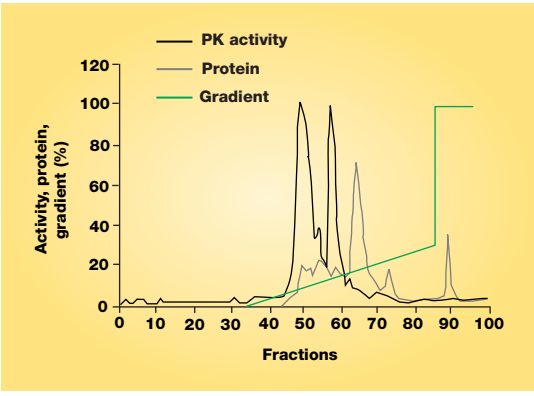
- **INCORPORATING UNO IN THIS PURIFICATION SCHEME ALLOWED THE USE OF A BUFFER PH, RESULTING IN INCREASED STABILITY OF THE PRODUCT**
- **THE STABILIZING AGENT, REQUIRED WHEN USING A CONVENTIONAL COLUMN, COULD BE OMITTED FROM THE UNO PROCESS**

Purification of Two Isoforms of Pyruvate Kinase from Nordic Krill Using an UNO™ Q-1 Column

Introduction

Pyruvate kinase (PK, E.C. 2.7.1.40) is one of the key regulatory enzymes of the glycolytic pathway in crustaceans. This enzyme catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate and the phosphorylation of ADP to ATP. In addition to the role of PK in aerobic and anaerobic energy metabolism in invertebrates, regulation of PK activity in response to changes in temperature and oxygen regimes was found to occur in marine mollusks. Others reported changes in PK enzyme characteristics with temperature in fish and the intertidal mussels, suggesting that modification of the biochemical properties of PK may also play a role in temperature adaptation. The Nordic krill (*Meganyctiphanes norvegica*) is a crustacean which thrives in the open sea under aerobic conditions and a wide range of temperatures. Purification of two isoforms of PK (PK I and PK II) from this species was required to facilitate kinetic and chromatographic studies of these enzymes relating to seasonal temperature adaptation.



Purification

Nordic krill were caught in February and July, deep-frozen and stored at -80 °C. For a single extract, two individuals (about 0.4 g) were homogenized in 2.7 ml extraction buffer (50 mM Tris-HCl, 60 mM KCl, 4 mM $MgSO_4$, pH 7.0) with an Ultra-Turrax homogenizer. The suspension was centrifuged and the supernatant was buffer-exchanged into column buffer by gel filtration chromatography. The buffer-exchanged extract was filtered and applied to an UNO Q-1 column (Bio-Rad catalog # 720-0001, see Figure 1). PK I and PK II were well-separated by this procedure at pH 8.0. The previous protocol, using a popular high resolution beaded support, required the use of pH 9.0 buffer to prepare the PK I species, at which pH the enzyme is less stable. The addition of PEP as a substrate stabilizing agent was also required in this pH 9.0 system but not for the UNO-based system. The purified enzyme obtained by this purification process was used to determine that the PK I of Nordic krill does, indeed, exhibit altered physicochemical and kinetic properties with the seasons and may contribute to the temperature adaptations of this species.

Reference

Vetter, R-A.H., and Buchholz, F., Comp. Biochem. Physiol., ***116A****, 1–10 (1997).*

Data courtesy of Dr. Rolph-Achim Vetter, Biologische Anstalt Helgoland, Meeresstation.

Fig. 1*. Sample: 5 ml extract; Column: UNO Q1 column; Buffer A: 20 mM Tris-HCl pH 8.0; Buffer B: A+1 M NaCl, pH 8.0; Gradient: 0% B in 10 ml, 0–30% B in 20 ml, 100% B in 5 ml; Flow rate: 4 ml/min.*

BIO-RAD
Bio-Rad Laboratories
Life Science Group

Website www.bio-rad.com **U.S.** (800) 4BIORAD **Australia** 02 9914 2800 **Austria** (01) 877 89 01 **Belgium** 09-385 55 11 **Canada** (905) 712-2771 **China** 86-10-62051650 **Denmark** 45 39 17 99 47 **Finland** 358 (9) 804 2200 **France** (01) 43 90 46 90 **Germany** 089 318 84-0 **Hong Kong** 852-2789-3300 **India** (91-11) 461-6103 **Israel** 03 951 4127 **Italy** 02 21609.1 **Japan** 03-5811-6270 **Korea** 82-2-3473-4460 **The Netherlands** 31 318-540666 **New Zealand** 64-9-4152280 **Singapore** 65-2729877 **Spain** (91) 661 70 85 **Sweden** 46 (8) 627 50 00 **Switzerland** 01-809 55 55 **United Kingdom** 0800-181134