STUDIES ON THE BINDING OF DOPA (3,4-DIHYDROXYPHENYLALANINE) TO tRNA

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1. Introduction

The amino acid L-dihydroxyphenylalanine (L-DOPA), the immediate precursor of dopamine, is administered in doses of up to several grams per day to patients suffering from Parkinson's disease [1–3]. Such high amounts are required to at least partially compensate [4] for the dopamine deficiency in certain regions of the brain of such patients [5–8]. The question arises whether L-DOPA, which is apparently only a transient intermediate in the biosynthesis of catecholamines and melanin, might also be incorporated into proteins under conditions of vast excess of L-DOPA. This possibility can be tested by assaying whether L-DOPA can be bound enzymatically to one or several species of transfer RNA. That this may indeed occur has been made likely by the results [9] demonstrating that tyrosyl tRNA synthetase from *E. coli* will catalyze the pyrophosphate–ATP exchange reaction not only in the presence of L-tyrosine but also with L-DOPA. In this communication we report the binding of L-DOPA to *E. coli* tRNA. However, similar experiments with the respective components from mouse cells gave no indication for the enzymatic activation of L-DOPA.

2. Materials and methods

2.1. *Materials*

E. coli tRNA was obtained from Schwarz/Mann Co. (Orangeburg, NY). Mouse liver tRNA was prepared as in [10] with the following modifications: total RNA was not extracted with 1 M NaCl but instead dissolved in equilibration buffer and directly applied to the DEAE-cellulose column. For the elution of tRNA from the column the concentration of NaCl was increased to 1 M. Ascites tRNA was a gift of Dr R. Nolan (Sandoz Forschungsinstitut, Vienna).

L-[^{3}H]DOPA was purchased from The Radiochemical Centre (Amersham). Specific activities of different samples ranged from 1.7–2.5 mCi/mmol. Labelled DOPA obtained after Jan. 1977 gave high blanks due to unspecific binding to tRNA of some unknown contaminant. This could partly be overcome by first treating the radioactive amino acid with ascorbate. For the experiments with mouse liver tRNA, 1 part [^{3}H]DOPA solution was mixed with 1 part 0.5 M ascorbate for 15 min at room temperature. Immediately before use, this mixture was adjusted to pH 6 with 0.1 N NaOH.

2.2. *Cell extracts*

For the determination of tRNA-amino acid acceptor activities crude enzyme preparations were used, derived either from *E. coli* D-10 or from mouse cells. In case of the *E. coli* extract the supernatant obtained after centrifugation at 100 000 × *g* (S-100) was passed over a DEAE-cellulose column to remove endogenous tRNA [11]. The mouse liver extract was prepared as in [12]; in one preparation the protamine sulfate and ammonium sulfate steps were omitted. The enzyme preparations were extensively dialyzed to remove trace amounts of unlabelled amino acids.

2.3. *Estimation of amino acid acceptor activities*

The assays were carried out in 100 μl incubation mixtures which were 40 mM in Hepes, pH 8, 25 mM

in KCl, 5 mM in $MgCl_2$, 1 mM in ATP, 25 μM in CTP, 1 mM in EDTA, 10 mM in dithioerythritol and contained 0.032 mg *E. coli* S-100 extract protein or 0.14–0.26 mg mouse liver S-100 extract protein, 0.035–4.4 A_{260} units tRNA, 10 μCi [^{3}H]DOPA or [^{3}H]tyrosine and, in some experiments, nonradioactive amino acids in quantities as indicated in tables 1 and 2. The mixtures were incubated for 15 min at 37°C and the reactions terminated by the addition of 3 ml of ice cold 10% trichloroacetic acid. The precipitated tRNAs were filtered under suction through Whatman GF/C filter discs. The filters were washed subsequently five times each with 5% trichloroacetic acid and finally twice with ethanol. The filters were then dried and counted in a toluene based scintillation fluid.

2.4. *Separation of tRNA by column chromatography*

For the separation of the tRNA mixture the RPC 5 technique [13] was followed. The plaskon-like material Voltalef® (Ugine Kuhlmann, Rhone) was employed as a solid support. The size of the column was 0.5 × 110 cm and it was operated under pressure at 37°C. 100 A_{260} units tRNA were applied and eluted with 2 × 200 ml NaCl gradient ranging from 0.5–0.9 M. Fractions, 4 ml, were collected. The tyrosine acceptor tests of the various fractions were as in [14].

2.5. *Identification of DOPA bound to tRNA*

After counting, the Whatman GF/C discs were washed free of scintillation chemicals. Subsequently, they were treated at 95°C for 90 min in 1 N HCl in the presence of 10 μg unlabelled DOPA. The solution was then filtered, dried and applied to Whatman 3 MM paper. DOPA was identified by descending paper chromatography (butanol:acetic acid:water = 4:1:2) and/or by paper electrophoresis at pH 1.8 (10% acetic acid adjusted with formic acid, 40 V/cm, 120 min), using authentic DOPA as marker.

3. Results and conclusions

In a first series of experiments the possibility that L-DOPA will be accepted by tRNA was assayed using both a crude enzyme and tRNA from *E. coli*. As shown in table 1, a significant amount of radioactive DOPA is bound to tRNA. To exclude a possible binding of a contaminant present in the radioactive DOPA, samples were hydrolyzed in 1 N HCl and subsequently fractioned by paper electrophoresis and chromatography. More than 80% total radioactivity present in the sample comigrated with authentic unlabelled DOPA.

