Enthalpy of Stacking in Single-Stranded Polyriboadenylic Acid[1]

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Abstract: The enthalpy of stacking in single-stranded polyriboadenylic acid has been measured calorimetrically by determining the heats of solution at two temperatures, and correcting the data with the aid of heat capacity measurements on the solid and on the solutions. In the course of this work it was necessary to demonstrate that the conformation of polyriboadenylic acid in concentrated solution is helical; this was done by means of optical rotation measurements in the visible region. The value obtained for the enthalpy of stacking, about -9 kcal/mole of adenine, is in agreement with that obtained from the application of a theory of noncooperative base stacking to melting data on oligomers of riboadenylic acid and on polyriboadenylic acid.

Several investigators[3–12] have shown that riboadenylic acid oligomers, as well as polyriboadenylic acid, form single-stranded, ordered structures in aqueous solution at pH 7. Various properties associated with these ordered structures, such as the ultraviolet absorption spectra,[10] optical rotatory dispersion,[6–8] and circular dichroism,[11] show a temperature dependence which indicates that the degree of ordering decreases with increasing temperature. Values[8,10–12] for the enthalpy of stacking, as determined from the melting curves, range from about -6.5 to -13 kcal/mole of interacting adenine bases. Because of the unusually large (and negative) value of this quantity, it appeared desirable to determine it directly by a calorimetric method.

The usual calorimetric method of obtaining the enthalpy of a thermal transition is to measure the variation of the heat capacity of a solution as a function of temperature. In the present case, because of the broadness, *i.e.*, noncooperativity, of the transition, as well as the difficulties in measuring the heat capacities of solutes in *dilute* aqueous solution, we used a less direct approach. By measuring the isothermal heat of solution at two temperatures, as well as the heat capacities of the solid and the polymer in solution, the enthalpy of stacking may be calculated. Although it is necessary to measure the heat capacity of the polymer in solution, in this procedure, this may be done at high concentrations, and thus a fairly precise enthalpy can be obtained.

Experimental Section

Materials. The potassium salt of polyriboadenylic acid (control no. 11531 and 17636) was obtained from Miles Chemical Co., Clifton, N. J., and was used without further purification. The commercial samples were prepared by exhaustive dialysis against EDTA and distilled water, followed by lyophilization. This material is expected to be relatively free of excess salt, although it probably contains some water of hydration. On the basis of ultraviolet light absorption, the material appears to be over 90% pure, the remainder probably being water. Heats of solution in 0.15 M KCl–0.10 M phosphate buffer, pH 6.83, were measured with polyriboadenylic acid (control no. 11531). Heat capacities and heats of solution in 0.10 M NaCl–0.01 M Tris buffer, pH 7.30, were measured with polyriboadenylic acid (control no. 17636).

The concentration of polyriboadenylic acid was determined spectrophotometrically with a Cary Model 14 recording spectrophotometer (in cells of 1-cm path length), using a molar extinction coefficient[6,13] at pH 7 of 1.01×10^4 l./mole cm at the absorption maximum (258 mμ). At high concentrations, cells of 1-mm path length were used with a Zeiss PMQII spectrophotometer.

The standards for heat capacity measurements were: potassium chloride (Mallinckrodt, analytical reagent grade), benzoic acid (Mallinckrodt, U.S.P. grade crystals), and urea (Mallinckrodt, N.F. crystals), recrystallized twice from ethanol.

Calorimetry. Calorimetric measurements were carried out with an adiabatic solution calorimeter modeled after one described by Benjamin,[14] and used previously in this laboratory.[15]

Heat of solution measurements were made by placing solid polyriboadenylic acid in a sample cell of about 3-ml volume. The solid was brought to thermal equilibrium in the calorimeter vessel containing both the cell and about 40 ml of buffer solution. The cell was broken and the heat of solution measured. These values were corrected for the heat liberated in blank experiments in which the sample cell contained only air.

