Hayatsu, H., and Ukita, T. (1967), *Biochem. Biophys. Res. Commun. 29*, 556.
Hayatsu, H., and Yano, M. (1969), *Tetrahedron Lett. 9*, 755, 1031.
Johnson, L., Hayashi, H., and Söll, D. (1970), *Biochemistry 9*, 2823.
Kaiser, I. L. (1969), *Biochim. Biophys. Acta 182*, 449.
Katz, S., and Comb, D. G. (1963), *J. Biol. Chem. 238*, 3965.
Kochetkov, N. K., Budowsky, E. I., Shibaev, V. N., Yelisuva, G. I., Grachev, M. A., and Demushkin, V. P. (1963), *Tetrahedron 19*, 1207.
Kondo, M., and Woese, C. R. (1969), *Biochemistry 8*, 4177.
Kyogoku, Y., Lord, R. C., and Rich, A. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 250.
Lipsett, M. N. (1965), *J. Biol. Chem. 240*, 3975.
Lipsett, M. N. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31*, 449.
Lipsett, M. N., and Doctor, B. P. (1967), *J. Biol. Chem. 242*, 4072.
Pal, B. C., Uziel, M., Doherty, D. G., and Cohn, W. E. (1969), *J. Amer. Chem. Soc. 91*, 3634.
Pleiss, M., Ochiai, H., and Cerutti, P. A. (1969), *Biochem. Biophys. Res. Commun. 34*, 70.
Saneyoshi, M., and Nishimura, S. (1970), *Biochim. Biophys. Acta 204*, 389.
Shulman, L. H. (1970), *Proc. Nat. Acad. Sci. U. S. 66*, 507.
Smith, T. D., Barnett, L., Brenner, S., and Russell, R. L. (1970), *J. Mol. Biol. 54*, 1.
Spinder, W. (1962), *in* Inorganic Isotope Synthesis, Herbert, R. H., Ed., New York, N. Y., Benjamin, p 89.
Taylor, E. C., and Chang, C. C. (1960), *J. Org. Chem. 25*, 148.
Walker, R. T., and RajBhandary, U. L. (1970), *Biochem. Biophys. Res. Commun. 38*, 907.
Weil, J. H., and Ebel, J. P. (1962), *Biochim. Biophys. Acta 55*, 836.
Yaniv, M., Favre, A., and Barrell, B. G. (1969), *Nature (London) 223*, 1331.
Zachau, H. G. (1969), *Angew. Chem., Int. Ed. Engl. 8*, 711, and references cited therein.
Ziff, E. B., and Fresco, J. R. (1968), *J. Amer. Chem. Soc. 90*, 7338.
Ziff, E. B., and Fresco, J. R. (1969), *Biochemistry 8*, 3242.

Interaction of Poly-L-tyrosine with Nucleic Acids. I. Formation of Complexes*

S. Friedman† and P. O. P. Ts'o

ABSTRACT: The interaction of poly-L-tyrosine, $(Tyr)_n$, with various nucleic acids in aqueous solution was studied. $(Tyr)_n$ solutions were mixed with various nucleic acids and then slowly neutralized to pH 7.5, both components of each reaction mixture being initially at pH 10.6 (ionic strength after mixing $\simeq 0.06$). Under these conditions, insoluble complexes are formed between $(Tyr)_n$ and denatured DNA, poly(A), poly(I), and poly(U), but not with poly(C) or native DNA. As the molar ratio of Tyr:nucleotide increases from 0 to 10, formation of these complexes increases linearly. At a ratio of 10, the complex formation is essentially complete; in the case of denature DNA and poly(I), at a ratio of Tyr:nucleotide = 14 approximately 15 and 20% of the respective nucleic acids remain uncomplexed. At neutral pH and for varying ratios of Tyr:nucleotide from 1 to 0.1, the 1:1 complex of poly(A) and $(Tyr)_n$ (concentration $\leq 5 \times 10^{-4}$ M) is the only soluble complex found for the various systems studied; it migrated as a single component in sucrose gradient electrophoresis. In the case of poly(A)–$(Tyr)_n$ system, the following situations can exist, depending on the concentration of $(Tyr)_n$ and the ratio of Tyr:A: (1) formation of a soluble complex only, (2) formation of a soluble and an insoluble complex, and (3) formation of an insoluble complex only.

The general features of this nonelectrostatic base-specific polypeptide–polynucleotide interaction are described.

Although the importance of protein–nucleic acid interactions has long been recognized, it is only recently that this phenomenon has been investigated systematically. These interactions play a key role in the expression and regulation of genetic information in living cells. Thus, DNA–histone, tRNA-activating enzyme, and DNA–RNA polymerase are examples of systems exhibiting these interactions. Because of the complexity of these interactions extensive use has been made of model systems. These studies have shed some light on the importance of the various forces implicated in such interactions, and how these forces affect the conformation of the macromolecules. Systems consisting of basic polypeptides and nucleic acids have been investigated intensively (Tsuboi *et al.*, 1966; Latt and Sober, 1967a,b; Olins *et al.*, 1968; Davidson and Fasman, 1969; Shapiro *et al.*, 1969). These systems form well-defined and very stable complexes, held together primarily by electrostatic forces (Tsuboi *et al.*, 1966; Olins *et al.*, 1968). Nevertheless, some specificity is exhibited as far as the nature and size of the components of a given system are

* From the Department of Radiological Sciences, The Johns Hopkins University, Baltimore, Maryland 21205. *Received January 18, 1971.* Presented in part at the 11th Annual Biophysical Society Meeting, Houston, Texas, 1967, Abstracts, p 87. This work was supported in part by grants from the National Science Foundation (GB-5483) and by a program project grant, National Institutes of Health (GM-10802-04).

