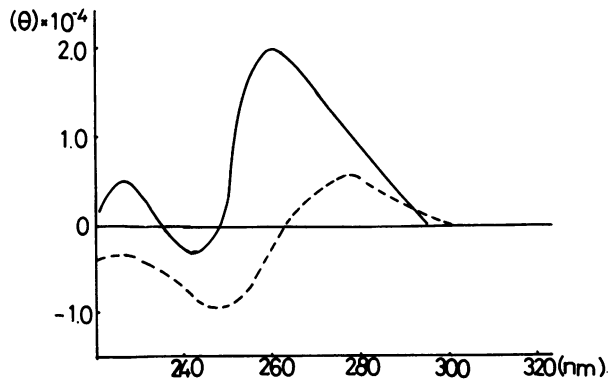Fig. 11. Mixing experiments of poly(Aa) with poly(U).

Fig. 12. CD spectrum of poly(Aa)·2poly(U) complex.

(solid line). The CD spectrum definitely differed from the calculated sum of the CD curves of the component nucleotides (dotted line).

Tm of poly (Aa)·2 poly(U)

Tm of poly (Aa)·2poly(U) complex was measured in the presence of 0.15M sodium ion at pH 7.0. As shown in Fig. 13, UV absorption steeply increased from 52° and reached a plateau at 55°. From this curve a Tm of 54° was obtained. This value is somewhat lower than that of poly (A)·2poly(U)[15], which was reported to be 60° in the same conditions. This melting curve suggested a simultaneous dissociation of the two poly (U) strands from the poly(A)·2poly(U) complex.

DISCUSSION

From the experiments described above the following points may be emphasized. Polymerization of 2'-amino-2'-deoxyADP proceeds rather slowly relative to that of poly(A) and the yield of poly (Aa) was 15%. The UV spectrum of poly (Aa) closely resembled that of poly (A), though its hypochromicity is smaller. This may imply that the introduction of the 2'-NH_2 group instead of OH of poly (A) inhibits to some extent the stacking of bases.
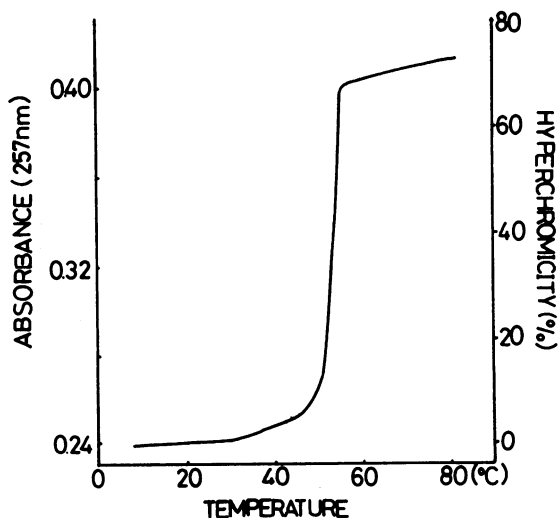

Fig. 13. Temperature-absorption profile of poly(Aa)·2poly(U) complex.

Acid titration showed that poly (Aa) must be protonated at
pH lower than 7.0. Since the amino group in the carbohydrate
moiety has a pK value around 7-8 and that of adenine is
3.5-4, this protonation must occur on the $2'-NH_2$ group.
From the thermal stability measurement and examination of
dependency of Tm on cationic strength, it may be deduced
that this protonated form of poly (Aa) would be stabilized
by an intrastrand interaction of $2'-NH_3^+$ and phosphate.
Therefore, the introduction of an amino group instead of a OH
to the 2'-position of adenosine in the polynucleotide array
led to an unusual structure, which has not been reported
previously. In the previous reports of polynucleotides
containing 2'-aminopyrimidinenucleotides[6,7] this type of
stable structure has not been detected, presumably due to
the relatively weak nature of stacking interactions in
pyrimidines.

Poly (Aa) forms a 1:2 complex with poly (U) as in the
case of poly (A)-poly (U) interaction. Its sharp melting
process and Tm value close to that of the poly (A)·2 poly (U)
case suggested a well-ordered three-stranded helical structure
probably stabilized by Watson-Crick-Hoogsteen type hydrogen
bonding. Again this is in sharp contrast with the fact
that poly (Ua) did not form any complexes with poly (A)[7].

From these studies, together with results obtained with
poly (Az)[3], it may be deduced that the effect of 2'-substi-
tuents in purine and pyrimidine polynucleotides is widely
different in nature and magnitude.

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