The cell used to measure heat capacities consisted of a 1.5-ml, thin-walled, glass cup wrapped with 1 ft of 10 ohms/ft manganin resistance wire and with two thermisters attached to the outside wall. The measuring thermistor, 2000 ohms (VECO 32A11), and that for automatic adiabatic control, 1000 ohms (VECO 31A11), were purchased from Victory Engineering Co., Springfield, N. J. The cell was covered with a small piece (about 10 mg) of parafilm. The total weight of the cell was 1.2 g, and its total heat capacity was 0.348 ± 0.004, 0.373 ± 0.003, and 0.386 ± 0.003 cal/deg at 25, 35, and 45°, respectively. Accurate results with standard solids were obtained only if the material was compressed. Solids were compressed at 100,000 psi pressure in a die normally used to make KBr pellets for infrared spectroscopy. The pellet was subsequently ground to a fine powder with a mortar and pestle and packed into the heat-capacity cell. Specific heats measured with 1 g of sample agree very well with values reported in the literature (Table I).

Optical Rotation. Measurements of optical rotation were made on a Cary 60 recording spectropolarimeter. The water-jacketed cell

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(14) L. Benjamin, *Can. J. Chem.*, **41**, 2210 (1963).

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Table I. Specific Heats of Standard Solids

Substance	Specific heat, cal/deg g Measured (this work)	Lit.
Potassium chloride	0.164 ± 0.010	0.164[a]
Benzoic acid	0.272 ± 0.014	0.287[b]
Urea	0.363 ± 0.014	0.371[c]

[a] P. G. Strelkov, E. S. Itskevich, V. N. Kostryukov, and G. G. Mirskaya, *Zh. Fiz. Khim.*, **28**, 645 (1954). [b] G. T. Furukawa, R. E. McCoskey, and G. J. King, *J. Res. Natl. Bur. Std.*, **47**, 256 (1951). [c] R. A. Ruehrwein and H. M. Huffman, *J. Am. Chem. Soc.*, **68**, 1759 (1946).

of 0.1- or 2.5-cm path length was connected to a Haake circulating constant-temperature bath. All optical rotation data were expressed in terms of specific rotation, $[\alpha]$, which equals $(100\alpha_{obsd}/lc)$ where α_{obsd} is the observed rotation, l the path length in decimeters, and c the concentration of polyriboadenylic acid (as determined spectophotometrically) expressed in grams/100 ml. No correction was made for changes in the index of refraction. Solvent blanks (0.05 *M* Tris–0.1 *M* NaCl, pH 7.5) were measured at all recorded wavelengths and temperatures. The change in rotation of polyriboadenylic acid with temperature was found to be reversible.

Results

Calorimetry. The heats of solution of polyriboadenylic acid at 25 and 45° are −3.14 ± 0.04 and −0.43 ± 0.13 kcal/mole of adenine in 0.10 *M* NaCl–0.01 *M* Tris buffer, pH 7.30. The corresponding values in 0.15 *M* KCl–0.10 *M* phosphate buffer, pH 6.83, are −3.17 ± 0.04 and −0.16 ± 0.12 kcal/mole. Each value is the average of two experiments using about 50 mg of sample and 40 ml of solvent. The heats of solution are independent of concentration, and Beer's law is obeyed in the range 0.37–1.34 g/l., suggesting that no aggregation occurs in the concentration range used in the calorimetric study.

The apparent heat capacity of polyriboadenylic acid in solution was obtained by measuring the total heat capacity of solutions, $C_{p,T}$, and subtracting the heat capacity of water added, mC_{p,H_2O}, where m is the total amount of water. These values are the average of two experiments made on consecutive days, using 0.65 g of poly A, and are precise to ±5% (Table II). The heat capacity of the polymer appears to be constant in solutions below concentrations of 1.4 g/g; hence, we assume that this value would not change significantly on further dilution. The difference between $C_{p,s}$, the heat capacity of the solid (first line of Table II), and $(C_{p,T} - mC_{p,H_2O})$, the apparent heat capacity of the polymer in solution (the remaining values in Table II), is $C_{p,s} - (C_{p,T} - mC_{p,H_2O}) = -0.02 \pm 0.03$ cal/deg g. This value is the average of the differences obtained at 25, 35, and 45° (all of which are within experimental error of the value −0.02), using the heat capacity of polyriboadenylic acid in solutions of concentrations 1.4, 0.9, and 0.65 g/g (the value at 1.4 g/g and 35° was rejected).