Table 1
DOPA-acceptor activity of *E. coli* tRNA

Exp. no.	Additions	pmoles [^{3}H]-DOPA bound
1	[^{3}H]DOPA	13.2
	[^{3}H]DOPA, 10 pmoles tyrosine	9.9
	[^{3}H]DOPA, 50 pmoles tyrosine	2.8
	[^{3}H]DOPA, 1 nmole tyrosine	0
2	[^{3}H]DOPA	15.5
	[^{3}H]DOPA, 19 amino acids	16.2
3	[^{3}H]DOPA	6
	[^{3}H]DOPA, tyrosine	0

Experiments 1 and 2: 0.3 A_{260} units of *E. coli* B tRNA was incubated as described in the experimental section in the presence of 4.8 nmoles (expt. 1) and 4.2 nmoles (expt. 2) L-[^{3}H]DOPA with and without the addition of nonradioactive amino acids. In experiment 1, L-tyrosine was added in amounts as indicated in the table. In experiment 2, a mixture of 19 amino acids minus tyrosine, 20 pmoles each, was added. The [^{3}H]tyrosine acceptor activity of the tRNA, assayed under similar conditions, was 17.2 pmoles.

Experiment 3: 0.035 A_{260} units of an enriched tyrosine specific tRNA, purified by RCP-5, was incubated in the presence of 5.9 nmoles L-[^{3}H]DOPA with and without 0.5 nmoles of nonradioactive L-tyrosine. In all three experiments, a blank of 2–2.2 pmoles was substracted from each measurement

Binding of labelled DOPA to tRNA was strongly inhibited in the presence of unlabelled L-tyrosine (exp. 1, table 1). The affinity for tyrosine must be at least two orders of magnitude higher than for L-DOPA. It appears that only L-tyrosine, but none of the other 19 amino acids, can suppress the activation of DOPA. In line with this is the observation that DOPA also binds to a fraction of tRNA enriched for $tRNA^{tyr}$ by RPC-5 chromatography and that this binding is again inhibited by L-tyrosine (exp. 3, table 1).

Similar experiments were performed with an extract from mouse liver and tRNA from the same source or from Krebs II mouse ascites cells. As shown in table 2, variable binding of radioactivity to tRNA was observed. However, this binding could not be

Table 2
Binding of radioactive DOPA to mouse tRNA

Exp. no.	Additions	[^{3}H]-DOPA bound (pmol)
1	[^{3}H]DOPA	8.17
	[^{3}H]DOPA, tyrosine	8.29
	[^{3}H]DOPA, no tRNA	5.30
2	[^{3}H]DOPA	6.60
	[^{3}H]DOPA, no enzyme	8.70

Exp. 1: Ascites tRNA (4.3 A_{260} units) was incubated in the presence of mouse liver extract and 4.8 nmol L-[^{3}H]DOPA with and without 1 nmol nonradioactive tyrosine.

Exp. 2: Mouse liver tRNA (3.6 A_{260} units) was incubated with radioactive DOPA (3.4 nmol) as above, in this case with a mouse liver extract from which endogenous tRNAs had been removed by treatment with protamine sulfate. For this experiment, the [^{3}H]DOPA has been pretreated with ascorbate (see section 2).

In a similar assay, the acceptor activity of ascites tRNA for [^{3}H]tyrosine was 32.3 pmol

prevented by adding unlabelled tyrosine to the samples or by incubating tRNA and [^{3}H]DOPA in the absence of enzyme (mouse liver extract). Moreover, after cleavage of the bound radioactivity with 1 N HCl and subsequent fractionation by paper electrophoresis at pH 1.8, less than 10% total label co-migrated with authentic DOPA, both in complete samples and in controls. We were thus observing unspecific, non-enzymatic binding of a contaminant, probably an oxidation product, present in the radioactive DOPA. This unspecific binding apparently increased with the age of the [^{3}H]DOPA preparation and batches of radioactive amino acid purchased in 1977 gave distinctly higher values than earlier ones. In all these experiments we could not obtain any evidence for the charging of L-DOPA to mouse tRNA with mouse liver activating enzymes. In exp. 2, table 2, the mouse liver extract had been freed of endogenous tRNA in order to avoid any possible contamination of the test system by tyrosine which could have been liberated from tyrosyl-tRNA. However, as the experiments of table 2 show, neither of the S-100 extracts containing or lacking endogenous tRNA promoted binding of L-DOPA to liver or ascites tRNA. The mouse liver extract was also inactive in assays with tRNA from *E. coli* (data not shown).

Our results demonstrate that L-DOPA can indeed be enzymatically bound to *E. coli* $tRNA^{tyr}$. It is noteworthy that the enzymatic binding of L-DOPA to tRNA was observed under the standard conditions used for assaying the acceptor activity of amino acids. Special additions, like ethanol etc. required to induce mischarging are not necessary for the binding of L-DOPA in the homologous *E. coli* system.

In the mouse system, enzymatic binding of DOPA to tRNA could not be demonstrated. Apparently this animal system has a more efficient way of discriminating between L-tyrosine and L-DOPA. Since a similar situation can be expected to exist in human cells, the formation of DOPA-containing proteins, even in the presence of 'unphysiologically' large concentrations of L-DOPA as used in the treatment of Parkinson's disease, is most unlikely.

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