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Affinity Precipitation of Enzymes

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Abstract

Lactate dehydrogenase has been purified by precipitation with a bis-ligand. The precipitating agent in this case was Bis-NAD. This approach of affinity precipitation is also applicable to other enzymes.

Index Entries: Affinity separation, of lactate dehydrogenase; separation, affinity, of lactate dehydrogenase; lactate dehydrogenose, affinity separation of; dehydrogenase, lactate, affinity separation of; precipitation, affinity, of lactate dehydrogenase; Bis-NAD, in affinity separation of lactate dehydrogenase; NAD, in affinity separation of lactate dehydrogenase.

Introduction

Affinity precipitation is a novel technique related to affinity chromatography (*1*). The essence of the technique is the specific interaction between an enzyme and a bisfunctional ligand (bis-ligand = two ligand molecules connected by a spacer. The spacer is sufficiently long to allow the two ligand units to interact simultaneously with two enzyme molecules).

Discussion

One example of affinity precipitation is the biospecific precipitation of lactate dehydrogenase. By making use of ternary complex formation between LDH, Bis-

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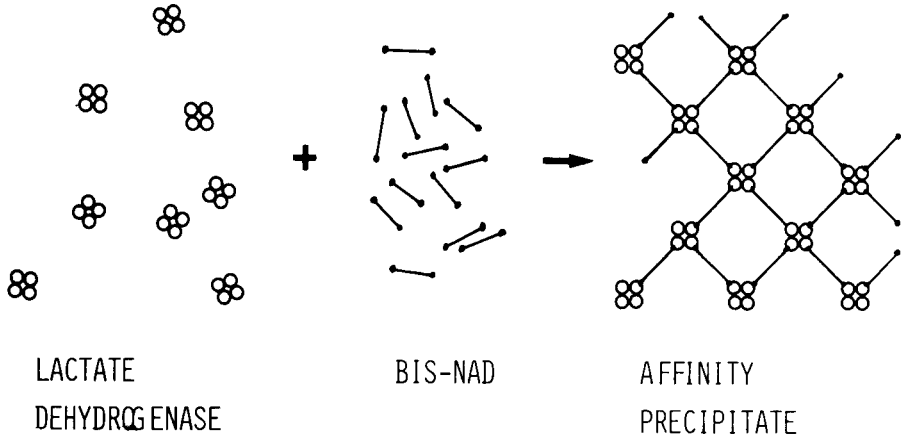

Fig. 1. Schematic visualization of affinity precipitation of lactate dehydrogenase.

NAD, and a third component (oxalate), strong complexes will form that eventually grow large enough to cause efficient precipitation of the enzyme (Fig. 1).

Affinity precipitation is a useful preparative technique. Lactate dehydrogenase was thus affinity precipitated directly from a crude extract of beef heart. A purification scheme is shown in Fig. 2, the details will be published elsewhere (*2*).

In a typical preparative affinity precipitation the starting crude extract contained 6.5 g protein (totally 200 mL) and the specific activity of the enzyme was 3 U/mg. In the final $(NH_4)_2SO_4$ precipitate, the amount protein was 100 mg and the specific activity was 150 U/mg. The total recovery of enzyme was 80% (or more).

In Table 1 affinity precipitation is shown to compare well with other techniques for purification/preparation of enzymes.

The main drawback is the narrow range of application. Affinity precipitation should, however, be applicable also to other enzymes or proteins than dehydrogenases if suitable bisfunctional ligands are identified.

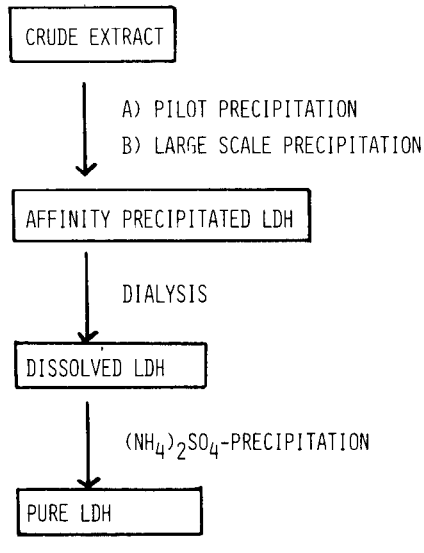

Fig. 2. Preparative affinity precipitation of lactate dehydrogenase, general outline.

TABLE I
Comparison of Enzyme Purification Methods

Method	Equipment demand	Speed of operation	Specificity	Range of application
Conventional (e.g., ion exchange, gel filtration, salt precipitation)	Medium/high	Slow	Low	Wide
Affinity chromatography	Medium	Medium/fast	High	Medium
Affinity precipitation	Low	Medium/fast	High	Narrow

Affinity precipitation could also be of value in contexts other than enzyme purification, for example, as an analytical tool, as an aid in morphological studies of proteins, and as a technique for reversible enzyme immobilization.

References

1. Larsson, P.-O., and Mosbach, K. (1979), *FEBS Lett.* **98,** 333.
2. Flygare, S., Griffin, T., Larsson, P.-O., and Mosbach, K., to be published.