Optical Rotations. Figure 1 shows the temperature dependence of the specific rotation of polyriboadenylic acid at several polymer concentrations in 0.05 *M* Tris–0.1 *M* NaCl, pH 7.5. The rotations were measured at 436 and 589 mμ, and the values for the most dilute solution compare well with those previously reported.[16]

Table II. Heat Capacity of Potassium Polyriboadenylate Solid and in Aqueous Solution[a]

Composition, g of poly A/ g of H₂O	Apparent specific heat, cal/deg g, at 25°	35°	45°
(Solid)	0.31	0.32	0.35
(3.3)[b]	(0.45)[b]	(0.46)[b]	(0.47)[b]
1.4	0.33	0.42	0.36
0.9	0.35	0.35	0.36
0.65	0.33	0.33	0.35

[a] pH measurements in these very concentrated solutions are considered unreliable, but in more dilute solution (*i.e.*, in water) the pH was about 6.5. It should be noted that the commercial sample is supplied as the potassium salt so that, although the pH and ionic strength may be somewhat different than it is in our heat of solution measurements, the state of ionization of the polymer is the same. [b] The material did not all dissolve in this experiment. If some of the insoluble material did dissolve *during* the heat capacity measurement a heat of solution would contribute to the measured heat capacity; hence, these data should not be taken into consideration in the interpretation of the remaining data.

Discussion

The thermochemical data may be evaluated with the aid of eq 1.

$$\begin{tikzcd}
(T_2)\ \text{solid} + m\text{H}_2\text{O} \arrow[r, "Q_2"] & \text{coil}\cdot n\text{H}_2\text{O} + (m-n)\text{H}_2\text{O} \arrow[r, "\Delta H_s"] & \text{helix}\cdot p\text{H}_2\text{O} + (m-p)\text{H}_2\text{O} \\
(T_1)\ \text{solid} + m\text{H}_2\text{O} \arrow[u, "\int_{T_1}^{T_2} C_{p,s}\text{d}T + m\int_{T_1}^{T_2} C_{p,H_2O}\text{d}T"] \arrow[r, "Q_1"] & \text{helix}\cdot p\text{H}_2\text{O} + (m-p)\text{H}_2\text{O} \arrow[ur, "\int_{T_1}^{T_2} C_{p,h}\text{d}T + (m-p)\int_{T_1}^{T_2} C_{p,H_2O}\text{d}T"'] &
\end{tikzcd} \tag{1}$$

In this scheme, T_1 and T_2 are the two temperatures $(T_2 > T_1)$ at which the isothermal heat of solution measurements were carried out, Q_1 and Q_2 being the heats of solution at T_1 and T_2, respectively; $C_{p,h}$ is the heat capacity of the hydrated helix, p is the water of hydration of the helix (*i.e.*, the ordered form), and n is the water of hydration of the coil (*i.e.*, the disordered form); ΔH_s is the (isothermal) heat of transition (at T_2), and the other symbols have been defined already. The scheme is written as if only pure helix and pure coil forms exist; actually, the per cent helix and coil in each step will be taken into account below. In addition, the conformation of the polymer in the solid state is not specified, since this information is not required to complete the thermodynamic cycle.

Addition of the enthalpy of each step in the proposed cycle yields for the isothermal enthalpy of transition, ΔH_s

$$\Delta H_s = Q_1 - Q_2 + \int_{T_1}^{T_2} (C_{p,h} - pC_{p,H_2O} - C_{p,s})\text{d}T \tag{2}$$

The total apparent heat capacity of a solution of helix is

$$C_{p,T} = C_{p,h} + (m - p)C_{p,H_2O} \tag{3}$$

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(16) P. O. P. Ts'o, G. K. Helmkamp, and C. Sander, *Biochim. Biophys. Acta*, **55**, 584 (1962).

