L-ASPARAGINE BIOSYNTHESIS BY NUTRITIONAL VARIANTS OF THE JENSEN SARCOMA

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Despite the wide occurrence of asparagine in mammalian tissue proteins, little is known of its biosynthesis. The report that asparagine was formed by reversible amide nitrogen transfer from glutamine to aspartic acid in liver slices (Mardashev and Lestrovoya, 1951) has not been confirmed and another explanation for the results has been advanced (Hsu, 1959). Recently, Arfin (1965) reported asparagine synthesis from aspartic acid and glutamine which disappears upon maturation in chick embryos. Levintow (1957) has shown conversion of glutamine amide nitrogen (N^{15}), but not N^{15}-NH_3, to asparagine amide nitrogen in growing cultures of HeLa cells.

A radiometric assay designed to study the biosynthesis of asparagine will be described in this report. This assay was applied to cell-free preparations of the Jensen sarcoma and its nutritional variants (McCoy et al., 1955), which in contrast to the parent cells do not require exogenous asparagine for proliferation in culture. The system included the pH 5 enzyme protein from the tissue, Mg^{++}, ATP, and a nitrogen source, glutamine or ammonium ion, the glutamine having the greater affinity for the enzyme.

METHODS

All tumors were carried as intramuscular transplants for seven days. The tumors were excised, freed of stromal and necrotic elements, homogenized in a Potter-Elvejhem homogenizer, and the pH 5 enzyme fraction isolated (Keller and Zamecnik, 1956). The pH 5 enzyme fraction was dissolved in a solution containing 0.05 M glutathione, 0.005 M EDTA, and 0.10 Tris buffer, pH 7.5 (Allen, Glassman, and Schweet, 1960) and used without further treatment. This fraction could be stored frozen over a period of several months without loss of activity.

Where applicable, the incubation mixture consisted of 1.5 mM L-aspartic acid-U-C^{14} (10^6 cpm/μm), 20 mM L-glutamine or NH_4Cl, 10 mM K-ATP, 10 mM $MgCl_2$, 100 mM Tris buffer (pH 8.0), and 1.5 mg protein in a final volume of 1.0 ml. The mixture was incubated for 30 minutes at 37° C and the reaction stopped by addition of 1.0 ml of cold 0.8 M perchloric acid. After centrifugation the supernatant was neutralized with KOH, centrifuged to remove the salt, and free asparagine-C^{14} isolated from the supernatant (Patterson, Orr, and McCoy, 1963). Paper ionophoresis was carried out at pH 5.9 using K-103 buffer (Kensco Model 50 Electrophoresis Apparatus). A partially purified guinea pig serum asparaginase preparation, previously described (Patterson et al., 1965), was prepared according to Meister (1955).

RESULTS AND DISCUSSION

The properties of the enzyme system are shown in Fig. 1. The reaction was linear for the first 45 minutes of incubation (Fig. 1-A) and for the amount of protein added to the system (Fig. 1-B). A pH optimum of 8.0 was found when Tris buffer was used (Fig. 1-C); phosphate buffers appeared to inhibit the reaction since at pH 7.5 it gave 35% of the activity observed when Tris buffer pH 7.5 was used. Fig. 1-D shows the enzyme was saturated at 2.0 mM

