SYNTHESIS AND RELATIONSHIP OF L-GLUTAMINYL-L-HISTIDYL-L-PROLINAMIDE TO THE THYROTROPIN RELEASING HORMONE

by Karl Folkers, Jaw-Kang Chang and Bruce L. Currie
The University of Texas at Austin, Texas

and

C. Y. Bowers, Alice Weil and A. V. Schally
Tulane University School of Medicine and
Veterans Administration Hospital
New Orleans, Louisiana

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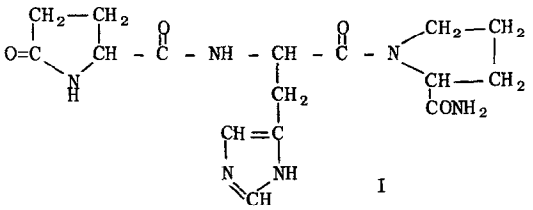
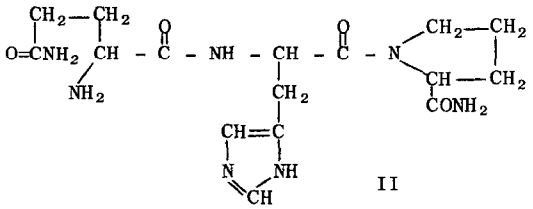
Summary. The new L-glutaminyl-L-histidyl-L-prolinamide (Gln-His-Pro(NH_2)) has been synthesized. This open glutaminyl-form has been compared with the cyclized pyro-form of (pyro)Glu-His-Pro(NH_2), thyrotropin releasing hormone (TRH). Gln-His-Pro(NH_2) is converted into (pyro)Glu-His-Pro(NH_2) under very mild conditions some of which are analogous to steps used in the isolation of TRH. Gln-His-Pro(NH_2) had up to five per cent of the activity of TRH although it is unknown whether this activity is intrinsic or due to contamination or conversion. There is no evidence that Gln-His-Pro(NH_2) is present in the hypothalamus, but if it were it could be a moiety of a larger molecule which is convertible into Gln- or (pyro)Glu-His-Pro(NH_2) or it could be a biosynthetic precursor or a metabolic transformation product of TRH. (Pyro)Glu-His-Pro(NH_2) shows the functions expected of the hormone.

The hypothalamus secretes a hormone which stimulates the anterior pituitary gland to secrete thyrotropin that in turn stimulates the thyroid gland to secrete its calorigenic hormones. By multi-step fractionation of hypothalamic extracts with the guidance of biological assays based largely on the release of thyrotropin, a substance, TRH, has been isolated and presumed to be the neurosecretory product. The recent knowledge (Folkers et al. (1)) that apparently L-pyroglutamyl-L-histidyl-L-prolinamide (I) exhibited the hormonal activities corresponding to that of the isolated porcine TRH directly led to additional data showing that the chemical and hormonal properties of the porcine TRH and synthetic (pyro)Glu-His-Pro(NH_2) are identical; in this manner, the structure of TRH was first established, and synthesis had also been achieved in this structural elucidation (Bøler et al., (2)). Concomitant structural and synthetic studies on ovine TRH led also to structure I (Burgus et al., (3)). Additional biological data on (pyro)Glu-His-Pro(NH_2) were reported (Bowers et al., (4)).

The structure of (pyro)Glu-His-Pro(NH_2) (I) has interesting features for studies on structure-hormonal activity relationships. In particular, the glutamic acid moiety is present in the cyclized or "pyro"-form rather than in the open glutaminyl-form as in II.



I



II

The L-glutaminyl-L-histidyl-L-prolinamide (II) has been synthesized by the following sequence of reactions. N-t-Butyloxycarbonyl-N^{im}-benzyl-L-histidine and prolinamide were coupled by the dicyclohexylcarbodiimide method. Treatment of the resulting protected dipeptide with hydrogen bromide in glacial acetic acid yielded N^{im}-benzyl-L-histidyl-L-prolinamide dihydrobromide which was then coupled with N-carbobenzoxy-glutamine by the mixed anhydride method to afford N-carbobenzoxy-L-glutaminyl-N^{im}-benzyl-L-histidyl-L-prolinamide. After characterization of these intermediates, hydrogenation of the protected tripeptide gave L-glutaminyl-L-histidyl-L-prolinamide (II), Gln-His-Pro(NH_2). Hydrolysis of Gln-His-Pro(NH_2) yielded the three amino acids, glutamic acid, histidine and proline; the R_f values for three chromatographic systems are in Table I.

Table I. R_f Values on Silica Gel G

Substance	R_f Values x 100 Solvent Systems*		
	I	II	III
Gln-His-Pro(NH_2)	14	38	52
(Pyro)Glu-His-Pro(NH_2) (TRH)	25	51	64

*I, n-Butanol/glacial acetic acid/ethyl acetate/water at a respective ratio of 1:1:1:1; II, n-Propanol/30% ammonia solution at a respective ratio of 70:30; III, Chloroform/methanol/30% ammonia solution in a respective ratio of 60:45:20.

The chemistry of glutamic acid served for one to predict that Gln-His-Pro(NH_2)(II) could probably be converted into (pyro)Glu-His-Pro(NH_2)(I).

The conversion of II to I was achieved. It was found that not only does the conversion of II to I take place, but only extremely mild conditions are required. Also, some of the conditions of fractionation which were actually used at given steps in the isolation of TRH from porcine and ovine hypothalami convert II to I. Examples of this conversion are as follows.