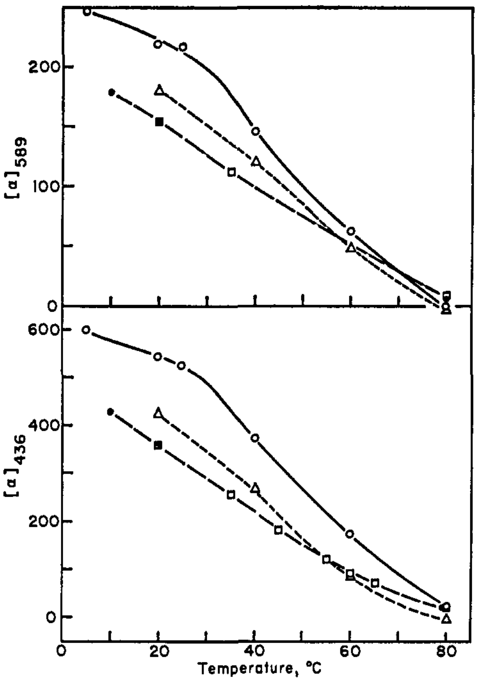

Figure 1. Specific rotation of polyriboadenylic acid in 0.05 *M* Tris–0.1 *M* NaCl, pH 7.5, *vs.* temperature: □, data from ref 16 using polymer concentration in the range 1–3 mg/ml; ●, polymer concentration 2.7 mg/ml; △, 36.8 mg/ml; ○, 138 mg/ml.

According to the data of Table II, the quantities in eq 3 may be assumed to be independent of temperature within experimental error. Substitution of eq 3 into eq 2 gives

$$\Delta H_s = Q_1 - Q_2 + \int_{T_1}^{T_2} (C_{p,T} - mC_{p,H_2O} - C_{p,s})dT \tag{4}$$

and the unknown quantity p drops out, so that we can use the quantities of Table II, in appropriate units, directly to correct the experimental values of $Q_1 - Q_2$ to obtain ΔH_s.

Between 25 and 45° the term $\int(C_{p,T} - mC_{p,H_2O} - C_{p,s})dT$ (from T_1 to T_2) is 0.1 kcal/mole and ΔH_s, in 0.10 *M* NaCl–0.01 *M* Tris buffer at pH 7.30, is -2.6 ± 0.4 kcal/mole of adenine. The corresponding value for ΔH_s in 0.15 *M* KCl–0.10 *M* phosphate buffer, pH 6.83, is -2.9 ± 0.4 kcal/mole of adenine. The heats of solution Q_1 and Q_2 make the major contribution to this value. An additional term, amounting to only about 4% of the final value, comes from the term containing the integral in eq 4.

The above calculation is valid provided there is no change of helix content during the heat capacity measurements. Thus, the heat capacity measurements were made at very high concentration, not only to achieve good precision in the measurement, but also to attain a condition where the helix does not melt. The addition of a diluent to a crystalline polymer will lower the temperature at which a phase transition will occur.[17] The phase change under consideration here is between a helix in aqueous solution and a random coil in aqueous solution. For such a system, Flory[18] has shown that, at high polymer concentrations, the competition for available space greatly diminishes the number of possible conformations from its value for the same polymer at high dilution, and thus the random state loses some of its statistical advantage. Hence, even though the melting point of polyriboadenylic acid is low in dilute solution, its melting point would be very high in concentrated solutions, *i.e.*, the helix would be below its melting point in the temperature range where the heat capacity measurements were made. The following observations suggest that this situation was achieved, *i.e.*, that no melting of the helix occurred during the heat capacity measurements. For ΔH_s not to be zero, the polymer conformation produced by heating a concentrated solution from T_1 to T_2 must be different from the conformation produced on dissolving the solid at T_2. If the melting point were the *same* in dilute and in concentrated solutions, then the heating from T_1 to T_2 would melt the helix and ΔH_s would be zero, contrary to observation. If the melting point in the concentrated solutions were only slightly higher than in dilute solution, the fraction of polymer melted would differ in the concentrated and dilute solutions, and thus lead to a marked dependence of the apparent heat capacity on the amount of water added and on temperature. Since the data of Table II indicate a negligible dependence of the apparent heat capacity on composition and temperature, we can rule out the possibility that any helix melted during the heat capacity measurements. Thus, although the melting temperature of the polymer is lowered by addition of diluent, we must assume that it is not lowered enough to approach the temperature at which the heat capacity measurements were made. These results also render unlikely the possibility of formation of a double-stranded helix (with a heat capacity different from the single-stranded structure), caused by the high polymer concentration or a lowering of the pH, since this equilibrium would be highly concentration dependent. In agreement with this, nmr data[3] indicate that, in concentrated solutions (although ten times more dilute than our solutions), a single-strand helix is formed in unbuffered solutions of polyriboadenylic acid in D_2O. Additional inaccuracy in the apparent heat capacities may be introduced by the long extrapolation to dilute solution and by the possible effect of the very high counterion concentration. However, since the heat capacity correction is so small (~4%), it is felt that such possible errors will not alter the value of ΔH_s significantly. It is not surprising that the apparent heat capacity of the solid and the polymer in solution are not very different since, even if a conformational change occurred on dissolving the polymer (*i.e.*, if the solid contained some amorphous regions), no change in the apparent heat capacity would result since the heat capacities of the helix and coil forms in solution are the same, as shown by the linearity of the van't Hoff plots for their interconversion.[10,11] This fact also allows us to use the enthalpy change ΔH_s (for the fraction of the transition which was observed) to calculate the enthalpy change, $\Delta H_{\text{calorimetric}}$, for the entire transition. $\Delta H_{\text{calorimetric}}$ is for an isothermal process, in contrast to the enthalpy obtained by measuring the variation of the heat capacity with temperature. As a result, our method is less likely to include enthalpy-producing

