these cultures assayed 329 and 326 d.p.m./mg., respectively. Radiochemical yields were 0.86 and 0.84%, and the ratio of specific activities was 0.37%. These figures were substantially lower than those obtained from the use of nicotinic acid and also of certain aliphatic intermediates (*e.g.*, alanine and β-alanine) with respect to label incorporated into the pyridine ring of nicotine. Thus we concluded[12] that quinolinic acid is probably not an important precursor of the pyridine ring of nicotine.

The results reported in the preceding paper with carbon-14 ring-labeled quinolinic acid necessitate a reappraisal of our conclusions. Using quinolinic acid-2,3,7,8-^{14}C,[32] we have obtained the following results.

Treatment of a portion of the carbon-14 labeled material with charcoal in hot water and recrystallization yielded quinolinic acid-2,3,7,8-^{14}C with a specific activity of 1006 mμcuries/mg. of carbon. The recrystallized and original quinolinic acid-2,3,7,8-^{14}C samples were supplied at about 0.01 mg./ml. to tobacco root culture fluid. After 7 days of incubation, the nicotine picrates obtained were found to assay 0.929 and 0.659 mμcurie/mg. of carbon, respectively. The respective radiochemical yields were 0.78 and 0.83%.

The latter figures can be corrected for predictable losses (*e.g.*, carbon atoms in positions 7 and 8) by doubling. Thus, the corrected radiochemical yields would be 1.56 and 1.66%, respectively. Similar corrections may be made for the incorporation of the tritium-labeled acid if we assume that the hydrogen atom on position 6 is lost during the formation of nicotine.[2a] In this case, radiochemical yield figures are multiplied by three-halves, assuming that the distribution of label on the ring of quinolinic acid was uniform. The resulting values are 1.17 and 1.24%, respectively. (For justification of the latter procedure, see Dawson, *et al.*[2a]

(32) Supplied by Dr. R. K. Gholson, Oklahoma State University.

Communications to the Editor

Studies on the Helix–Coil Transition by Polarization of Fluorescence Measurements[1]

Sir:

Polarization of fluorescence measurements are ideally suited to the study of the rigidity of macromolecules[2-4] and its changes during the helix–coil transition. It is more sensitive to changes in rigidity than the usual hydrodynamic measurements[5] and can be applied to molecules of all sizes. The rotational relaxation time and the degree of polarization of fluorescence at a given temperature reflect the over-all rigidity of the molecule. The transition temperature T_T, *i.e.*, the temperature at which $(1/p + {}^1/_3) = (1/p_0 + {}^1/_3)\cdot(1 + RT\tau/V\eta)$ deviates from a linear dependence upon T/η to follow the exponential equation $(1/p + {}^1/_3) = Ke^{+aT/\eta}$, measures the stability of the internal structure.[5-7] T_T is a function of the kinetic energy of the molecule and not of the viscosity of the medium up to 9 cp.[6] The rotational relaxation time $\rho_h{}^5$ and the equivalent volume of the rotating segment $V_e{}^5$ were calculated from the straight line portion of the curve (at 5°). The values of these two parameters for a rigid sphere were calculated from the molar volumes of the polypeptides.

The helix–coil transitions in poly Glu^{97}Lys^3, poly-Lys (No. 2), and poly Glu^{63}Lys^{37} (No. 3)[8] were studied in order to investigate the rigidity and stability of the glutamic acid helix, the lysine helix, and the helix in a copolymer containing both glutamic acid and lysine. Poly Glu^{97}Lys^3 was used as a model for the glutamic acid helix, because a few lysine residues were necessary to introduce the fluorescein isothiocyanate or 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS) dye. The polarization of fluorescence was studied as a function of pH and temperature in a modified Brice-Phoenix light-scattering apparatus as previously described[5]; all studies were performed in 0.2 *M* NaCl and 0.1 *M* buffer (citrate, phosphate, or carbonate). As the polymer undergoes a transition from the rather flexible coil at neutral pH to the more rigid helical conformation at pH 4 for glutamic acid and pH 11 for lysine, p, $\rho_h{}^5$, $\rho_h{}^5/\rho_0{}^5$, and $V_e{}^5$ increase. The data are summarized in Table I.

The helices of poly Glu^{97}Lys^3 and poly Glu^{63}Lys^{37} (No. 3) show a higher degree of polarization and a longer rotational relaxation time than that of poly Lys (No. 2), and the $V_e{}^5$ of the glutamic acid helices is larger. The midpoint of the helix–coil transition in poly Glu^{97}Lys^3 by polarization measurements (pH 4.5 in 0.2 *M* NaCl + 0.1 *M* phosphate or citrate buffer) is lower than that obtained from titration (pH 5.0 in 0.2 *M* NaCl)[9] and from optical rotation (pH 5.1 in 0.2 *M* NaCl).[9-12] This suggests that the glutamic acid residues assume the helical conformation before the helix reaches its maximal rigidity. The degree of

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(5) T. J. Gill, III, *Biopolymers*, 3, 43 (1965).
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(7) The symbols used in these equations are: p, the degree of polarization at temperature T; p_0, the limiting degree of polarization as $T/\eta \to 0$; R, the gas constant; V, the molar volume; η, the viscosity; τ, the lifetime of the excited state of the conjugated fluorescent dye; and K and a, constants in the exponential equation.
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Table I. The Helix–Coil Transition in Synthetic Polypeptides Measured by Polarization of Fluorescence Methods