† Present address: Department of Biochemistry, Faculty of Science, Laval University, Quebec, Can.

concerned (Latt and Sober, 1967a,b; Shapiro *et al.*, 1969). More complex model systems have been investigated, *i.e.*, DNA–RNase (Felsenfeld *et al.*, 1963; Sekine *et al.*, 1969; Raju and Davidson, 1969) which, under certain conditions, result in the formation of both soluble and insoluble complexes (Sekine *et al.*, 1969). The same study also showed that both native and denatured DNA competitively inhibit the activity of pancreatic RNase. Moreover, the binding of RNase to denatured DNA is believed to involve both electrostatic and more specific interactions, namely, the interplay of the protein with the pyrimidine residues of the nucleic acid (Raju and Davidson, 1969). For the above system, these authors reported an equilibrium binding constant per site of protein of 9.7×10^4 l./mole at ambient temperature. A recent investigation clearly demonstrated that the bases are directly involved in the binding of both polyribo- and polydeoxyribonucleotides to ribosomes, probably through hydrogen bonding (Salas and Bollum, 1969). The successful isolation of some repressors has opened up a new avenue in the domain of protein–nucleic acid interactions. Thus, the binding of a given repressor (a protein) to a specific region of DNA coding for its operator has been reported; the binding results in the formation of a soluble complex (Riggs and Bourgeois, 1969; Pirrotta *et al.*, 1970). It is evident that the majority of the model systems studied so far consist of either basic polypeptides or proteins and nucleic acids. The interactions in these systems are thus predominantly of an electrostatic nature. So far, no study has been reported on the interaction of an uncharged polypeptide with a nucleic acid. In search of a model system involving nonelectrostatic and base-specific interaction between proteins and nucleic acids, the poly-L-tyrosine–nucleic acid system was the only one found to yield promising results. In the present study, we report the interaction at neutral pH of $(Tyr)_n$, with $r(A)_n$, $r(I)_n$, $r(U)_n$, and denatured DNA, as demonstrated by formation of insoluble complexes, but not with $r(C)_n$ or native DNA.[1] Moreover, of all the systems studied, the $r(A)_n$–$(Tyr)_n$ system is the only one which forms, under certain conditions, a 1:1 soluble complex at neutral pH.

Materials and Methods

Poly-L-tyrosine, $(Tyr)_n$, was purchased from Pilot Chemicals Inc., Watertown, Mass.; hexa-L-tyrosine, $(Tyr)_6$, and the polyribonucleotides were obtained from Miles Laboratories Inc., Elkhart, Ind. Highly polymerized calf thymus DNA was secured from Sigma Chemical Co., St. Louis, Mo. Tritiated water was purchased from Packard Instruments Inc., Chicago, Ill. Throughout this study, deionized water was used and all of the chemicals were of reagent grade.

Preparation of DNA, Polyribonucleotide, and Polypeptide Solutions. DNA. The sodium salt of native calf thymus DNA (n-DNA) was dissolved in HMP and then heat denatured (d-DNA).

POLYRIBONUCLEOTIDES. All the polyribonucleotides were extensively dialyzed against HMP buffer at 4°, using treated dialysis tubing. The concentrations of the DNA and the various polyribonucleotide solutions were determined spectrophotometrically (Ts'o *et al.*, 1962; Sarkar and Yang, 1965).

POLY-L-TYROSINE. The polymer (mol wt 90,000) was first treated according to a previously published procedure (Fasman *et al.*, 1964), with a slight modification. After dialyzing the sodium salt of $(Tyr)_n$ against 0.01 N HCl at ambient temperature (instead of 40° as in Fasman's procedure), the suspension of the polymer was then dialyzed against deionized water until a negative chloride test was obtained. The suspension was then lyophilized at 25° under reduced pressure.

$(Tyr)_n$ solutions were prepared by dissolving the polymer in 0.1 N NaOH. The solutions were stored at 4° and were constantly protected against light. The concentrations of the solutions were determined by measuring the absorbance at 294 mμ at pH >13 and are expressed in monomeric units using $\epsilon_{294} = 2.25 \times 10^3$ (Fasman *et al.*, 1964).

HEXA-L-TYROSINE. The purity of $(Tyr)_6$ was verified by thin-layer chromatography. The hexapeptide was dissolved in HMP buffer and the concentration of the solution was determined by measuring the absorbance at 275 mμ using $\epsilon_{275} = 1.3_2 \times 10^3$ as determined in this study.

Mixing of $(Tyr)_n$ and n-DNA Solutions. In the case of n-DNA, two procedures were employed. In one case, 2 ml of a solution of n-DNA in HMP was added to an equal volume of $(Tyr)_n$ solutions of various concentrations of pH 11. The pH after mixing was 10.6. A Radiometer pH meter Model r22, equipped with microelectrodes (glass electrode no. 2222B) was used throughout this work. The solutions were then incubated for 1 hr at 25°, dialyzed against HMP buffer at 4°, and then centrifuged as described below. In the other case, n-DNA and $(Tyr)_n$ solutions were first dialyzed at 4° against 0.05 M glycine–NaOH buffer (pH 10.6). After mixing equal volumes of the two, the pH was slowly lowered to 7.5 by adding a solution of monobasic sodium phosphate, followed by centrifugation (see below).