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[17] L. Mandelkern, "Crystallization of Polymers," McGraw-Hill Book Co., Inc., New York, N. Y., 1964, Chapter 3.

[18] P. J. Flory, *J. Polymer Sci.*, **49**, 105 (1961).

processes which are not coupled to the conformational change.

In addition to the indirect arguments given above, which suggest the existence of helix at high polymer concentration, the optical rotation data directly demonstrate the increase in helix melting temperature with polymer concentration (Figure 1). The highest concentration (138 mg/ml) used in Figure 1 is still approximately ten times more dilute than that used for the heat capacity measurements since technical difficulties prevented the use of the more concentrated solutions. In the 138-mg/ml solution the conformation of the polymer at 25° seems to be essentially all helical. This is shown by the fact that, in the 138-mg/ml solution, the slope of the $[\alpha]$ *vs.* temperature curve (Figure 1) levels off markedly at 25°, and the final value at low temperature is approximately that which would be expected for a solution of helix on the basis of the rotation of the dilute solution and its helix content as determined by ultraviolet absorption and circular dichroism. The shift in the melting curves with concentration is unlikely to be caused by the formation of double-stranded helix since this conformation (at low pH) exhibits a higher rotation ($[\alpha]^{20}{}_{589}$ 374°, $[\alpha]^{20}{}_{436}$ 873°) and melts very sharply at 62°.[16] Since, at a concentration of 138 mg/ml, the conformation of polyriboadenylic acid is helical at 25°, then in the solutions used to measure heat capacity, which are about ten times more concentrated, the conformation is almost certainly helical at 25°. As a result the enthalpy of the helix–coil transition would not contribute to the measured heat capacity at 25°; since the heat capacity did not change with temperature from 25 to 45° (Table II) it is very probable that the conformation of the polymer at 45° was also helical during the heat capacity measurements.

The demonstration that the temperature at which the helix melts depends on its concentration indicates that polymers crystallized from concentrated solution may have a different conformation than in dilute solution. This effect may also be important *in vivo* where localized high concentrations of macromolecules may be found.

The remaining information necessary to calculate the enthalpy of stacking is the change in the fraction of bases stacked in going from 25 to 45° in the dilute solution measurements. For this information we have used the data published by Leng and Felsenfeld[10] on ultraviolet hypochromism and the data of Brahms, *et al.*,[11] on circular dichroism. Table III gives $\Delta H_{\text{calorimetric}}$, calculated from a ΔH_s value of -2.6 ± 0.4 kcal/mole for going from 45 to 25°. Using the data of Brahms, *et al.*,[11] we calculate a $\Delta H_{\text{calorimetric}}$ which is larger than $\Delta H_{\text{van't Hoff}}$ by somewhat more than the experimental error. This result appears rather unlikely since $\Delta H_{\text{van't Hoff}}$ will increase with the cooperativity factor whereas $\Delta H_{\text{calorimetric}}$ is independent of it; thus[19] $|\Delta H_{\text{calorimetric}}| \leq |\Delta H_{\text{van't Hoff}}|$.

