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Artificial Cell-Microencapsulated Phenylalanine Ammonia-Lyase

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ABSTRACT

Phenylalanine ammonia-lyase (PAL) is immobilized in collodion artificial cells. Once technical problems associated with the encapsulation of this enzyme were solved, the enzyme kinetics were compared to PAL in free solution. Microencapsulated PAL has an apparent enzyme activity that is 20% of the activity of enzyme in free solution. The K_m for both free and immobilized PAL is 475 μM. The V_m for the microencapsulated PAL is 9 μM/min, whereas that of PAL in free solution is 55 μM/min.

Index Entries: Phenylalanine; phenylalanine ammonia-lyase, encapsulated; microcapsules, of phenylalanine ammonia-lyase; artificial cells, of microencapsulated phenylalanine ammonia-lyase.

INTRODUCTION

Classical phenylketonuria (PKU) is the result of a genetic deficiency of the enzyme, phenylalanine hydroxylase, which normally degrades the amino acid phenylalanine. This deficiency is characterized by an elevated level of phenylalanine. In liver failure, there is an elevation of three aromatic amino acids, one of which is phenylalanine. An enzyme that degrades phenylalanine is available commercially in the form of phenylalanine ammonia-lyase (PAL). The present study is to microencapsulate PAL inside collodion artificial cells and to study the in vitro enzyme kinetics.

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MATERIALS AND METHODS

L-Phenylalanine (Sigma Chemical Co.) was dissolved in 0.1*M* Tris-HCl buffer, pH 8.5. Phenylalanine ammonia-lyase (PAL) (EC 4.3.2.5) was obtained in two forms: (1) from Sigma Chemical Co. in a 60% glycerol Tris-HCl, pH 7.0, medium with an activity of 1.9 μmol/min/mg and (2) from P-L chemicals in a 10 m*M* phosphate buffer (pH 7.0) with an activity of 1 μmol/min/mg.

Preparation of Semipermeable Microcapsules

Collodion semipermeable aqueous microcapsules containing PAL were prepared according to the method described (*1–3*).

Phenylalanine Ammonia-Lyase Activity

The spectrophotometric method of Shen and Abell (*4*) was used to determine PAL activity.

To test for leakage of the enzyme from the microcapsules, a 0.5 mL volume of PAL-containing microcapsules was suspended in 0.5 mL Tris-HCl, pH 8.5, at 4°C. At regular intervals an aliquot of the supernatant was tested for enzyme activity.

RESULTS AND DISCUSSION

Microencapsulation of PAL in 60% glycerol presented a major problem, since the glycerol prevented proper formation of the artificial cell membranes resulting in leakage of PAL. The glycerol was removed using gel chromatography before microencapsulation. In this form, microencapsulation could be carried out successfully. However, gel chromatography to remove glycerol markedly decreased the enzyme activity of the PAL.

The next approach was to use the less purified enzyme in phosphate buffer (P-L Chemical). Direct microencapsulation of this enzyme preparation could be carried out successfully. The assay showed that microencapsulated PAL has an apparent activity that is 20% of the enzyme in free solution. There was no enzyme activity in the supernatant, indicating no enzyme leakage. Enzyme kinetics (K_m and V_{max} values) were determined for both PAL in solution and the microencapsulated PAL (Fig. 1). The K_m of 475 μ*M* is the same for both free and microencapsulated PAL (Table 1). The V_{max} of the PAL-loaded microcapsule is 9 μmol/min, while that of PAL in free solution is 55 μmol/min (Table 1). Oral administrations to phenylketonuria rats resulted in significant decreases in blood phenylalanine levels.

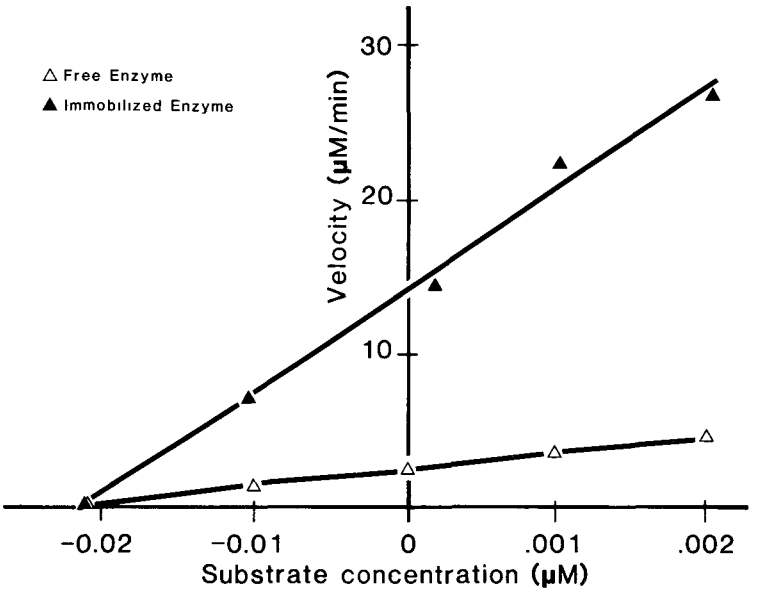

Fig. 1. Enzyme kinetics of PAL enzyme in solution (△) and in collodion membrane microcapsules (▲).

TABLE 1
Enzyme Kinetics of Phenylalanine Ammonia-Lyase

	K_m, μM	V_{max}, μmol/min
Free enzyme	475	55
Microencapsulated enzyme	475	9

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REFERENCES

1. Chang, T. M. S. (1964), *Science* **146,** 524.
2. Chang, T. M. S., MacIntosh, F. C., and Mason, S. G. (1966), *Can. J. Physiol. Pharmacol.* **44,** 115.
3. Chang, T. M. S., *Artificial Cells,* 1st edn., Charles C Thomas, Springfield, IL, 1972, p. 207.
4. Shen, R. S., and Abell, C. W. (1977), *Science* **197,** 665.