Mixing of $(Tyr)_n$ and d-DNA or Polynucleotide Solutions. Equal volumes of $(Tyr)_n$ and various polyribonucleotide $(N)_n$ solutions, all at pH 10.6, were mixed at room temperature ($\mu \simeq 0.06$). The solutions were then slowly neutralized and the supernatants were centrifuged for 15–20 min at 15,000 rpm at 4°, using a Sorvall S-2 centrifuge. It must be emphasized that in order to obtain reproducible results, the neutralization must be carried out very slowly with adequate stirring (*e.g.*, ≃7 min to titrate 4.0 ml of solution). In some cases, as a check, the alkaline solutions were dialyzed again HMP buffer at 4°. The supernatants were then analyzed for polyribonucleotides or DNA and $(Tyr)_n$ by ultraviolet spectroscopy and by the Lowry technique, respectively. In the case of the Lowry technique, the absorbance was read at 650 mμ on a Beckman DU spectrophotometer.

These polynucleotides were never exposed to a pH higher than 10.6 (pH 11.0 in the case of n-DNA) for a period longer than 15 min at room temperature. No detectable increase in ultraviolet absorption was found for the polyribonucleotide solution after this short exposure to pH 10.6. Thus, the extent of hydrolysis, if any, must be negligible.

Determination of the Ratio of Tyrosine to Nucleotide in the Insoluble Complexes. The nucleic acids in the various complexes can be resolubilized by heating the latter in HMP buffer at 100°. Analysis of the complexes were carried out as follows: the complex was first washed three times with HMP, after which a known volume of HMP was added to the precipitate. The suspension was then heated for 10–15 min at 100°. After cooling, the poly-L-tyrosine remains insoluble in this neutral HMP solution, while the nucleic acid is released from the insoluble complex and dissolves in the buffer. The suspension was then centrifuged and the absorbance of the super-

[1] Abbreviations used are: poly-L-tyrosine, $(Tyr)_n$; poly(A), $r(A)_n$; poly(I), $r(I)_n$; poly(U), $r(U)_n$; poly(C), $r(C)_n$; HMP, phosphate buffer (pH 6.8) consisting of 0.0025 M disodium hydrogen phosphate and 0.005 M sodium dihydrogen phosphate; n-DNA, native DNA, d-DNA, heat-denatured DNA.

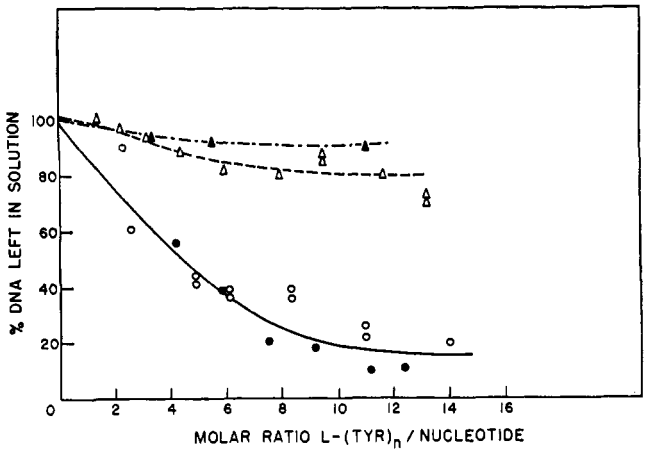

FIGURE 1: Precipitation curves for the n-DNA–$(Tyr)_n$ and d-DNA–$(Tyr)_n$ systems: concentration of DNA fixed at 9.10×10^{-4} M of nucleotides while concentration of $(Tyr)_n$ varied from 1.37×10^{-3} to 1.45×10^{-2} M; (▲) n-DNA (titration)–DNA and $(Tyr)_n$ first dialyzed against 0.05 M glycine–NaOH buffer (pH 10.6); (△) n-DNA (dialysis); (○) d-DNA (titration); (●) d-DNA (dialysis). DNA solution and $(Tyr)_n$ solution were mixed at pH 10.6 and then brought down to neutrality (see Materials and Methods section).

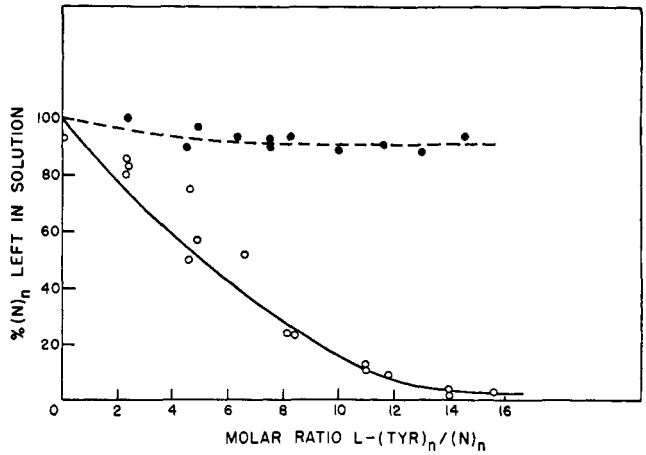

FIGURE 2: Precipitation curves for the $r(C)_n$–$(Tyr)_n$ and the $r(U)_n$–$(Tyr)_n$ systems: concentrations of $r(C)_n$ and $r(U)_n$ fixed at 9.90×10^{-4} M (●) and 9.15×10^{-4} M (○), respectively; concentration of $(Tyr)_n$ varied over the same concentration range as in Figure 1.

