THE STIMULATION OF TRYPTOPHAN PYRROLASE ACTIVITY BY RNA FRACTIONS ISOLATED AT VARIOUS TEMPERATURES

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Several RNA preparations possess the capacity to direct or stimulate the synthesis of a specific protein in a cell-free system. The addition of TMV RNA to a cell-free system from E. coli results in the synthesis of a TMV-like protein (Tsugita et al., 1962); the addition of an RNA fraction from "wild-type" Neurospora crassa to a cell-free system from a mutant of the same organism results in the formation of tryptophan synthetase (Wainwright and McFarlane, 1962); and the addition of E. coli RNA to a cell-free system from E. coli results in an increase in β-galactosidase activity (Novelli and Eisenstadt, 1963). The report of the extraction at 65°C of an RNA fraction with a DNA-like nucleotide composition (Georgiev and Mantieva, 1962), prompted the present authors to investigate the possibility of a variation in the capacities of RNA fractions isolated at different temperatures to act as templates for the synthesis of a specific protein in a cell-free system. The present communication describes the results of such studies using tryptophan pyrrolase as the specific protein.

MATERIALS AND METHODS

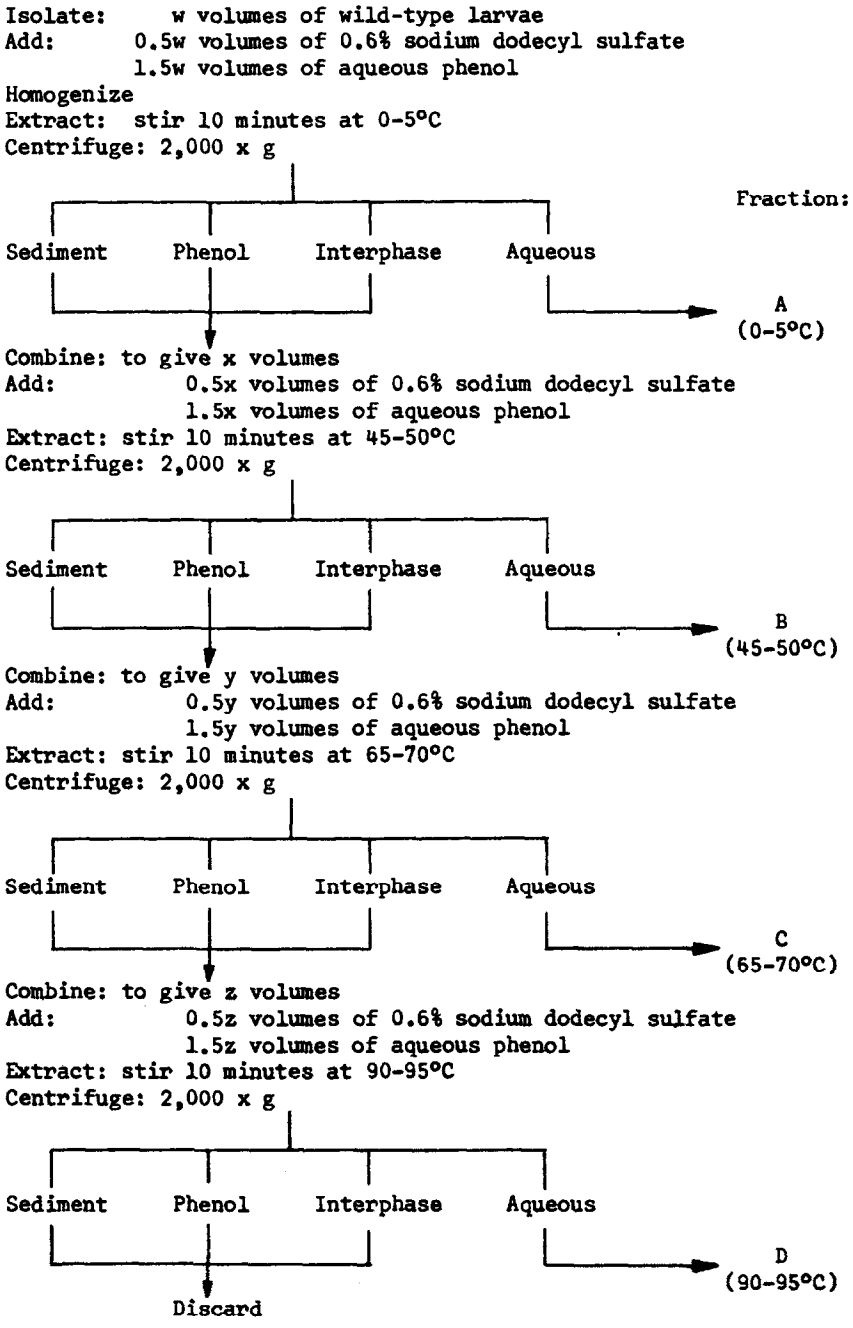
For the isolation of the RNA fractions, 50 to 100 grams of live, wild-type Drosophila larvae were suspended in 0.5 volumes of cold 0.6% sodium dodecyl sulfate and homogenized with a TenBroeck tissue grinder at 0-5°C in 1.5 volumes of freshly distilled, water-saturated phenol.