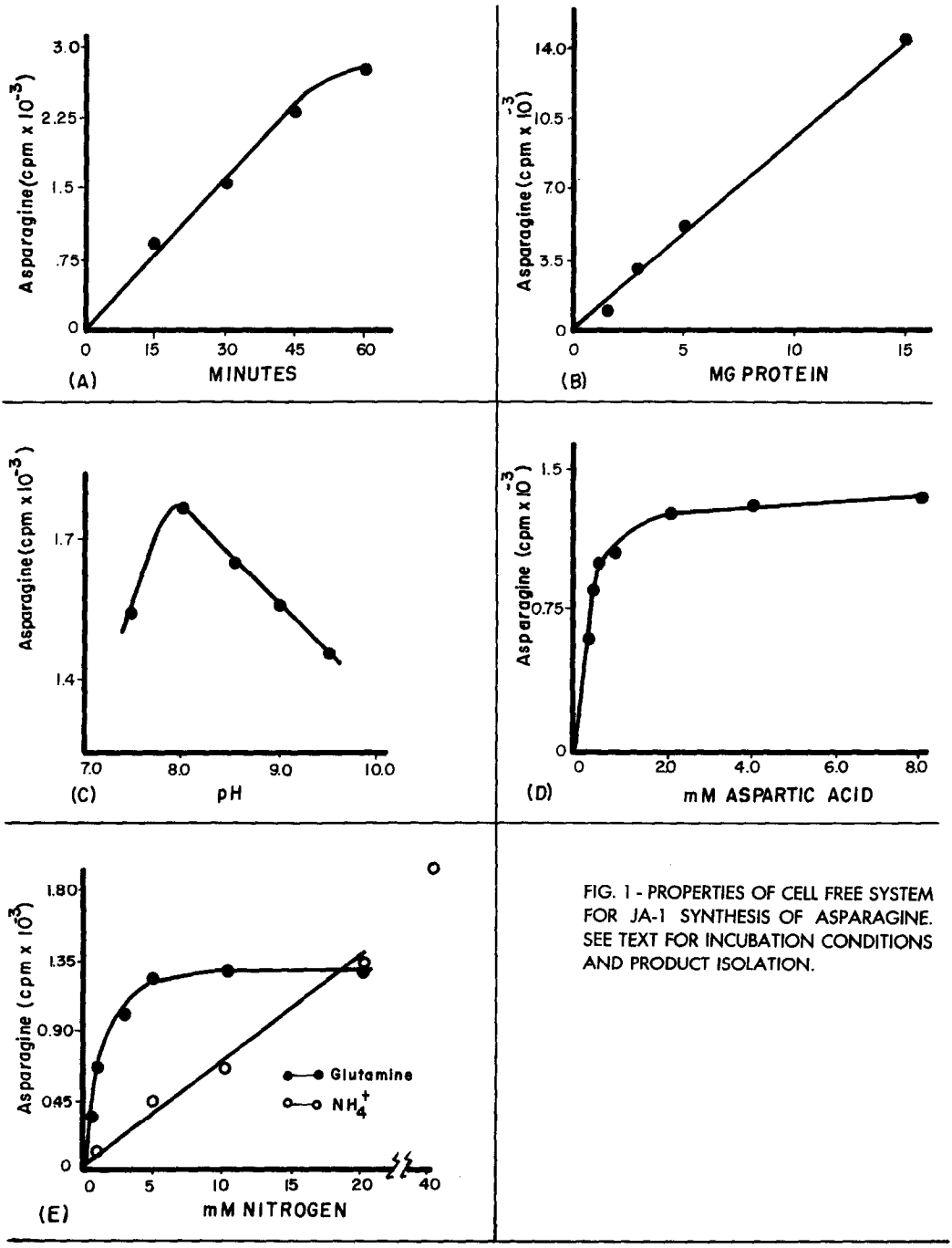

FIG. 1 - PROPERTIES OF CELL FREE SYSTEM FOR JA-1 SYNTHESIS OF ASPARAGINE. SEE TEXT FOR INCUBATION CONDITIONS AND PRODUCT ISOLATION.

aspartic acid. Glutamine or ammonium ion served as a nitrogen source (Fig. 1-E). Glutamine was the more effective nitrogen donor, however, since

5.0 mM glutamine saturated the system while 20.0 mM ammonium ion was required for an equivalent activity. Both appeared to be functioning as alternate substrates since the addition of 10 or 20 mM NH_4^+ did not give an additive increase in asparagine biosynthesis at saturation levels (10 or 20 mM) of glutamine. Although this would be indicative of a single enzyme, other possibilities have not been excluded and are presently under investigation.

Enzyme activity was destroyed by heating at 45° for 10 minutes in the absence of substrate. If Mn^{++} was substituted for Mg^{++} at 1.0 mM or 10 mM, 29% and 16%, respectively, of the original activity was observed. Concentrations above 10 mM of either ATP or Mg^{++} inhibited the reaction.

The product of the reaction had an ionic mobility in electrophoresis similar to that of authentic asparagine and was resolved from aspartic acid-C^{14}. When asparaginase was added to the incubation mixture at time 0 and 30 minutes and incubated an additional 30 minutes, over 90% of the product was destroyed (Table I). Similar effects on the product were seen when either

TABLE I

EFFECT OF ASPARAGINASE ON PRODUCT OF REACTION*

Asparaginase	Time (mins)	Substrate	
		Glutamine	NH_4Cl
–	30	1222	1173
+	30†	67	52
–	60	2281	2254
+	60‡	209	126

*Values expressed as cpm in asparagine/mg protein.
†Asparaginase added at time 0.
‡Asparaginase added at time 30 minutes.

glutamine or NH_4Cl were used as the nitrogen donor. Addition of asparagine to the incubation mixture, followed by the column isolation procedure, showed the radioactive and ninhydrin peaks to coincide. The specific activity of the fractions under the peak remained constant.

Table II shows the range of enzyme activities obtained in the parent Jensen sarcoma, two nutritional variants, and liver, and the effect of substrate deletions. The nutritional variants showed a 3- to 6-fold increase over that

TABLE II

ASPARAGINE BIOSYNTHESIS BY JENSEN SARCOMA AND NUTRITIONALLY VARIANT CELLS*

Tissue	Deletions from Substrate			
	None	Gltm	ATP	Mg^{++}
Jensen	213- 299	20- 67	0	0
JA-1	882-1798	118-134	0	0-19
JA-2	875-1667	68-163	0-6	0
Liver	97- 120	0- 13	0-7	0

*Each value represents a pool of tissue from three animals. Values expressed as cpm in asparagine/mg protein.

found in the Jensen sarcoma. These activities appear to reflect the asparagine requirement of the tissues in culture. Similar differences were seen between the Walker 256 (434 cpm/mg protein) and its nutritional variant (1339 cpm/mg protein). In one experiment the Novikoff hepatoma, which does not require asparagine, when carried as an intraperitoneal transplant showed a 68-fold higher activity than the JA tumors.

From preliminary studies it appears that JA tumor cells cultured in media devoid of asparagine have higher enzymic activity than when the cells

are cultured in the presence of asparagine. This effect is under investigation. If such an effect is operative in vivo, then an explanation for the previous difficulties in detecting this enzyme in fresh tissues may be forthcoming.

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