natant was measured at the λ_{max} of the nucleic acid. Pulverized NaOH was then added to the supernatant plus precipitate until the latter passed into solution. The solution was then transferred to a 5-ml volumetric flask and 0.1 N NaOH was added to bring the solution to the mark. The resultant solution was then analyzed for tyrosine by the Lowry technique. Alternatively, the precipitate was dissolved in a known volume of 0.1 N NaOH and the ratio of tyrosine to nucleotide was determined by the Lowry technique and the orcinol method for the ribose polynucleotides (Ashwell, 1957).

Sucrose Gradient Electrophoresis. For the sucrose gradient electrophoresis experiments, an apparatus similar to the one described by Olivera, Baine, and Davidson (Olivera *et al.*, 1964), was used which has been described elsewhere (Tsuboi *et al.*, 1966). A linear density gradient of 6–27% (w/v) sucrose in supporting electrolyte was used throughout this work. The pH, concentration of buffer, current, voltage, and duration of the runs are given below (see the appropriate figures). In each case, 1 ml of solution ($OD_{257} \simeq 5.0$ and 4% in sucrose), was layered on top of the sucrose gradient column. Tritiated water was used to mark the origin, *i.e.*, 1 drop of $[^3H]H_2O$ (2.5×10^6 cpm/ml) was added to the 1-ml sample. At the end of the experiment, approximately 45 1.1-ml fractions were collected and were then analyzed for $r(A)_n$ and tyrosine by ultraviolet spectroscopy and the Lowry technique, respectively. In a separate experiment, the fraction at the peak was analyzed for $r(A)_n$ and tyrosine, the latter being determined by measuring the absorbance at 294 mμ at pH >13 using an appropriate blank and, at the same time, making the necessary correction for the $r(A)_n$ present in the solution. This enabled us to determine the ratio of tyrosine to A in the peak position with a high degree of accuracy. The tritiated water was counted in a Packard liquid scintillation counter using a *p*-dioxane counting solution.

Results

n-DNA–$(Tyr)_n$; d-DNA–$(Tyr)_n$. The results for these systems are given in Figure 1. d-DNA interacts with $(Tyr)_n$ and forms an insoluble complex having a tyrosine to nucleotide ratio of 10. As the molar ratio of tyrosine:nucleotide increases from 0 to 10, the complex formation increases linearly. However, at a ratio of Tyr:nucleotide ≥ 10, the complex formation is essentially complete and about 15% of the DNA remains in solution. DNA can be resolubilized quantitatively from the complex by heating it in HMP at 100°.

There is little or no interaction between n-DNA and $(Tyr)_n$ as over 90% of the DNA remains in solution after all of the $(Tyr)_n$ was precipitated. As can be seen in Figure 1, the per cent n-DNA remaining in solution is somewhat higher when n-DNA and $(Tyr)_n$ are first dialyzed against a glycine–NaOH buffer (pH 10.6) and then mixed together. The solubility of $(Tyr)_n$ in aqueous solution at pH 7.5 ($\mu \simeq 0.06$) is very low, $<10^{-6}$ M.

$r(U)_n$–$(Tyr)_n$; $r(C)_n$–$(Tyr)_n$. Figure 2 shows the results obtained for these two systems. In the case of the $r(U)_n$–$(Tyr)_n$ system, the shape of the curve is essentially the same as the one for the d-DNA–$(Tyr)_n$ system, except that all of the $r(U)_n$ precipitates out of solution at a ratio of Tyr:nucleotide ≥ 10.

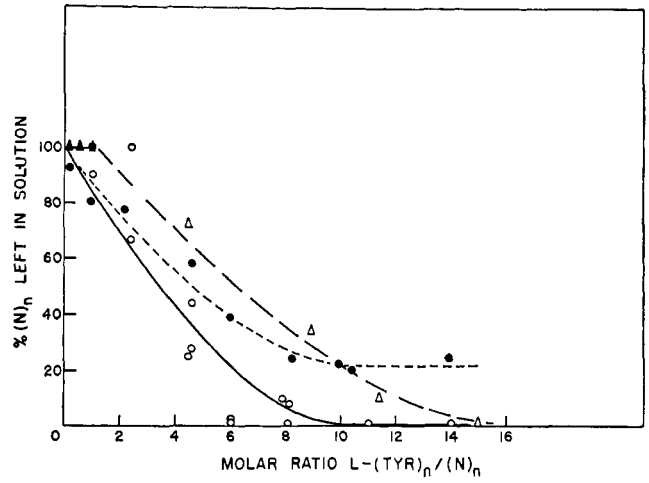

FIGURE 3: Precipitation curves for the $r(I)_n$–$(Tyr)_n$ and the $r(A)_n$–$(Tyr)_n$ systems: concentrations of polynucleotides fixed at 9.70×10^{-4} M for $r(I)_n$ (●), and at 4.83×10^{-4} M (△) and 9.10×10^{-4} M (○) for $r(A)_n$; [different concentrations of $r(A)_n$ used for Tyr:A ≤ 1 (▲) see Table II]; concentration of $(Tyr)_n$ varied from 4.83×10^{-4} to 1.30×10^{-2} M.