The resulting homogenate was stirred for 10 minutes at 0-5°C and centrifuged at 2,000 x g for 10 minutes. After removing the aqueous layer, the interphase was combined with the phenol layer and sediment and the mixture re-extracted by stirring for 10 minutes at 45-50°C in an additional 0.5 volumes of 0.6% sodium dodecyl sulfate and 1.5 volumes of water-saturated phenol. Following a 10 minute centrifugation at 2,000 x g, the aqueous layer was again removed and the process repeated, first at 65-70°C and finally at 90-95°C (Figure I). To determine the effect of the increasing phenol and sodium dodecyl sulfate and the increased total extraction time, the above procedure was repeated four times at 0-5°C. Adjustment of the various aqueous layers to 2% with respect to potassium acetate, the addition of two volumes of cold 95% ethanol, and storage at -20°C for 12 hours, brought about the precipitation of RNA. After collection by centrifugation and several washes with cold 95% ethanol, the RNA was dissolved in 0.05 M Tris, pH 7.8, dialyzed for 18-24 hours at 0-5°C against three changes of 0.05 M Tris, pH 7.8, and stored in the dark at 0-5°C until used.

In order to obtain initial activities of tryptophan pyrrolase which would be as low as possible, the cell-free system was prepared from a mutant (vermilion) of Drosophila melanogaster which has low levels of this enzyme (Baglioni, 1960). Approximately 50 grams of live, vermilion Drosophila larvae were homogenized at 0-5°C with 3-6 strokes of a loose fitting (0.5 mm clearance) Dounce tissue grinder in an equal volume of a buffer containing, in micromoles/ml; Tris, pH 7.8, 50; magnesium acetate, 10; potassium chloride, 100; 2-mercaptoethanol, 6; and phenylthiourea, 1.0. The clear supernatant, obtained after filtration through cheesecloth and centrifugation at 15,000 x g for 15 minutes in a Spinco Model L preparative ultracentrifuge, was added to an equal volume of an incubation medium to give a reaction mixture which contained, in

FIGURE I

ISOLATION OF THE RNA FRACTIONS



micromoles/ml: Tris, pH 7.8, 100; magnesium acetate, 10; potassium chloride, 100; mercaptoethanol, 3; phenylthiourea, 0.5; ATP, 1.0; GTP, 0.25; and creatine phosphate, 5. Sufficient creatine phosphokinase was

added to convert the creatine phosphate present.

TABLE I

THE STIMULATION OF TRYPTOPHAN PYRROLASE ACTIVITY BY RNA FRACTIONS

The various RNA fractions were adjusted to the same concentration and added to identical portions of the reaction mixture. The resulting systems, each of 10.0 ml total volume, and each containing 35 ug of one of the "added RNA" fractions, were incubated at 37°C on a Dubnoff metabolic shaker for 30 minutes, at which time aliquots were withdrawn and assayed for tryptophan pyrrolase activity by the method of Knox and Auerbach (1955). In order to minimize a possible variation due to cofactor release (Feigelson and Greengard, 1960), hematin was added to a final concentration of 1.25 ug/ml. The activity of a control (0.104± 0.005 umoles kynurenine/hour/ml), containing Tris in the place of any "added RNA", was subtracted from all experimental values obtained with the RNA fractions. Activities are reported as the mean ± one standard deviation.

Fraction	Isolation Temperature of the RNA Fraction Added to the Reaction Mixture (° C)	Increase in Tryptophan Pyrrolase Activity* (umoles kynurenine/hour/ml)
A	0-5	0
B	45-50	0
C	65-70	0.044±0.019
D	90-95	0.127±0.046

*No increase in tryptophan pyrrolase activity was observed in any of the four sequential fractions when the isolation temperature for each fraction was 0-5°C.

DISCUSSION

From the data in Table I, it is evident that RNA fraction D, isolated at 90-95°C, had two to three times the capacity to effect an increase in tryptophan pyrrolase activity as fraction C, isolated at 65-70°C, while fractions A and B isolated at 0-5°C and 45-50°C, respectively, had no stimulatory ability. Whether these increases in tryptophan pyrrolase activity represent actual de novo synthesis or merely some type of activation or release of the apoenzyme is not yet clear. For example, although the complete inhibition of the increased tryptophan pyrrolase activity by RNase treatment (see Table II) is suggestive of

TABLE II

REQUIREMENTS FOR THE RNA STIMULATION OF TRYPTOPHAN PYRROLASE ACTIVITY

Experimental conditions were the same as those described with Table I. The 90-95°C RNA fraction was added to all experimental systems to a final concentration of 3.5 ug/ml.

System Tested	Increase in Tryptophan Pyrrolase Activity (umoles kynurenine/hour/ml)
Complete	0.091
-ATP	0.038
-Energy Regenerator	0.050
-GTP	0.070
-"Added RNA"	0
+RNase	0
Zero Time Control	0

de novo synthesis rather than activation or release, the dependency of the increased activity on ATP, GTP, and an energy regenerating system could be indicative of either de novo synthesis or of an energy dependent activation or release of the apoenzyme from the particulate fraction of the cell (Pitot and Cho, 1961). Since the various reaction mixtures differed only in the nature of the RNA fractions added, the increased tryptophan pyrrolase activities, whether due to activation, release or synthesis of the apoenzyme, must nevertheless be a reflection of the action of the RNA fractions added to the cell-free system.

SUMMARY

In the sequential extraction of RNA fractions at various temperatures, the fraction obtained at 90-95°C had the greatest capacity to stimulate tryptophan pyrrolase activity.

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Erratum

Vol. 17, No. 3 (1964), in the communication "Enzymic Synthesis of D-Phenylalanyl-L-Prolyl-L-Valine, A Peptide Sequence Present in Gramicidin S," by Shiro Tomino and Kiyoshi Kurahashi, pp. 288-293:

Page 289, paragraph 1, line 7, "...centrifuged at 10,500 x g..." should read: "...centrifuged at 105,000 x g..."