Table III. Enthalpy of Stacking in Single-Stranded Polyriboadenylic Acid

	Leng and Felsenfeld[10]	Brahms, *et al.*[11]
Fractional decrease in stacking (25–45°)	0.276	0.195 (0.281)[a]
$\Delta H_{\text{calorimetric}}$, kcal/mole	-9.4 ± 1.4	-13.3 ± 2.0 (-9.3 ± 1.4)[a]
ΔS, eu[b]	-29	$-44\ (-30)$[a]
$\Delta H_{\text{van't Hoff}}$, kcal/mole	-13.0	$-7.9\ (-11)$[a]

[a] See text. [b] $\Delta S = (RT \ln K + \Delta H_{\text{calorimetric}})/T$. K is taken from ref 10 and 11.

One of the assumptions[11] made to calculate the fraction of stacked bases was that the circular dichroism of the high-temperature or coil form is zero. If, instead, the circular dichroism curve of polyriboadenylic acid in 98% ethanol[11] is taken as that of the coil form, the values shown in parentheses are obtained. These values are consistent with our calorimetric data.

The method of obtaining heats of transition from heats of solution, described in this paper, should be generally applicable to other systems. Using the calorimetric data of Rawitscher, *et al.*,[13] Stevens and Felsenfeld[20] estimated a value of between -5 and -8 kcal/mole of adenine for the enthalpy of stacking in polyriboadenylic acid. This method uses the temperature dependence of the enthalpy of acidification of neutral solutions of polyriboadenylic acid, and a number of assumptions must be made. First, the ΔC_p between single- and double-stranded helix is assumed to be zero, although the former is a less rigid chain[6] and may thus have a higher heat capacity. In addition, a large and somewhat uncertain correction must be applied for the heat of ionization. It is assumed that the only group partially protonated in going from pH 5 to 3.7 is the N_1 proton of adenine, *i.e.*, the group titrated in the transition region, although the NH_2 group of adenine has a pK near this range. The enthalpy of ionization is also assumed to be independent of the fraction of groups ionized on the polymer. Despite these assumptions their value is not very different from ours.

Poland, *et al.*,[8] and Applequist, *et al.*,[12] using statistical mechanical analyses, calculated that the formation of an ordered base pair in polyriboadenylic acid is essentially independent of the state of its neighbors and is thus a noncooperative process. This conclusion agrees with our finding that the calorimetric isothermal ΔH agrees with that obtained from ultraviolet and circular dichroism data using a simple two-state theory and the van't Hoff equation (with ΔH independent of temperature). From analysis of the temperature dependence of the optical rotatory dispersion for a series of riboadenylic acid oligomers, Poland, *et al.*,[8] obtained a value, equivalent to our $\Delta H_{\text{calorimetric}}$, of -6.5 kcal/mole interaction. This value is somewhat lower than the one we find from our calorimetric data, but part of the problem may again be the difficulty of assigning high- and low-temperature limits to the optical rotatory dispersion data. Using the data of Fresco, Blake, and Doty[21] on the ultraviolet absorption of polyriboadenylic acid and its oligomers, Applequist and Damle[12] calculated a value of -9.4 kcal/residue mole for the enthalpy of stacking. This value is in excellent agreement with our data.

The high negative enthalpy of stacking is particularly unusual because of the effect of solvent. The stacked configuration forms more readily in water than it does in ethanol,[11,22] and it forms because of a favorable

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[19] J. N. Vournakis, D. Poland, and H. A. Scheraga, *Biopolymers*, in press.

[20] C. L. Stevens and G. Felsenfeld, *ibid.*, 2, 293 (1964).

[21] J. R. Fresco, R. Blake, and P. Doty, unpublished data quoted in ref 12.

[22] J. Massoulié and A. M. Michelson, *Compt. Rend.*, **259**, 2923 (1964).

enthalpy change. This is unusual because most noncovalent interactions favored in water are facilitated because of a favorable entropy change, resulting from disruption of solvent structure. However, this change in stability may not directly involve the forces stabilizing the helix in water but may result instead from the phosphate–phosphate repulsions which should be greater in the helix than in the coil where they are more separated.[19] Thus, in water, the negative charges are shielded by the solvent, whereas in ethanol they may become a major destabilizing factor. The exact nature of the stacking interaction in polyriboadenylic acid awaits further investigation.