TABLE I: Formation of Soluble and Insoluble Complexes in 0.5 M HMP–0.05 M NaCl (pH 7.5).

Systems	Insoluble Complex	Soluble Complex[a]
n-DNA–$(Tyr)_n$	−	−
d-DNA–$(Tyr)_n$	+	−
$r(U)_n$–$(Tyr)_n$	+	−
$r(C)_n$–$(Tyr)_n$	−	−
$r(I)_n$–$(Tyr)_n$	+	−
$r(A)_n$–$(Tyr)_n$	+[b]	−[b]
	−[b]	+[b]

[a] A negative result indicate that the per cent of $(Tyr)_n$ solubilized is negligible <1%. [b] Formation of soluble and insoluble complexes depends on concentration of $(Tyr)_n$ and ratio of Tyr:A (see text).

$r(C)_n$, on the other hand, does not interact with $(Tyr)_n$, *i.e.*, it does not form either a soluble or an insoluble complex with this polypeptide. As $(Tyr)_n$ precipitates out of solution, $r(C)_n$ simply remains in the supernatant.

$r(I)_n$–$(Tyr)_n$; $r(A)_n$–$(Tyr)_n$. The results for these two systems are given in Figure 3. $r(I)_n$ interacts with $(Tyr)_n$ and forms only an insoluble complex. Approximately 20% of $r(I)_n$ remains in the supernatant, even at a ratio of Tyr:I = 14. In each of the systems considered so far, an analysis of their supernatants for $(Tyr)_n$ reveal that they contain a negligible amount of the polypeptide (less than 1%) (See Table I).

The curve obtained for the $r(A)_n$–$(Tyr)_n$ system, using a constant concentration of $r(A)_n$ of 9.10×10^{-4} M, is similar to that of the $r(U)_n$–$(Tyr)_n$ system. However, if a lower concentration of $r(A)_n$ of 4.83×10^{-4} M is used, the curve is displaced to the right and exhibits a plateau region for Tyr:A = 0 to 1 (Figure 3). Therefore, under certain conditions, *i.e.*, Tyr:A ≤ 1 and concentration $(Tyr)_n \leq 5 \times 10^{-4}$ M, all the $(Tyr)_n$ is solubilized. (See Table II.) In the absence of $r(A)_n$, the solubility of $(Tyr)_n$ is less than 1×10^{-6} M; the amount of $(Tyr)_n$ solubilized must therefore be complexed with $r(A)_n$. At neutral pH and concentration $(Tyr)_n \leq 5 \times 10^{-4}$ M, the highest ratio of Tyr:A than can exist in solution is 1. The stoichiometry of the soluble complex is therefore 1:1. The solubility of $(Tyr)_n$ was determined by the Lowry technique after centrifuging the complex at 15,000 rpm and 4° for 15 min. In some cases, the maximum concentration of the soluble complex could not be obtained and the $(Tyr)_n$ not solubilized precipitated out of solution. An examination of Tables III and IV reveals that, depending on the ratio of Tyr:A and on the concentration of the $(Tyr)_n$, some of the polypeptide is solubilized by $r(A)_n$, while the remaining $(Tyr)_n$ forms an insoluble complex with $r(A)_n$ having a ratio of Tyr:A = 10. However, the ratio of Tyr:A remaining in solution is roughly 1, thus indicating that the polypeptide is complexed with $r(A)_n$ in a 1:1 ratio. Formation of the 1:1 (A + Tyr)$_n$ complex thus increases greatly the solubility of $(Tyr)_n$ at neutral pH.

Sucrose Gradient Electrophoresis. The existence of the soluble complex is further demonstrated by sucrose gradient electrophoresis. Figure 4 shows the electrophoretic pattern for the complex. It can be seen that, at neutral pH, $(Tyr)_n$ travels with $r(A)_n$, as over 90% of the $(Tyr)_n$ is recovered under the $r(A)_n$ peak. Analysis of the fraction at the peak reveals that the ratio of Tyr:A is equal to 1, just as in the solution layered on the sucrose gradient column. Since $(Tyr)_n$ is insoluble at pH 7.5 it cannot be used as a control in this experiment. However, the oligopeptide, hexa-L-tyrosine, is fairly soluble at neutral pH, and it can therefore be used as a control provided it does not interact with $r(A)_n$ at neutral pH. It was

TABLE II: Solubilization of $(Tyr)_n$ by $r(A)_n$ (Tyr:A ≤ 1).[a]

Molar Ratio Tyr:A	% $r(A)_n$ Left in Soln	% $(Tyr)_n$ Left in Soln
0.13[b]	100	100
0.24	100	97
0.49	100	94
1.00	100	98

[a] Concentration of $(Tyr)_n$ kept constant at 4.83×10^{-4} M while concentration of $r(A)_n$ varied; results represent averages of at least two experiments. [b] Concentration of $(Tyr)_n$ used 5.50×10^{-4} M.