When Gln-His-Pro(NH_2)(II) was dissolved in glacial acetic acid and the mixture was refluxed for two minutes, (pyro)Glu-His-Pro(NH_2) was formed; the conversion took place slowly at room temperature in glacial acetic acid (System A). When a solution of Gln-His-Pro(NH_2) in 2 N acetic acid was allowed to stand at room temperature, conversion to (pyro)Glu-His-Pro(NH_2) was evident after several hours and was quantitative after four days (System B). When Gln-His-Pro(NH_2) was dissolved in mixture of methanol and aqueous 30% ammonia and the mixture heated at reflux for two hours, (pyro)Glu-His-Pro(NH_2) was again obtained in high yield. When Gln-His-Pro(NH_2) was dissolved in 0.1% acetic acid-n-butanol-pyridine, respective ratio of 11:5:3; (System C) and the mixture allowed to stand at room temperature for three days, analysis by TLC showed 60% conversion to (pyro)Glu-His-Pro(NH_2). The Systems A, B and C were used by Schally et al. (5) in their isolation of porcine TRH, and Systems B and C were used by Guillemin et al. (6) in their isolation of ovine TRH, and at one or more steps of the extensive fractionation procedures of these groups of investigators.

Gln-His-Pro(NH_2) has been assayed for hormonal activity in comparison with synthetic TRH. Gln-His-Pro(NH_2) was assayed in mice by the T_3-TRH method of Bowers et al.(4,7,8) which quantitatively measures the change, as Δcpm, in I^{125} in the blood before and two hours after the intravenous injection of the sample. The levels of I^{125} are proportional to the amount of the TSH released from the pituitary, the Δcpm for each dosage is an average of the changes in the levels of I^{125} in the blood from five mice. Gln-His-Pro(NH_2) and (pyro)Glu-His-Pro(NH_2) are easily separated on glass fiber paper (Gelman) impregnated with Silica Gel in a system ($CHCl_3$:CH_3OH:NH_4OH, 60:50:5; R_f-TRH, 0.45 and R_f-Gln-His-Pro(NH_2), 0.1). A sample of Gln-His-Pro(NH_2) which chromatographically showed no TRH in this system was administered to the mice. A dosage of 250-500 nanograms elicited Δcpm of 3250-6680 in comparison with Δcpm of about 4000-6000 for about 10 nanograms of TRH; Gln-His-Pro(NH_2) showed up to about 5% of the activity of TRH assuming nearly zero content of TRH, but one percent of TRH in the sample could account for all of the observed Δcpm. When this assay solution was stored over weeks in a refrigerator at a concentration of 1μg/3μl in 0.01 N acetic acid a significant conversion to (pyro)Glu-His-Pro(NH_2) was chromatographically observed. It is evident that

Gln-His-Pro(NH_2) is not readily convertible into TRH during its short transit in the blood stream before reaching the anterior pituitary gland.

These data on the facile conversion of Gln-His-Pro(NH_2) to (pyro)Glu-His-Pro(NH_2) and under certain conditions analogous to some steps used in the isolation of TRH from hypothalamic glands demonstrate that if Gln-His-Pro(NH_2) were present in the tissue, it would have been converted to (pyro)Glu-His-Pro(NH_2) during isolation. However, there is no evidence to indicate that Gln-His-Pro(NH_2) is present as a natural constituent of hypothalamic tissue. Hypothetically, Gln-His-Pro(NH_2) could be a moiety of a larger molecule in the hypothalamus which is convertible to Gln-His-Pro(NH_2) or to (pyro)Glu-His-Pro(NH_2) or it could be a biosynthetic precursor of or a metabolic transformation product of (pyro)Glu-His-Pro(NH_2).

(Pyro)Glu-His-Pro(NH_2) has repeatedly been shown in our studies and those of others that it is not only extraordinarily potent but exhibits physiological behavior expected of the hypothalamic hormone releasing TSH.

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REFERENCES

1. Folkers, K., Enzmann, F., Bøler, J., Bowers, C.Y., and Schally, A.V., Biochem. Biophys. Res. Commun., 37 (1), 123 (1969).
2. Bøler, J., Enzmann, F., Folkers, K., Bowers, C.Y., and Schally, A.V., Biochem. Biophys. Res. Commun., 37 (4), 705 (1969).
3. Burgus, R., Dunn, T.F., Desiderio, D., and Guillemin, R., C. R. Acad. Sc. Paris, 269, 1870 (1969).
4. Bowers, C. Y., Schally, A. V., Enzmann, F., Bøler, J., and Folkers, K., Endocrinology (1970) in press.
5. Schally, A. V., Redding, T. W., Bowers, C. Y., and Barrett, J. F., Journal of Biological Chemistry, 244 (15), 4077 (1969).
6. Guillemin, R., Sakiz, E., and Ward, D. N., Proc. Soc. Exptl. Biol. Med., 118 (4), 1132 (1965).
7. Bowers, C. Y., Schally, A. V., Reynolds, G. A., and Hawley, W. D., Endocrinology, 81, 741 (1967).
8. Bowers, C. Y., and Schally, A. V., (J. Meites, ed.) Proceedings of NIH Conference on Hypothalamic Hypophysiotropic Hormones, Tucson, Arizona (1969) in press.