Polymer	pH	p^a	T_T, °C.	$\rho_h^5 \times 10^8$, sec.[b]	ρ_h^5/ρ_0^5	$V_e^5 \times 10^{-3}$, cc./mole
Poly $Glu^{97}Lys^{3c}$	3.27	0.185	21	5.2	0.37	26
	5.35	0.022	12	0.8	0.06	4
	9.58	0.010	<0			
Rigid sphere			None	14		71
Poly Lys (No. 2)[d]	7.20	0.053	13	0.7	0.04	4
	9.13	0.053	17	1.0	0.06	5
	10.03	0.061	30	2.4	0.15	12
	11.00	0.087				
Rigid sphere			None	16		83
Poly $Glu^{63}Lys^{37}$ (No. 3)[e]	4.67	0.088	32	7.5	0.38	38
	7.20	0.049	10	0.9	0.05	5
	9.72	0.049	7	0.6	0.03	3
	11.00	0.044				
Rigid sphere			None	20		100

[a] Measured with DNS-labeled polypeptides. [b] Measured with fluorescein-labeled polypeptides. [c] Mol. wt. 125,000. [d] Mol. wt. 105,000. [e] Mol. wt. 152,000.

polarization at pH 4 can be 85% reduced by 9 *M* urea, and this reflects destruction of the helix.

The modest increases in ρ_h^5, ρ_h^5/ρ_0^5, and V_e^5 in going from the coil to the helical form of poly Lys (No. 2) reflect a helical structure that is less rigid than that of poly $Glu^{97}Lys^3$. The midpoint of the helix–coil transition by polarization measurements (pH 9.6 in water) is approximately the same as that obtained from titration (pH 9.4 in water),[13] but lower than that from optical rotation (pH 10.0 in water).[13] This suggests that the helical form of poly Lys reaches its maximal rigidity before all of the residues have gone into the helical conformation.

When poly Lys (No. 2) in solution at pH 10.03 is heated and then cooled, it shows a melting out of rigid structure at 24°, a transition to a more rigid structure at 50°, and a return to the original, less rigid structure at 30°; the process is reversible. These changes in rigidity agree with the α-helix to β-conformation change observed in solid films.[14]

The helix–coil transition in poly $Glu^{63}Lys^{37}$ (No. 3) shows that the glutamic acid residues clearly form the dominant helix. The midpoint of the transition by polarization measurements (pH 5.2 in 0.2 *M* NaCl + 0.1 *M* phosphate or citrate buffer) is higher than that obtained by titration (pH 4.42 in 0.15 *M* KCl),[15] but the same as that from optical rotation in polypeptides of comparable composition (pH 5.2 in 0.2 *M* NaCl[12] and water[16]). Thus the changes in rigidity and conformation occur at the same time. There is no evidence for a lysine helix at alkaline pH values either by polarization of fluorescence or optical rotation; in fact, both p and $[\alpha]^{12}D$ decrease. The degree of polarization of the copolymer at pH 4 can be 85% reduced by 9 *M* urea; this reflects destruction of the glutamic acid helix.

The same conclusions concerning the relationship between changes in rigidity and helical content in the different polymers can also be substantiated by comparing the pH values at which the maximal rigidity (polarization of fluorescence) and maximal helical content (optical rotation) occur. The pH values of maximal polarization, complete titration, and maximal optical rotation, respectively, under the same conditions used to obtain the data about the midpoints of the transitions[17] are: 3.5, 2.8, and 4.5 for poly $Glu^{97}Lys^3$; 10.7, 11.0, and 11.8 for poly Lys (No. 2); and 4.2, 3.0, and 4.0 for poly $Glu^{63}Lys^{37}$ (No. 3).

The equivalent volume of the rotating segment V_e^5 in all polypeptides is considerably larger than that of DNS plus the lysine side chain (0.25×10^3 cc./mole) or fluorescein plus the lysine side chain (0.34×10^3 cc./mole). Thus the rotational unit causing depolarization is a segment of the macromolecule and not just the dye conjugated to it.

The intrinsic viscosity of poly Lys (No. 2) at 25° in 0.2 *M* NaCl + 0.1 *M* buffer (citrate or phosphate) increases monotonically and reversibly from 0.70 dl./g. at pH 7.26 to 1.65 dl./g. at pH 0.80, but the degree of polarization of the DNS-labeled polypeptide at the same temperature and salt concentration does not change. The increase in viscosity in acid solution is largely suppressed by 3.0 *M* NaCl. This phenomenon has, most likely, an electrostatic basis and indicates that polylysine expands in acid solution.

Polarization of fluorescence is a very sensitive hydrodynamic method that is useful in measuring structural transitions, including the helix–coil transition, in polypeptides. It is of particular interest when used in conjunction with spectroscopic methods to correlate the extent of conformational change and the change in hydrodynamic properties of the molecule.

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Thomas J. Gill, III, Gilbert S. Omenn
Laboratory of Chemical Pathology
Harvard Medical School, Boston, Massachusetts
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An Authenticated Perchlorate Complex

Sir:

There has recently been much interest in compounds containing the so-called weakly coordinating ligands,