TABLE III: Solubilization of $(Tyr)_n$ by $r(A)_n$ (Tyr:A >1).[a]

Tyr:A Molar Ratio	mmoles of $r(A)_n$ Left in Soln $\times 10^4$	% $r(A)_n$ Left in Soln	mmoles of $(Tyr)_n$ Left in Soln $\times 10^4$	% $(Tyr)_n$ Left in Soln	(Tyr:A) (M) in Soln
4.4	14.1	72	8.0	9.7	0.57
9.0	6.20	33	9.0	5.2	1.4
11.4	1.82	9.5	3.7	1.6[b]	2.0[b]
15.1	0	0	0.51	0.2	

[a] Concentration of $r(A)_n$ kept constant at 4.83×10^{-4} M while concentration of $(Tyr)_n$ varied; results represent averages of at least two experiments. [b] Results quite approximate due to extremely low concentration of $(Tyr)_n$.

TABLE IV: Solubilization of $(Tyr)_n$ by $r(A)_n$ (Tyr:A ≥ 1).[a]

Tyr:A Molar Ratio	mmoles of $r(A)_n$ Left in Soln $\times 10^4$	% $r(A)_n$ Left in Soln	mmoles of $(Tyr)_n$ Left in Soln $\times 10^4$	% $(Tyr)_n$ Left in Soln	(Tyr:A) Molar in Soln
1.0	36.4	100	30.3	83.4	0.83
2.4	24.1	67	15.2	17.6	0.63
4.6	13.3	36	12.9	8.1	0.97
6.0	0.26	0.8[b]	0.46	0.2[b]	1.8[b]
8.1	1.35	3.8	1.31	0.6[b]	0.97[b]
11.0	0.18	0.5[b]	1.96	0.6[b]	1.1[b]
14.0	0.18	0.5[b]	2.54	0.6[b]	1.4[b]

[a] Concentration of $r(A)_n$ kept constant at 9.10×10^{-4} M while concentration of $(Tyr)_n$ varied; results represent averages of at least two experiments. [b] Results quite approximate due to extremely low concentrations of $(Tyr)_n$ and/or $r(A)_n$.

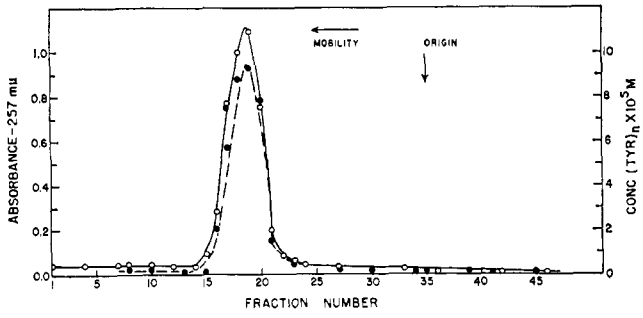

FIGURE 4: Sucrose gradient electrophoresis pattern of (A + Tyr)$_n$ complex (concn of r(A)$_n$ ≅ concn of (Tyr)$_n$ = 4.94×10^{-4} M) in half-diluted HMP (pH 6.8); 2100 V, 4 mA, 4 hr; (○), r(A)$_n$; (●) (Tyr)$_n$.

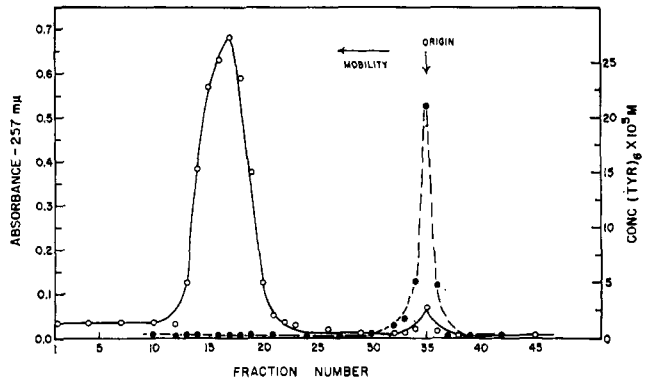

FIGURE 5: Sucrose gradient electrophoresis pattern of a 1:1 molar solution of r(A)$_n$ + (Tyr)$_6$: (concn of r(A)$_n$ = concn of (Tyr)$_6$ = 4.20×10^{-4} M) in half-diluted HMP (pH 6.8); 2100 V, 4 mA, 4.25 hr; (○), r(A)$_n$; (●) (Tyr)$_6$.

found experimentally that (Tyr)$_6$ does not migrate with r(A)$_n$ in sucrose gradient electrophoresis. As can be seen in the electrophoretic pattern for the r(A)$_n$–(Tyr)$_n$ system (Figure 5), (Tyr)$_6$ does not travel with r(A)$_n$ but remains near the origin.