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Syntheses and Pharmacological Properties of Selenium Isologs of Oxytocin and Deamino-oxytocin[1–3]

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Abstract: Isologs, differing from oxytocin and deamino-oxytocin only in the replacement of the sulfur in position 1 or in position 6, have been synthesized, purified by countercurrent distribution, and tested for pharmacological activities. For the synthesis of the isolog 1-hemi-L-selenocystine-oxytocin, N-carbobenzoxy-Se-benzyl-L-selenocysteine was employed. This intermediate was obtained by the resolution of N-acetyl-Se-benzyl-DL-selenocysteine with hog acylase to yield Se-benzyl-L-selenocysteine which was subsequently carbobenzoxylated. The deamino-1-hemiseleno-oxytocin was synthesized with the use of Se-benzyl-β-selenopropionic acid in place of N-carbobenzoxy-Se-benzyl-L-selenocysteine. In addition, 6-hemi-L-selenocystine-oxytocin and its deamino analog were prepared. For the synthesis of the intermediate tetrapeptide N-carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide, the tripeptide L-prolyl-L-leucylglycinamide was acylated with *p*-nitrophenyl N-carbobenzoxy-Se-benzyl-DL-selenocysteinate, and the resulting diastereoisomeric tetrapeptides were resolved by fractional crystallization. The replacement of one sulfur atom by a selenium atom in position 1 or 6 yielded highly potent isologs of oxytocin and deamino-oxytocin. Upon bioassay, 1-hemi-L-selenocystine-oxytocin was found to possess 362 ± 9 units/mg of rat oxytocic activity, 351 ± 15 units/mg of rabbit milk-ejecting activity, 361 ± 18 units/mg of avian vasodepressor activity, 3.1 ± 0.2 units/mg of rat pressor activity, and 5.7 ± 0.6 units/mg of rat antidiuretic activity. The deamino-1-hemiseleno-oxytocin exhibited 560 ± 34 units/mg of oxytocic activity, 248 ± 8 units/mg of milk-ejecting activity, 613 ± 38 units/mg of vasodepressor activity, 1.7 ± 0.2 units/mg of pressor activity, and 24.0 ± 1.5 units/mg of antidiuretic activity. The comparable values for 6-hemi-L-selenocystine-oxytocin and deamino-6-hemiseleno-oxytocin were 405 ± 5, 398 ± 6, 385 ± 15, 3.8 ± 0.2, and 3.4 ± 0.1 units/mg and 492 ± 5, 397 ± 8, 622 ± 18, 1.1 ± 0.1, and 15.5 ± 1.0 units/mg, respectively.

The synthesis of oxytocin[4] (Figure 1) provided the foundation for investigating contributions of structural features of the molecule to its biological activity. One point of focus in our studies of structure–activity relationships involves questions about the role of the disulfide bond of neurohypophyseal hormones. In view of the chemical and biological analogies and diversities which exist between organic sulfur and selenium compounds,[5] it appeared desirable to investigate from a synthetic and biological standpoint the effect of the replacement of one of the sulfur atoms by a selenium atom in oxytocin and deamino-oxytocin. Thus the synthesis and pharmacological evaluation of 1-hemi-L-selenocystine-oxytocin (1-seleno-oxytocin), 6-hemi-L-selenocystine-oxytocin (6-seleno-oxytocin), and their corresponding deamino analogs were undertaken.[6]

For the syntheses of the key intermediates of the analogs presented herein, the *p*-nitrophenyl ester method[7] was used as introduced in the stepwise syn-

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(2) R. W. was responsible for the chemical, and W. Y. C. for the pharmacological aspects of the work presented here.

(3) The following abbreviations have been incorporated into the text: $CHCl_3$, chloroform; C_6H_{12}, cyclohexane; PrOH, 1-propanol; 2-PrOH, 2-propanol; BuOH, 1-butanol; AcOH, acetic acid; DMF, dimethylformamide; THF, tetrahydrofuran; DCC, N,N′-dicyclohexylcarbodiimide.

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