Discussion

At neutral pH and at low ionic strength ($\mu \simeq 0.06$), (Tyr)$_n$ interacts strongly with d-DNA, r(A)$_n$, r(I)$_n$, and r(U)$_n$ to form insoluble complexes. However, there is no interaction between (Tyr)$_n$ and n-DNA or r(C)$_n$, as the latter two do not form either soluble or insoluble complexes. In addition, under certain conditions, r(A)$_n$ is the only nucleic acid that can form a soluble complex with (Tyr)$_n$. The d-DNA–(Tyr)$_n$- and the r(A)$_n$–(Tyr)$_n$-insoluble complexes have a Tyr:nucleotide ratio of 10. This is probably also the composition of the other insoluble complexes, since the formation of the complexes seems to be complete at a ratio of Tyr:nucleotide = 10, just as in the case of the d-DNA–(Tyr)$_n$ and r(A)$_n$–(Tyr)$_n$ systems. The (Tyr)$_n$ appears to be strongly bound to the various nucleic acids, as these insoluble complexes must be heated at 100° for 10–15 min in order to liberate quantitatively the various polynucleotides from the precipitates to the solution. Upon cooling back to room temperature, the nucleic acids remain in solution. However, after heating, 99% or more of (Tyr)$_n$ remains in the precipitate, as shown by the Lowry technique. This is to be expected since (Tyr)$_n$ is insoluble at neutral pH in HMP buffer and the nucleic acids cannot solubilize the polypeptide precipitate under these conditions.

An examination of Figure 1 reveals that there is a slight interaction between n-DNA and (Tyr)$_n$, especially in the case of the undialyzed solutions where only 80% of the n-DNA remains in the supernatant at a ratio of Tyr:nucleotide ≥ 8. This is most likely due to some denaturation of the DNA, either during the process of dissolution or as a result of the nucleic acid being exposed to high pH while being mixed with (Tyr)$_n$. Support for the above argument is found in the fact that, in the case of dialyzed n-DNA, over 90% of the nucleic acid is left in solution, even at a ratio of Tyr:nucleotide ≥ 10. It can therefore be concluded that n-DNA does not interact with (Tyr)$_n$.

In the case of the r(I)–(Tyr)$_n$ system, 20% of the nucleic acid remains uncomplexed at a ratio of Tyr:I = 14. It is well known that r(I)$_n$ can form a triple helix; however, this requires a relatively high salt concentration (Sarkar and Yang, 1965). Since the salt concentration used here is fairly low ($\mu \simeq 0.06$), the formation of a triple helix is unlikely. It is possible that some aggregation of r(I)$_n$ is taking place resulting in the following interactions: (Tyr)$_n$–r(I)$_n$ ≃ r(I)$_n$–r(I)$_n$ < (Tyr)$_n$–(Tyr)$_n$. At present, a satisfactory explanation of the properties of the r(I)$_n$–(Tyr)$_n$ system cannot be advanced. r(C)$_n$ does not interact with (Tyr)$_n$, as neither a soluble nor insoluble complex is formed for this system. The absence of interaction between r(C)$_n$ and (Tyr)$_n$ is not yet understood.

The r(A)$_n$–(Tyr)$_n$ system is very sensitive to (1) the concentration of (Tyr)$_n$ and (2) the ratio of Tyr:A. Depending on these two factors, the following situation can exist: (1) formation of a soluble complex only (Table II); (2) formation of a soluble and an insoluble complex in the same solution (Tables III and IV); and (3) formation of an insoluble complex only (Tables III and IV, Tyr:A = 11.4, 14.0, and 15.1). All of the above results depend on the delicate balance between the (Tyr)$_n$–(Tyr)$_n$ and (Tyr)$_n$–r(A)$_n$ interactions. An increase in the concentration of (Tyr)$_n$ above the critical value of 5×10^{-4} M, coupled with an increase in the Tyr:A ratio beyond one favors the strong (Tyr)$_n$–(Tyr)$_n$ interaction and results in the formation of either (1) a soluble and an insoluble complex or (2) an insoluble complex only. The existence of the 1:1 (Tyr)$_n$–r(A)$_n$-soluble complex at neutral pH and low ionic strength has been demonstrated by two techniques, namely solubility and sucrose gradient electrophoresis. The latter technique clearly demonstrates that, at neutral pH, the (A + Tyr)$_n$ complex migrates as a single component having a 1:1 stoichiometry.

(Tyr)$_n$ was found to precipitate out of solution at pH 10.3 [concentration (Tyr)$_n$ = 7×10^{-3} M and $\mu \simeq 0.06$]. In the r(N)$_n$–(Tyr)$_n$ systems, where r(N)$_n$ = r(A)$_n$, r(U)$_n$, or d-DNA, the insoluble complexes appear at approximately the same pH. Fasman *et al.* (1964) reports that the apparent pK_a of (Tyr)$_n$ in 0.2 M NaCl solution is approximately 11.5. The ionic strength of our solutions is approximately 0.06; therefore, the apparent pK_a must be somewhat higher than 11.5. We can therefore safely assume that at the pH at which the insoluble complexes are formed, the polypeptide is essentially un-ionized. A previous ultraviolet study (Friedman and Ts'o, 1971) showed that at pH 10.6 ($\mu = 0.1$), the degree of ionization of (Tyr)$_n$ is small. The interaction of (Tyr)$_n$ with the various nucleic acids is, therefore, not of an electrostatic nature. In fact, the ionized (Tyr)$_n$ carries a negative charge; thus the electrostatic interaction in this case would hinder the interaction of this polypeptide with the negatively charged nucleic acids.

In order to understand the interaction mechanism of this polypeptide with nucleic acids, a knowledge of the conformation of $(Tyr)_n$ in aqueous solution at pH 10.6 is required. The properties of $(Tyr)_n$ were therefore investigated between pH 10.6 and 12.75 by means of circular dichroism, optical rotatory dispersion, and ultraviolet spectroscopy (Friedman and Ts'o, 1971). Based on the latter study, it is proposed that in aqueous solution at pH 10.6, $(Tyr)_n$ exists in a helical conformation with only a small fraction of its side chains ionized. These results are different from those reported previously (Fasman *et al.*, 1964; Beychok and Fasman, 1964) and are discussed elsewhere (Friedman and Ts'o, 1971; Damle, 1970; Patrone *et al.*, 1970). On the basis of an infrared study, Patrone *et al.* (1970) have assigned a β-anti-parallel structure to $(Tyr)_n$ in D_2O at pD 11.6.

At present, there is insufficient information to understand, in detail, the interaction mechanism of $(Tyr)_n$ with the various nucleic acids; research is still in progress. Nevertheless, the following general features of this polypeptide–polynucleotide interaction can readily be recognized. (1) The interaction is nonelectrostatic in nature and highly specific in terms of different bases. For example, $(Tyr)_n$ can form insoluble complexes with $r(U)_n$ but does not interact at all with $r(C)_n$. Also, $(Tyr)_n$ can form a soluble complex with $r(A)_n$ but not with $r(I)_n$. In an earlier study, Akinrimisi and Ts'o (1964) have found that tyrosine can be solubilized to a greater extent in water through its interaction with purine. The results of this study suggest that the interaction of purine with tyrosine is stronger than that with tryptophan, phenylalanine, alanine, leucine, histidine, and glycine. (2) The interaction is dependent on the conformation of the nucleic acid. For example, $(Tyr)_n$ can form insoluble complexes with d-DNA but not with n-DNA. This would be expected since both the polypeptide and the polynucleotide are in the helical conformation and the bases in DNA are paired and buried inside the duplex. However, we have found that $(Tyr)_n$ can form a soluble 1:1 complex with the $r(A)_n \cdot r(U)_n$ duplex (but not with the $r(I)_n \cdot r(C)_n$ one); the existence of this complex can be demonstrated by sucrose gradient electrophoresis (S. Friedman and P. O. P. Ts'o, 1971, unpublished data). It is therefore concluded that $(Tyr)_n$ can interact with an helical nucleic acid duplex under certain conditions. (3) This interaction requires the $(Tyr)_n$ to have a sufficient size, as the interaction does not take place with $(Tyr)_6$. (4) The $(Tyr)_n$–$(N)_n$ interaction is governed by strong $(Tyr_n$–$(Tyr)_n$ and by $(N)_n$–$(N)_n$ interactions. The delicate balance existing between these interactions is best illustrated by the $(Tyr)_n$–$r(A)_n$ system.

Acknowledgments

We thank Professor M. Tsuboi for carrying out preliminary studies, Dr. Christian Sander for many interesting discussions, and Drs. Robert S. Umans and Marvin Silver for comments on the manuscript. S. F. is indebted to Professor D. Shugar for stimulating discussions concerning some aspects of this work and for reading the manuscript. Finally, the technical assistance of Miss Andrée Pedneau in certain parts of this work is gratefully acknowledged.

References

Akinrimisi, E. O., and Ts'o, P. O. P. (1964), *Biochemistry 3*, 619.
Ashwell, G. (1957), *Methods Enzymol. 3*, 73.
Beychok, S., and Fasman, G. D. (1964), *Biochemistry 3*, 1675.
Damle, V. N. (1970), *Biopolymers 9*, 937.
Davidson, B., and Fasman, G. D. (1969), *Biochemistry 8*, 4116.
Fasman, G. D., Bodenheimer, E., and Lindblow, C. (1964), *Biochemistry 3*, 1665.
Felsenfeld, G., Sandeen, G., and Von Hippel, P. H. (1963), *Proc. Nat. Acad. Sci. U. S. 50*, 644.
Friedman, S., and Ts'o, P. O. P. (1971), *Biochem. Biophys. Res. Commun. 42*, 510.
Latt, S. A., and Sober, H. A. (1967a), *Biochemistry 6*, 3293.
Latt, S. A., and Sober, H. A. (1967b), *Biochemistry 6*, 3307.
Olins, D. E., Olins, A. L., Von Hippel, P. M. (1968), *J. Mol. Biol. 33*, 265.
Olivera, B. M., Baine, P., and Davidson, N. (1964), *Biopolymers 2*, 245.
Patrone, E., Conio, G., and Brighetti, S. (1970), *Biopolymers 9*, 897.
Pirrotta, V., Chadwick, P., and Ptashne, M. (1970), *Nature (London) 227*, 41.
Raju, E. V., and Davidson, N. (1969), *Biopolymers 8*, 743.
Riggs, A. D., and Bourgeois, S. (1969), *Biophys. J. 9*, A-84.
Salas, J., and Bollum, F. J. (1969), *J. Biol. Chem. 244*, 1152.
Sarkar, P. K., and Yang, J. T. (1965), *Biochemistry 4*, 1238.
Sekine, H., Nakamo, E., and Sakaguchi, K. (1969), *Biochim. Biophys. Acta 174*, 202.
Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8*, 3219.
Ts'o, P. O. P., Helmkamp, G. K., and Sander, C. (1962), *Biochim. Biophys. Acta 55*, 584.
Tsuboi, M., Matsuo, K., and Ts'o, P. O. P. (1966), *J. Mol. Biol. 15*, 256.